DOCTOR OF PHILOSOPHY

The Design, Build and Validation of a Realistic Artificial Mouth Model for Dental Erosion Research

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Award date:
2015

Awarding institution:
University of Dundee

THE DESIGN, BUILD AND VALIDATION OF A REALISTIC ARTIFICIAL MOUTH MODEL FOR DENTAL EROSION RESEARCH

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PhD THESIS

THE DESIGN, BUILD AND VALIDATION OF A REALISTIC ARTIFICIAL MOUTH MODEL FOR DENTAL EROSION RESEARCH

A thesis submitted to the

University of Dundee

for the degree of

DOCTOR OF PHILOSOPHY

by

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December 2015

Restorative Dentistry

Dundee Dental Hospital and School

University of Dundee

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This thesis is dedicated in loving memory to my father

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to:

- Prof. Graham Chadwick and Dr. Andrew Mason for granting me the opportunity for this study, and for providing encouraging support and valuable advice. Without their guidance, this undertaking would have been impossible.
- Mrs. Jacqueline Cox, Ms. Karen Howat, Dr. Maggie Florence, Mr. Christopher 0 Pratt, Mrs. Valerie Wilson, Mr. Evangelos Sotiropoulos and Mr. Amer Alwarea for technical assistance and general support of this work.
- My family, friends, and most especially, Rayhana, my beloved wife, for her continued encouragement and support throughout.

DECLARATION

I hereby declare that, all the work described in this thesis is my own original work and that I have consulted all the references cited. This work has been carried out in the dental materials and biochemistry laboratories of Dundee Dental School, under the supervision of Prof. RG Chadwick and Dr. AG Mason.

Signature ……..

Date……..

AS Qutieshat

CERTIFICATE

I hereby certify that Abubaker S. Qutieshat has fulfilled the condition of Ordinance 39 of the University of Dundee and is qualified to submit this thesis for the degree of doctor of philosophy.

Professor R. G. Chadwick

Professor of Operative Dentistry and Dental Materials Science, Hon Consultant in Restorative Dentistry

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ABSTRACT

This work investigated the design parameters necessary for the build and use of an *in vitro* artificial mouth model built for dental erosion research. It also ascertained the working knowledge of dentists concerning the Human Tissue Act (HTA) and explored an alternative tissue for erosion-testing to human enamel. The design inputs for the artificial mouth were acquired by an innovative observational study conducted upon human volunteers and used in the decisions made in the setting of the fluids' kinematic behaviour and how the associated devices were to function. This novel system was sought to mimic the interaction of saliva and the dental substrate during the process of consuming an erosive beverage. The model allows researchers to gather data using customizable experimental diets without the technical burden of dealing with a non-realistic regime. The design and build of the artificial mouth model along with its associated equipment and parameters are described and a manual for operation of the model is appended. The device is designed on a fully adjustable multitask basis in which the operator can set several variables such as the desirable salivary kinematic behaviour, offensive beverage flow rate, and volume of consumption. This, subsequently, allows the samples preloaded on the system to be tested for surface characteristics (i.e. surface hardness and surface profilometry) to determine the extent of erosion if any. The model also allows the resultant solution to be analysed for traces of calcium and phosphate ions. To validate the capabilities of the artificial mouth system a set of diets was performed repeatedly. The high degree of agreement and the consistency of results showed that the model is able to mimic realistic scenarios and is capable of producing reliable, reproducible and accurate outcomes. Ostrich eggshell proved to be a potential alternative erosion substrate which is fortuitous as the lack of knowledge on the HTA had meant human enamel was less readily available.

CHAPTER 1

1. Introduction, Aims and Objectives

This work started off with a clear perception of the problems involved with the use of human teeth in dental laboratory research. The implications of the Human Tissue Act for the collection of teeth were therefore investigated to determine whether there was a need for an alternative tissue. However, there is a reason to suppose that it was, in view of the decline in the number of published dental articles as a result of work undertaken in the United Kingdom utilising human teeth under *in vitro* conditions since 2006 which was the year when the Act came into force.

Many papers are reported in the dental literature that seek to evaluate the erosiveness of foods and drinks by laboratory assessment. Commonly these use a range of accepted laboratory techniques to reach their conclusions. Often, in order to obtain measurable effects, the regimes adopted to expose the tooth tissues to the food/beverage under investigation are severe representing many cumulative exposures. They thus do not represent what could be considered as normal eating/drinking behaviour but atypical behaviours of greater erosive risk. If more realistic testing regimes are to be developed it is important that an assessment of normal eating and drinking behaviour is made. Surprisingly the literature contains few reports of this and where it does it is concerned with the consumption of hard rather than soft drinks. As the first stage in the development of an artificial mouth for erosion testing; this work sought to determine normal drinking behaviour, by means of an observational study conducted upon human volunteers.

In this thesis, an artificial mouth model system that adopts a realistic erosion testing regime for dental erosion research was designed, built and validated. This novel system was sought to mimic the interaction of saliva and the dental substrate during the process of consuming an erosive beverage. The model allows researchers to gather data using customizable experimental diets without the technical burden of dealing with a non-realistic regime. The design and build of the artificial mouth model along with its associated equipment and parameters are described and a manual for operation of the model is appended.

The device is designed on a fully adjustable multitask basis in which the operator can set several variables such as the desirable salivary kinematic behaviour, offensive beverage flow rate and volume of consumption. This, subsequently, allows the samples preloaded on the system to be tested for surface characteristics (i.e. surface hardness and surface profilometry) to determine the extent of erosion if any. The model also allows the resultant solution to be analysed for traces of calcium and phosphate ions.

Photo taken by RG Chadwick, October, 2014.

In this work, and using the novel realistic artificial mouth model; the suitability of a tissue, that was never been implemented in dental research, the Ostrich eggshell, to substitute human enamel *in vitro* was assessed. The ostrich (Struthio camelus) egg is the largest among all other avian species with a shell thickness of about 2 mm. Compared to other avian species, Ostrich eggshell lacks the cuticle layer or any shell accessory material which renders its outer most layer suitable for immediate surface assessment without the extra burden of having to pre-prepare the surface. In addition,

the vertical crystal layer is characterized by an amorphous crystalline structure with no evidence of porosities. As a result of this and the fact that the egg shell can reach up to 2 mm thick in thickness, it has the potential to be considered as a favourable substrate upon which several surface tests can be conducted. This unique eggshell composition allows for better control when preparing test samples and eases their cutting into desirable shapes and sizes benefiting from its dense structure and convenient thickness.

To validate the artificial mouth model, experimental diets were performed repeatedly using specimens prepared from ostrich eggshells, and compared to those obtained from extracted human teeth specimens for the same simulated experimental setup. In addition, other applications such as examining the efficacy of potential protective agents against dental erosion and assessing the effect of different beverages were also performed. The high degree of agreement and the consistency of results showed that our model is able to mimic realistic scenarios and is capable of producing reliable, reproducible and accurate outcomes. This capability, the author believes, allows for a much more realistic and natural data representation, and provides a solution to the inconsistent experimental settings found in previous *in vitro* models which would in turn not only widen the scope of *in vitro* dental erosion research but also improve the reproducibility and comparability of the results.

To date, this is the first study to report human drinking behaviour values for carbonated beverages in a social environment. In addition, Saltus is the first *in vitro* artificial mouth model that simulates natural human drinking behaviour in terms of test beverage flow rate and quantity; temperature; consumption time period; as well as normal physiological stimulated and unstimulated saliva flow rates.

The aims and objectives of this work were to;

- Assess the level of knowledge and understanding of the Human Tissue Act among UK dentists and its implications for dental research.
- Determine the normal drinking behaviour by means of an observational study conducted upon human volunteers.
- Inform a realistic erosion testing regime based on human drinking behaviour values.
- Design and build an artificial mouth model for dental erosion research.
- Validate the artificial mouth system.
- Assess the suitability of using ostrich eggshell as an alternative to human enamel in erosion testing.

CHAPTER 2

2. Literature Review

Much has been written about dental erosion. This literature review focuses upon the test methods used to evaluate it with a summary of the essential points about the condition, aetiology, risk factors and protective factors. The main body of this thesis is concerned with erosion testing; this generally uses a substrate that is commonly human enamel or bovine enamel, but as it is perceived to be getting difficult to obtain these, the Human Tissue Act is reviewed together with a potential alternative substrate that does not require ethical approval. Any erosion testing regime should also take into account the effects of saliva and so this review also covers saliva physiology and biochemistry.

2.1 Dental Erosion

Dental erosion can be defined as a cumulative lifetime process where tooth structures, enamel and dentine, are lost as a result of chemical attack other than from those chemicals produced intraorally by cariogenic bacteria (Chadwick, 2006). This differentiates it from dental caries where the damaging acid is produced by carbohydrate fermentation by microorganisms that reside in dental plaque.

Occurrence of the main oral disease, dental caries, is inversely proportional to increased oral health awareness (Touger-Decker and Van Loveren, 2003). As such awareness has increased over the years a considerable decrease of tooth decay in many societies has been seen. As a result, ever-increasing attention is targeted towards the dental wear process that includes dental erosion (Lussi et al., 2004) as

investigatory resources, previously used for caries, are freed up. An understanding of dental erosion is complicated by its multifactorial nature. Therefore, there is no single factor that can be considered as the determinant indicator for the occurrence of dental erosion.

2.1.1 Epidemiology

Generally, dental erosion is more commonly evident during late adolescence and early adulthood. This coincides with the time when patients start to be more enthusiastic about their appearance and oral health. In the current literature, dental erosion appears to be prevalent ranging from 5 to 100 % among children and adolescents, and from 76 and 100 % among adults (Van't Spijker et al., 2008, Jaeggi and Lussi, 2006). Clearly, its occurrence can be a serious problem especially if the process of erosion continues throughout adulthood resulting in cumulative loss of considerable amounts of tooth structure where preventive measures are not successful. In contrast, tooth wear that involves the wear processes of erosion, abrasion and attrition has been found to be clinically common and positively associated with age (Van't Spijker et al., 2008, Milosevic and Lo, 1996).

2.1.2 Aetiology and risk factors

The sources of acids that risk erosion may be from within the body (intrinsic) or from external sources (extrinsic).

I. Intrinsic factors

Any voluntary or non-voluntary human action that results in the release of acid from the gastrointestinal tract to the oral cavity is categorized as intrinsic. This permits internal acids to come into contact with tooth structure. Such intrinsic acids may reach

the oral cavity via the gastrointestinal tract by a variety of ways namely vomiting, regurgitation, reflux and rumination. Some eating disorders are associated with vomiting such as anorexia nervosa and bulimia nervosa (Scheutzel, 1996).

II. Extrinsic factors

Different extrinsic factors might act either singly or in combination.

A. Extrinsic factors: Dietary

While a food or drink that has a pH of 7 is considered neutral, many foods and beverages are in fact acidic. The acid content in convenience food and drinks is important for flavour, taste perception, product stability and shelf life (Kilcast and Subramaniam, 2011).

A wide variety of soft drinks have been associated with the development of dental erosion; namely carbonated drinks, still and dilutable drinks, fruit juices, smoothies and sports and energy drinks (Rees et al., 2005a, Ehlen et al., 2008, Hunter et al., 2008, Rees et al., 2007, Blacker and Chadwick, 2013). In addition to the aforementioned drinks there are some acidic alcoholic drinks including wines, ciders and alcopops (Hughes and Rees, 2008, Rees, 2003). Healthy lifestyle foods such as certain herbal teas (Phelan and Rees, 2003), fruits and berries together with salad dressings and vinegar conserves may also produce erosion if consumed in excess (Lussi et al., 2004).

A recent systematic review (Salas et al., 2015) concludes that consumption of soft drinks, acidic snacks/sweets and acidic fruit juices increases the odds of an individual developing dental erosion. In recent years there has been a very large increase in the sales of such drinks. This has been attributed to the increased availability and affordable price of a greatly expanded range of heavily marketed drinks. Consumers

tend to stick to traditional favourites and become less experimental with the continuously increasing prices of food and drink. The ones looking for greater value for their money can find a huge variety of multipack offers that suits them. All in all, according to the British Soft Drink Association, carbonated drinks are affordable and sales are expected to keep growing not only in the UK but also in the whole world (BSDA, 2015).

In order to reduce the incidence of certain illnesses such as diabetes, cardiovascular disease, stroke and carcinomas; various national campaigns were launched through which people were encouraged to consume at least five portions of fruit and vegetables each day. These campaigns came after the recommendations of the World Health Organization where a minimum consumption of 400 g of fruit and vegetables per day was advocated. Such campaigns were implemented in the USA, the United Kingdom and Germany via the so called “5-a-day” campaigns. Australia adopted a similar concept but different terminology; “go for 2 & 5” campaign (World Health Organization, 2015).

The increased popularity of fruits and vegetables is largely due to the increased awareness of their health benefits. They contain high levels of fibre, vitamins, antioxidants and minerals which will altogether contribute to better health especially if they were consumed as part of a balanced diet; one low in saturated fats, sugars and salt (Sanders, 2004). However, a higher risk of dental erosion is associated with excessive fresh fruit and vegetables consumption especially with subjects who are living on a raw food diet (Ganss et al., 1999).

B. Extrinsic factors: Medication

Some medications possess low pH and high titratable acidity values. If they contact tooth structure, it can therefore potentially have an erosive effect. Clearly, a frequent and an extended use of acidic medications is presumably required for such an effect to occur as cumulative prolonged contact with the teeth is required for the erosive process to manifest. This is also influenced by the form (chewable tablets, suspensions, liquids, lozenges etc.) that the medicament is taken in. This is because this affects the surface area of the medicament and influences the rate at which it dissociates and clears from the mouth (Giunta, 1983, Lussi et al., 1998, Nunn et al., 2001). Moreover, some inhalers are responsible for reducing salivary flow, a major protection mechanism, which therefore increases the risk of dental erosion occurring (Manuel et al., 2009).

C. Extrinsic factors: Environmental

Several industrial and mining occupations pose risks of dental erosion. These risks are decreasing dramatically with time as a result of the stricter Health and Safety regulations that now govern mines and factories (Bartlett et al., 1994, Amaechi and Higham, 2005). Other occupations might also pose a risk of dental erosion such as professional wine and tea tasters due to the prolonged time of acidic exposure to teeth (Wiegand and Attin, 2007).

D. Extrinsic factors: Behavioural

This factor is entirely associated with an individual's own preferences and habits. Some healthier lifestyles are associated with increased physical activity which leads to increased fluid consumption to maintain fluid balance (Casa et al., 2005). Unfortunately, these fluids can be acidic and potentially erosive such as citrus

flavoured drinks high-in-electrolyte and high-caffeine energy drinks. Other lifestyles, such as vegetarianism, involve the consumption of higher quantities of erosive citrus fruits and vegetables (Linkosalo and Markkanen, 1985). Excessive consumption of fruity-flavoured yoghurts and herbal teas may also be involved in such lifestyles (Lussi et al., 2012a, Phelan and Rees, 2003). On the other hand, drinking of mixers (vodka/ energy drink) might be risky if taken to extremes especially if taken with certain euphoric drugs such as ecstasy, which aggravate the situation by increasing their consumption to counter the dry mouth effect of such drugs (Michel et al., 2010). Consumption in moderation is therefore key to maintaining the health-state of dental tissues by reducing risk (Chadwick, 2006; Serra et al., 2009).

The preferred method of drinking (i.e. sipping, gulping, using a straw) has been shown to influence acidic clearance patterns and thus which teeth are affected by dental erosion (Lussi et al., 2004). Keeping the drink in the oral cavity for a prolonged period of time before swallowing is also associated with a higher incidence of erosion for this increases the exposure time. Frothing the drink and holding it in the mouth also affects its reactive potential with the dentition (Johansson et al., 2002). Clearly, special attention should be given to the duration of exposure and the frequency and duration of multiple exposures when assessing erosion risk.

In the laboratory, when assessing the erosivity of certain foods or drinks, the quantity, duration and frequency of intake are the most important factors to be considered amongst more generic factors that affect this such as chemical and biological ones (Lussi et al., 2004, Moynihan et al., 2002, Jarvinen et al., 1991). Excessive and frequent consumption are most likely to produce erosion in a susceptible individual. For instance, those who sip a carbonated drink over a long period of time increase the

length of time the salivary pH is below the critical pH, raising the erosive risk period, compared to those who consume it relatively quickly (Lussi et al., 2004).

Carbonated beverage intake frequency is an important factor to consider for a better understanding of dental erosion caused by such drinks. Acidic drinks consumed at the same time as consuming food (i.e. main meals) are less harmful than those consumed solely in between meals for such a practice limits the number of acid hits per day. Adopting the pattern of intermittent or continuous sipping is also more damaging to tooth structure than consuming the whole amount at once for it prolongs the at risk period (Shenkin et al., 2002, Dale, 2002) Moreover, in a study assessing the parameter Dupre's Work of Adhesion (defined as "the energy required to pull apart two adhering materials" (Fox and Zisman, 1950)) for different beverages (Ireland et al., 1995), blackcurrant and Coca-Cola had a greater Dupre's work of adhesion value (i.e. stickiness to tooth surface) than saliva and water. Therefore, they would be cleared less readily by either saliva or water owing to their ability to be retained on tooth surface (Ireland et al., 1995). On the other hand, Diet coke and unsweetened orange juice were found to possess a lesser Dupre's Work of Adhesion value rendering them more easily cleared by saliva or water (Ireland et al., 1995).

Excessive tooth-brushing, usually by health-conscious individuals, can aggravate dental erosion by means of removing the protective pellicle. The protective pellicle can minimize the effect of acidic exposure for it is believed that the presence of plaque delays tooth to acid contact (Amaechi et al., 1999b).

Some carbonated beverage consumers partake in "coke-swishing", a term that describes the act of moving the beverage back and forth in the mouth to reduce its carbonated content, to minimize the non-desired sensation of carbonation in the

mouth and throat. Yet, subjects that tend to swish are not usually heavy consumers of carbonated beverages for this act prolongs the time period required to finish one unit of beverage (Abrahamsen, 2005). Swishing usually enhances the development of dental erosion as it replenishes the acidic solution on the surface layer adjacent to enamel. In this regard the citric and phosphoric acids, commonly found in soft drinks, can act as a chelating agent capable of binding enamel or dentine via their constituent minerals (Calcium). The greater the surface area exposed to acidic drinks, the greater the chelating activity observed (Zero and Lussi, 2005).

E. Extrinsic factors: Biological

When it comes to the prevention of erosion, saliva is the most important biological parameter to be considered. Saliva dilutes and clears erosive substances from the oral cavity. It also buffers and neutralizes acids whose presence may hamper the process of remineralisation. It also provides a unique reservoir of calcium and phosphate ions to aid remineralisation (Lussi et al., 2004). This clearing and neutralizing effect is usually referred to as “salivary clearance” or “oral clearance capacity”. The higher the salivary flow rate the better the buffering capacity and the faster the clearance (Ongole and Praveen, 2014). The salivary rinsing action facilitates the elimination of food remnants and drink residues; it also accelerates the dilution of acids via the so called “saliva diluting effect” (Vissink, 2009). Water content of saliva has the ability to dilute the imbibed liquid both before and after it contacts the enamel (Speirs, 1984).

Saliva is also responsible for repairing the affected tooth structure by providing the required organic and inorganic material to compensate the loss caused by erosion. This eventually “re-hardens” the softened tooth structure by remineralisation (Gedalia et al., 1991). Other biological factors that might influence the outcome of an erosive

attack are the salivary gland health-state and function; the anatomy of teeth surfaces and the associated soft tissues of the oral cavity; the efficacy and extent of movement of the tongue and the associated mucosa; and the swallowing rate and pattern (Lussi et al., 2004). These factors influence both saliva quantity and the manner it flows around the mouth. It has also been suggested that remineralisation *in vitro* can get the tooth surface hardened again after a period of 6 hours (Lussi et al., 2004).

2.2 Soft drinks

People usually wish to experience a refreshing taste when consuming a beverage hence the ever-increasing popularity of fizzy drinks. This special taste is due to the presence of a variety of acids that possess low pK_a (pK_{a1} = 2.15 - 4.10). Citric, phosphoric, malic, ascorbic, lactic, tartaric, succinic and carbonic acids can all be found either singly or in combination in carbonated beverages that are available to today's consumers (Parry et al., 2001). The pK_a value indicates how readily an acid dissociates in an aqueous environment into its ionic components.

The concept of pH was first introduced by Sorensen, a Danish biochemist, who in 1909 was investigating the acidity of solutions using a normal hydrogen electrode (Sørensen, 1909). Nowadays, this concept is the gold standard of acidity level determination.

An acid is "a substance which when added to water produces H^+" (Masterton and Hurley, 2015). While a base is "a substance which when added to water produces OH^-" (Masterton and Hurley, 2015). Generally, acids are either weak or strong. A weak acid is one which dissociates only partially when in water while a strong acid completely dissociates in water (Masterton and Hurley, 2015). The following formulae illustrate this:

$$Weak\ Acid \qquad HA_{aq} \underset{\leftarrow}{\overset{\rightarrow}{}} {H^+}_{aq} + {A^-}_{aq}$$

$$Strong\ Acid \qquad HA_{aq} \rightarrow {H^+}_{aq} + {A^-}_{aq}$$

In the case of a weak acid in aqueous solution this coexists in equilibrium with its dissociated components. The relationship between the concentrations of the dissociated components of (H^+ and OH^-) determines whether the solution is acidic or basic. A solution is considered acidic when $[H^+] > [OH^-]$, whereas the solution is basic when $[OH^-] > [H^+]$ (Masterton and Hurley, 2015). In general, the acidity level of solutions is usually expressed in terms of the pH value (i.e. $-\log_{10} [H^+]$) rather than a direct measure of H^+ ion concentration (Masterton and Hurley, 2015). The use of [ ] indicates concentration so $[H^+]$ signifies H^+ concentration.

Carbonated drinks refer to drinks that are made predominantly from carbonated water to which juice or flavourings have been added. Carbonated beverages have carbonic acid formed by dissolving carbon dioxide in the solution hence the name “carbonated” (Kitchens and Owens, 2007). These are distinct from naturally carbonated beverages.

The characteristic acidic content of such beverages is believed to be the major factor in their erosive potential (von Fraunhofer and Rogers, 2004). The aforementioned polybasic acids can become very erosive to tooth structure due to their calcium chelating effect and their ability to maintain pH values in the oral cavity below the threshold point at which enamel apatite dissolution occurs (von Fraunhofer and Rogers, 2004).

2.2.1 Historical background

Early agricultural communities may have made different juices by squeezing fruits (Wolf et al., 2008). Although the early history of juice is hard to investigate it has been speculated that the first modern juice may have been lemonade. The earliest English reference to lemonade was in 1663 which, though thought to be quintessentially English, actually originated in Italy making its way to England via France (Emmins, 1991).

It's well known nowadays that citrus juices prevent scurvy as a result of their vitamin-C content. As late as the sixteenth century, this information was unknown until the value of juice was recognized by Elizabethan seamen (Emmins, 1991). However it took until the 1790s that the medicinal value of lemon juice in treating scurvy was officially recognized. As a result, grog (rum, lime juice, water and sugar) became by law a part of the seaman's diet (Emmins, 1991). This tradition was upheld until 1970 by the Royal Navy when its issue was discontinued as the Royal Navy had much sophisticated equipment and weaponry on board and needed sober sailors to operate it (axfordsabode.org.uk, 2015)!

Artificially carbonated water was first created in the late 1760s. It was first sold to the public by Thomas Henry, a Manchester apothecary in the 1770s. He made it by combining mineral salts, carbon dioxide gas and water (Wolf et al., 2008, Emmins, 1991). Its production was stimulated by the desire to make naturally carbonated waters by replication more readily available to the population for reasons of health promotion and no doubt profit! Initially distilled water was infused with carbon dioxide (Priestley, 1772) but later this approach was improved and commercialized by Jacob Schweppe who began selling seltzer in Geneva in 1789 and then established factory

scale production in London in 1792. Surprisingly, it was not before 1833 that the first reference to effervescent lemonade, made from this product, is found. (Emmins, 1991, Wolf et al., 2008).

Although the fizzy drink Dr. Pepper had been in existence since 1885 and has the distinction of being able to be served hot; a landmark in the history of soft drinks was when J.S Pemberton created Coca-cola in 1886 by combining kola (a caffeine-containing fruit from Africa) with coca (the parent plant of cocaine). Pepsi, its modern day rival, appeared in 1896 (Wolf et al., 2008, Emmins, 1991).

2.2.2 Soft drinks industry and consumption

Today soft drinks have become enshrined in the culture of the young with rhymes being recited by primary school children (Chadwick M. – personal communication). An example of a such rhyme is:

"*Coca cola went to town*

Pepsi cola shot him down

Dr Pepper fixed him up into 7-Up"

(recalled by Matthew Chadwick)

The European Food Safety Authority recommends drinking adequate amounts of water of 2 litres for females and 2.5 litres a day for males (European Food Safety Authority, 2010). A huge variety of soft drinks are available, that consist mainly of water, rendering them adequate for hydration making this target relatively easy and convenient for the consumer to achieve (BSDA, 2015).

6,380 million litres of carbonated drinks were sold in the UK in 2014 with an estimated value of 9 billion pounds sterling (BSDA, 2015).

The annual consumption of soft drinks in the UK is 232.9 litres per person, of these carbonated drinks are the most consumed soft drinks. These account for 43.1% of total individual fluid consumption. The UK annual consumption of carbonated drinks, per head of population, is around 100.5 litres demonstrating 5% volume growth compared with 2008. 49% of carbonates sold in 2014 were low-calorie and no-added-sugar variants. Cola is the most preferred flavour (55%) (BSDA, 2015). These figures indicate that carbonated drink consumption among the UK population is approximately 640 ml per day which is almost equivalent to 2 cans of beverage per day (based on the amount *per capita* of drinks sold for human consumption therefore non-consumers could not be excluded).

Outwith the UK comparable figures were found in a report assessing carbonated drinks consumption in the US among 13 to 18 year olds, where the consumption rate of carbonated drinks was surveyed and subsequently analysed (Jacobson, 2005). The mean consumption rate among males was 710 ml per day and among females 483 ml per yielding an overall consumption of 597 ml per day.

In this work consumption rates were also calculated, excluding non-consumers, to better reflect the actual consumption among consumers. The aforementioned values are calculated based on the overall population which includes non-consumers of fizzy drinks so non-consumers were excluded and consumption rates were recalculated to rates per consumer. Thereby, consumption rate was reported to be 910 ml per day for males and 654 ml per day for females yielding an overall consumption of 796 ml per day (Jacobson, 2005).

The average consumption of carbonated drinks in the US among different age groups based on collective nationally representative surveys of food and drink consumption of the US population also yields similar figures (Table 2-1) (Popkin, 2010).

Table 2-1. Daily carbonated drink consumption in the US population. Adopted from Popkin, 2010 (Popkin, 2010).

Age group (years)	2-6	7-12	13-18	19-39	40-59	>60
Consumption (ml) per day	206	342	606	520	307	150

Excessive consumption of carbonated beverages increases the odds of dental erosion more than threefold among 12 year olds and is also a strong predictor of the degree of damage caused by erosion among 14 year olds (Dugmore and Rock, 2004). A more recent study bears this out (Salas et al., 2015) but also indicates that included risk factors as suggested by Chadwick (Chadwick et al., 2005) control an individual's susceptibility.

2.2.3 Coca-Cola

In a prevalence study that assessed the consumption of different beverages in Iceland (Jensdottir et al., 2004), Coca-Cola was the most commonly consumed carbonated beverage and was found to be a highly credible factor among the extrinsic factors that have the potential to cause dental erosion. Interestingly, Coca cola was the only beverage that showed a positive association with subjects suffering from dental erosion regardless of the affected site (i.e. affected incisors and/or molars). Its consumption was significantly more frequent in those subjects ($p < 0.05$) (Jensdottir et al., 2004). The risk of developing dental erosion was nearly 3-fold higher in subjects drinking Coca-Cola more than thrice a week ($p<0.05$) in comparison to less frequent intakes of Coca Cola. Moreover, a gender difference was also noticed in relation to

beverage choice with males consuming more Coca-Cola than females ($p<0.05$) which, in turn, might explain the gender differences seen in several dental erosion studies where males were more likely to exhibit dental erosion (Jensdottir et al., 2004).

The risk of developing dental erosion has been shown to be 3-fold higher when more than 1000 ml of any carbonated acidic beverage per week (i.e. >3 cans) is consumed (Jensdottir et al., 2004). In addition, subjects with high consumption rate of Coca-Cola were shown to have an increased overall consumption of other carbonated drinks (Jensdottir et al., 2004).

Coca-Cola's pH, Titratable acidity (to pH 5.5 and 7.0), buffering capacity and ion concentrations according to the literature are shown in table 2-2. It is clear that this drink, used in this thesis to bring about erosion, has erosive potential.

Table 2-2. Coca cola pH, Titratable acidity (to pH 5.5 and 7.0), buffering capacity and ion concentrations according to literature.

Coca cola**	pH	NaOH mmol/l to 7.0	NaOH mmol/l to 5.5	Buffering capacity β mmol/l x pH	[Ca] mmol/l	[Pi] mmol/l
(Lussi et al., 1993)	2.6	34.0	14.0		0.84	5.43
(Larsen and Nyvad, 1999)	2.40	25.0	9.0		0.26*	5.47
(Lussi et al., 2000)	2.6	34.0	14.0		0.84	5.43
(Lippert et al., 2004)	2.66	18.0			0.81	5.52
(Attin et al., 2005)	2.53	48.0			0.94	5.24
(Jensdottir et al., 2005)	2.59	46.2	15.2	10.0	0.12*	2.68*
(Zero and Lussi, 2005)	2.6	34			0.8	5.4
(Francisconi et al., 2008)	2.6	41.8	20.0		0.84	5.43
(Hara and Zero, 2008)	2.46	32.0			0.45	5.47
(Jager et al., 2008)	2.47				0.87	4.76
(Rios et al., 2008a)	2.6	41.8	20.0		0.84	5.43
(Cochrane et al., 2009)	2.39	23.17	8.99		0.15*	5.99
(Poggio et al., 2010b)	2.44			11.2	0.52	5.67
(Cochrane et al., 2012)	2.45	23.36	8.25		0.36*	5.25
(Jager et al., 2012)	2.47		8.88		0.87	4.80
(Lussi et al., 2012b)	2.45	17.5		9.6	1.08	5.04

* Odd calcium and phosphate concentrations likely attributed to differences in water supplies used in the manufacturing process of beverages.

**Ingredients as stated by manufacturer: Carbonated water, sugar, colour (caramel E150d), phosphoric acid, natural flavourings including caffeine.

2.3 pH and Titratable acidity

In order to more fully understand how acidity affects erosive capacity of foods and beverages it is helpful to review both pH and titratable acidity. The pH or acidity level can be calculated from the hydrogen ion concentration of a food or beverage using the formula:

$$pH = -\log_{10} [H^+]$$

The pH scale ranges from 0-14 where a pH level above 7 is basic, with the most basic level at 14, and anything with a pH level below 7 is acidic. When the pH is 7, the solution is considered to be neutral (Masterton and Hurley, 2015).

Each acid has a specific dissociation constant (K_a). This indicates the ease at which an acid in aqueous solution dissociates into H^+ and OH^- ions. Acids can be categorized according to their H+ content as mono-, di- and tri-protic acids; where monoprotic acids contain one H^+, diprotic acids contain two H^+ and triprotic acids contain three H^+ (Licker, 2003). This also applies to the number of dissociation constants so monoprotic acids have one dissociation constant and so on. When comparing acids, pK_a is used rather than K_a. pK_a is the $-\log_{10}$ of K_a. The stronger the acid, the higher the amount of acid dissociation and the higher the pK_a value (Licker, 2003).

Generally, beverages of low pH values have greater potential to cause dental erosion. The erosive capability of acidic beverages can thus be predicted to a certain degree by their pH level; the erosivity increases as the pH decreases (Jensdottir et al., 2005, Larsen and Nyvad, 1999). A more important factor however, which researchers believe to better reflect the erosive potential of a certain food or drink, is titratable acidity. Titratable acidity of a solution is a measure of acidity expressed as the volume of alkali

(typically 0.1 molar NaOH) required to neutralize the solution (typically 25ml) to pH 7. The higher the quantity the more potentially erosive a solution is (Chadwick, 2006) . It is preferable that this is expressed as the standardised titratable acidity (STA) to make simple inter-study comparisons (Syed and Chadwick, 2009). It should be pointed out that the test is not fully predictive of erosiveness and should be used in conjunction with other laboratory tests to make an assessment of erosive potential.

The pH value can provide an indication as to the initial hydrogen ion concentration but it cannot indicate whether undissociated acid is present or not. The pH value is quite informative for the first few minutes of the erosive process, while titratable acidity is more indicative when the total acid content of a beverage is of concern; therefore it is considered a reliable indicator in prolonged periods of erosion (Hara and Zero, 2008, Jensdottir et al., 2006a).

Buffering of an acidic solution manifests in its ability to maintain sufficient hydrogen ion supply at low pH levels (Shellis et al., 2010). This role is dependent upon other factors such as the exposure time and ratio of volume of beverage to tooth surface area. Exposure of tooth structure to low volumes of acidic solution (i.e. low ratio of solution to tooth surface area) have been demonstrated to have a significant correlation between tooth tissue loss, buffering capacity and titratable acidity when acidic exposure is maintained over a prolonged period, while pH is significantly correlated with tooth tissue loss when shorter exposure periods are observed (Manton et al., 2010). Consequently, when using relatively larger volumes of acidic solutions, the pH value is better than buffering capacity and titratable acidity when predicting erosion (Jensdottir et al., 2005). However, the ratio of the total volume of acidic solution to the area of tooth structure exposed and acidic exposure time are mutually

important factors that both pH and titratable acidity are dependent upon (Lussi et al., 2012b).

At a certain point of time during the process of consuming an erosive beverage, the undissociated acid in the beverage/saliva solution corresponds to the buffering capacity. At this point it maintains enough hydrogen ion concentration to favour demineralisation of the exposed tooth (Zero, 1996). The time period needed for the saliva to neutralise the acid is directly proportional to the buffering capacity. In other words, in the presence of an acid in the oral cavity, tooth structure keeps losing its minerals until a certain pH value is reached where the dissolution process stops (Lussi et al., 2012b). While buffering capacity deals with a certain pH value, titratable acidity measures the total hydrogen ion available over a broad range of pH values instead.

Overall, when a beverage is being consumed for an extended period of time, pH and titratable acidity combined can serve as a good measure of erosive potential (Manton et al., 2010). Yet, to add to the confusion, both pH and titratable acidity might fail as predictors of erosive potential in certain circumstances (Ehlen et al., 2008). An *in vitro* study to assess the associations among pH, titratable acidity and tooth substance loss due to erosion failed to show any (Ehlen et al., 2008).

2.3.1 Critical pH

The critical pH of saliva can be referred to as the acidity level at which saliva becomes saturated with respect to the tooth structure's ionic constituents. As a general rule, a pH value of 5.5 has been suggested as a threshold above which it is considered safe with regard to loss of enamel mineral and below which demineralisation of enamel occurs (Larsen and Pearce, 2003). This however relates to dental caries and not dental erosion.

Ideally, the pH of the oral cavity recovers when a drop beyond the critical pH occurs, as a result of acidic intake, via compensatory mechanisms including the role of saliva. Prolonged periods of low pH levels or frequent pH fluctuations below the critical pH can result in more rapid tooth structure dissolution.

Where fluoride has been incorporated into the apatite crystal structure, however, the resultant fluorapatite is considered more erosion-resistant when compared to hydroxyapatite. A study by Larsen and Pearce (2003) showed that the critical pH for fluorapatite is below 4.5 (Larsen and Pearce, 2003).

During resting periods, with the lack of external stimuli, there is a slow and continuous salivary flow that manifests itself as a thin film covering the oral tissues. This flow is referred to as “unstimulated saliva” or “resting saliva”. Stimulated saliva, on the other hand, is produced as a result of external stimuli such as gustatory, olfactory, mechanical and pharmacological. This flow is conditional and exhibits a significantly higher flow rate (De Almeida et al., 2008).

Stimulated saliva is activated at certain time points which are usually related to food and drink consumption. For the majority of the day, resting saliva bathes the oral cavity and is considered by some a more influential factor for the integrity of tooth structure. On the other hand, the role of stimulated saliva cannot be underestimated for its direct and immediate role in clearing and buffering a consumed erosive food or drink (De Almeida et al., 2008).

Carbonate concentration, an important factor in the buffering capacity of saliva, is considerably lower in resting (1-3 mmol/l) compared to stimulated saliva (40 mmol/l). This coincides with the potential rise in pH arising from the shift from resting (5.5-7) to

stimulated saliva (up to 8). On the other hand, the pH in certain areas in the oral cavity can be as low as 4, this low value is usually found in areas of plaque build-up. As a result, a range of pH from 4 to 8 can be found in the oral cavity at different sites at the same time (Larsen and Pearce, 2003). Interestingly, when an acidic beverage is consumed, plaque pH level may not drop as low as the pH of the drink (Honório et al., 2008) perhaps indicating that a covering of plaque protects against erosion.

Larsen and Pearce (2003) investigated several aspects of saliva and found that stimulated whole saliva has a slightly higher pH value compared to resting whole saliva (7.28 ± 0.21 vs 7.07 ± 0.46). It has also a higher calcium but lower phosphate content (1.11 ± 0.21 vs 0.86 ± 0.46 mmol/l and 3.72 ± 0.73 vs 7.01 ± 4.12 mmol/l respectively) (Larsen and Pearce, 2003).

When saliva is marginally supersaturated with respect to enamel apatite, the apatite will most probably survive an erosive attack, while if it is considerably supersaturated it will not only survive but has the capacity to lay down mineral. In contrast, if saliva is unsaturated with respect to enamel apatite it will start losing its ionic constituents upon erosive attack (Larsen and Pearce, 2003). In summary, erosion occurs when tooth structure is exposed to an aqueous phase of acid which is undersaturated with respect to enamel apatite minerals.

The vulnerability for dental erosion varies widely among individuals owing to differences in physiological and biological aspects of the oral cavity (namely salivary flow rate, pH, buffering capacity, the formation of an acquired pellicle) as well as behavioural and habitual ones such as the frequency and pattern of acidic drinks consumption (Zero, 1996).

2.4 Protective Factors

Many factors protect the teeth from dental erosion. These mainly concern the pellicle and saliva.

2.4.1 The acquired pellicle

The acquired pellicle partially protects the integrity of tooth structure from the process of erosion by offering a selective permeability barrier in between the superficial tooth structure and the oral cavity which will, to a certain degree, govern the penetration and accessibility of acids into the enamel and dentine. This regulatory role serves in part as a physical protection barrier that the acids have to overcome in order to affect the enamel apatite (Hara et al., 2006).

The acquired salivary pellicle is mainly composed of proteins. It can readily be formed even after its removal following regular oral hygiene habits or by chemical dissolution. Moreover, it does not take longer than a few minutes of oral environment exposure for its organic structure to be detected on tooth surfaces. Since it is a protein based layer, the growth and maturation of salivary pellicle is regulated by the degree of equilibrium between the resorption and adsorption of its proteins upon the tooth surface which can be reached within 2 hours (Lussi and Jaeggi, 2008). It is worth mentioning that any procedure that tends to remove or reduce the thickness of the acquired pellicle layer might facilitate the erosive process by undermining the protective role the pellicle offers (Zero and Lussi, 2005).

When an acidic solution gets access to the oral cavity, the acquired pellicle is the enamel's first direct defence line. This is because acid has to diffuse through the whole

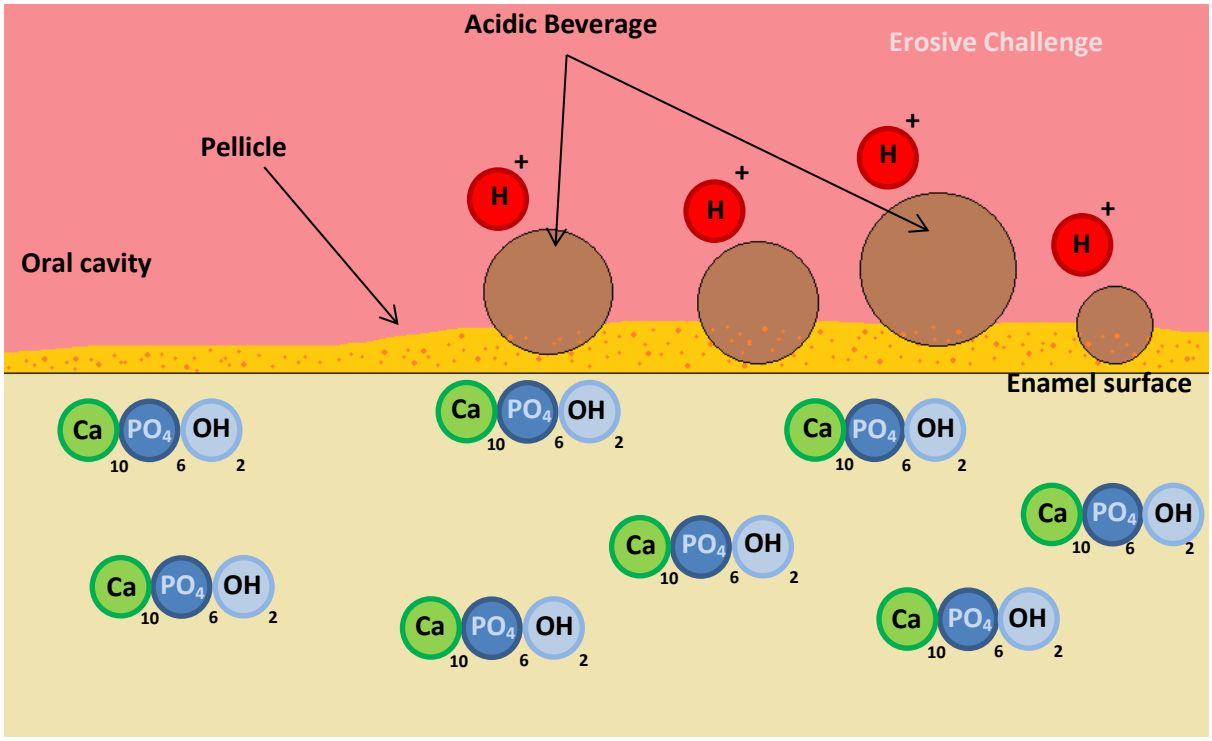
thickness of the pellicle to reach the tooth surface for erosion to be brought about (Meurman and Frank, 1991, Lussi and Hellwig, 2001, Lussi and Jaeggi, 2008)

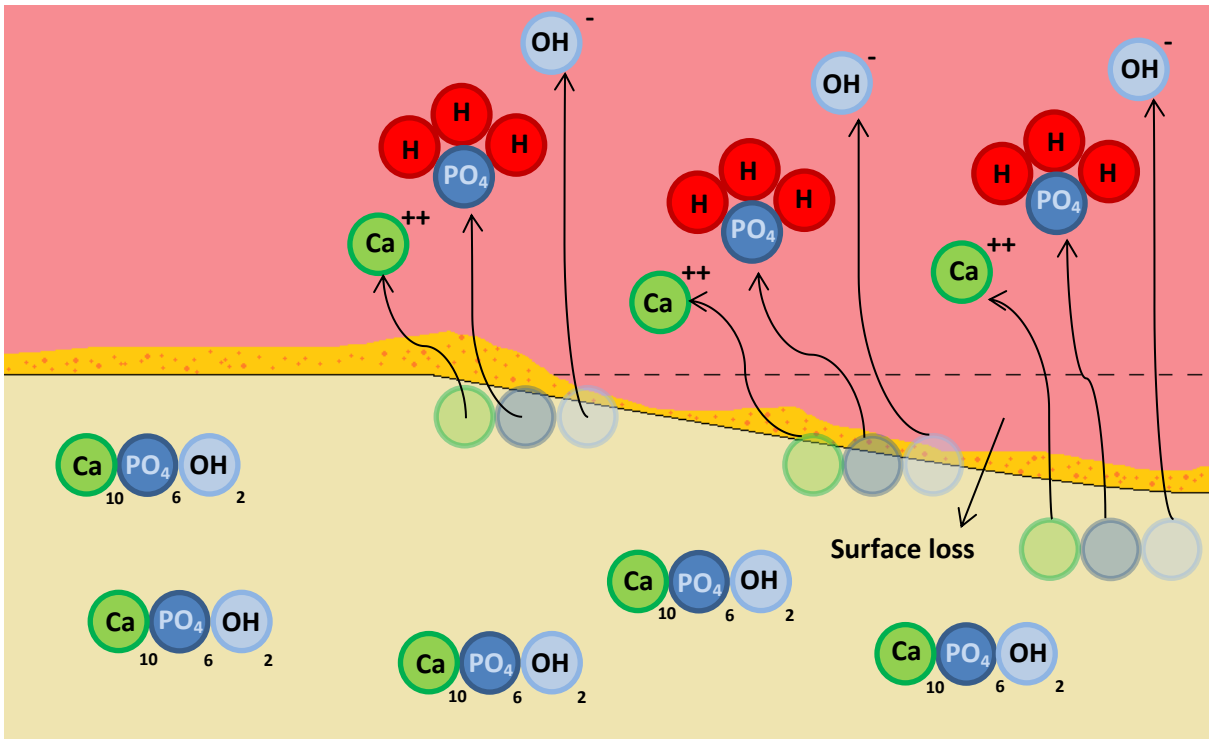
The acquired pellicle thickness differs among individuals and even at different sites in the same individual. This thickness is inversely related to the degree of dental erosion. The more mature the pellicle, the greater its protective barrier (Magalhaes et al., 2009).

Once penetrated, hydrogen ions from the acid start demineralising the tooth apatite crystals leaving a honey comb ultrastructural appearance owing to the enamel prism sheath and core being differentially dissolved. This allows further demineralisation of the enamel's subsurface layer by granting more access to the unionized acid component which will eventually reach the interprismatic areas. Subsequently, an outward flow of the dissolved enamel apatite, namely calcium and phosphate ions, raises the pH level in the area immediately adjacent to the tooth enamel surface for here all the acid is consumed in the erosive chemical reaction (Lussi and Jaeggi, 2008) (figure 2-1).

Generally, acidic solutions are made up of hydrogen ions, acid anions and acid undissociated molecules. The quantity of each of these constituents is dictated by the pH of the solution and its pKa constant. Apart from the role of the hydrogen ion in dissolving the enamel apatite, the acid anion can also complex with calcium detaching it from apatite crystals. Acid anions differ in their capacity to complex with calcium. This is dependent upon the structure of the molecule itself and its capacity to attract other ions such as calcium (Lussi and Jaeggi, 2008).

Figure 2-1. A diagram illustrating the loss of calcium and phosphate ions from enamel as a result of an erosive challenge





$$Ca_{10}(PO_4)_6(OH)_2 \xrightarrow{H^+} 10Ca^{+2} + 6H_3PO_4 + 2H_2O$$

Approximately one third of calcium in enamel apatite has the potential to complex with acid anions at concentrations found commonly in acidic juices and beverages, therefore undermining the supersaturated state of saliva and favouring the outward flow of minerals which will eventually result in tooth structure loss by the process of erosion (Lussi and Jaeggi, 2008).

Fluoride treatment has been demonstrated to enhance erosion resistance owing to the fact that fluoride ions can be incorporated into the crystalline structure of enamel apatite rendering it more acid-resistant by alteration of its crystal structure (Lussi and Jaeggi, 2008).

The protective effect of fluoride is probably attributed to the precipitation of calcium-fluoride compounds on the surface of the affected tooth surfaces. Yet, whether or not fluoride actually prevents dental erosion is debatable (Wiegand et al., 2009). On the other hand, the preventive role of fluoride has been demonstrated under *in vitro* and *in situ* conditions using agents containing high concentrations of fluoride (Lagerweij et al., 2006, Ganss et al., 2004); clinical studies are still to be undertaken to confirm this (Magalhães et al., 2009).

The acquired pellicle can be considered protective against erosive attacks especially in people who frequently consume soft drinks on an hourly basis for the pellicle is known to facilitate subsurface remineralisation (Gelhard et al., 1979).

2.4.2 Human Saliva: an overview

It is essential to our wellbeing that we understand the multifunctional nature of saliva. The composition and function of human saliva is not as simple as it might seem on first inspection.

I. Problems in human natural saliva

The difficulties of utilising natural saliva in laboratory investigations of erosion cannot be underestimated for:

I. There is a wide variation in the composition of saliva among individuals and inter-individual differences are also present. Therefore, only "typical" concentrations are aimed for when preparing a recipe for artificial saliva.
II. Saliva contains exogenous materials such as cellular debris and microorganisms along with their associated substances which might cause unreliable behaviour and results (Higgins et al., 1973, Jenkins, 1978, Lavelle, 1975).
III. The presence of mucin, which reversibly binds ionic constituents, is prone to generating interferences with the determination of either dissolved, ionized or total concentrations of constituents (Feldötö et al., 2008).
IV. Their instability is of its dissolved carbon dioxide with respect to the partial pressure in the oral cavity (Sellman, 1949).
V. There are a large number of methods for analysis of saliva, but a comprehensive method is still to be found for overcoming the problems of its instability (Lavelle, 1975, Jenkins, 1978).
VI. It is essentially impossible to collect saliva under conditions which are equivalent to natural physiological conditions. In other words, it will vary according to the methods of stimulation and collection adopted (Darvell, 1978, Darvell, 1975).

2.5 Erosion testing regimes: towards a more realistic *in vitro* model

It is important that past advances and challenges are known when developing a new approach to erosion testing.

2.5.1 Prologue

A search was undertaken using the Scopus academic search engine for dental erosion studies carried out under *in vitro* and *in situ* conditions published in the last two decades using the keywords “dental erosion”, “beverage erosion”, “drink erosion”, “erosion substrate”, “demineralisation” “remineralisation”, “pH cycling”, “*in vitro*”, “*in situ*”, “enamel”, “dentin”, “profilometry”, “hardness”.

The articles were thoroughly reviewed, classified and consequently summarised in order to more fully understand and appreciate the different experiment designs. The methodology and results of each were carefully assessed. Such studies are summarised in table 2-3 and their key findings in table 2-4 where surface loss values, relative hardness percentage change and other findings are reported. Surface loss values were recalculated by the author to per hour values to allow for comparison between studies. More detailed comments on these studies are found in the literature review that follows.

Table 2.3. Summary of the most relevant ***in vitro*** (color-coded blue) and ***in situ*** (color-coded orange) studies assessing erosive beverages and foods that were published in the past 2 decades.

Author	Acid source of concern	Substrate (Sample size)	Acidic exposure time	Artificial saliva	Temp.	Stirring / agitation	Vol.	Exposure method	Dem. Rem. Cycling (per day)	Erosion assessment	Condition/ special features	Abrasion	pH
Steffen, 1996 (Steffen, 1996)	Various	Various human teeth (n=9)	72 hrs	No	NA	No	Repl. 24 hrs	Immersion	No	SEM	*In vitro*	No	No
Amaechi et al. 1999 (Amaechi et al., 1999a)	Orange juice	Bovine and human/ permanent and primary (n=10)	Total 30 min/day Total 24 days	Modified from Hanes and Whitford 1992	4,20,37 ˚C	Yes	20 ml	Immersion	Dem. Rem. Cycles (Dem. 5 min Rem. 2 hrs) x6 Rem. overnight	Transverse micro-radiography	*In vitro*	No	pH 3.85
Larsen et al 2002(Larsen and Nyvad, 1999)	Various	Human premolar and molars / enamel (n=3)	24 hrs 1 week	No	NA	Yes	1.5 litres	Immersion	No	Micro-radiographs Quantimet 550+	*In vitro*	No	Coke pH 2.4
Maupome et al. 1999 (Maupome et al., 1999)	Coca cola	Human primary and permanent	5,25,50 min/day Total 8 days	Natural saliva (whole or clarified)	NA	Yes or No	NA	Immersion	Dem. Rem. Cycles Acquired pellicle (Dem. 5 min Rem. Nat. saliva) x1 x5 x10	Microhardness	*In vitro*	No	pH 2.6
Hughes et al. 2000 (Hughes et al., 2000)	Citric acid 0.1and 0.6	Third molar teeth/ enamel (n=5)	10 min x3	No	35 ˚C	No	250 ml	Immersion	No	Profilometry	*In vitro*	No	pH 2.8
Lussi et al. 2000 (Lussi et al., 2000)	Various Coca cola (degassed) Sprite (degassed)	Human primary and permanent teeth (n=5)	3 min	No	37 ˚C	Yes	10 ml	Immersion	No	Microhardness	*In vitro*	No	pH 2.6
Hammadeh and Rees 2001(Hammadeh and Rees, 2001)	Various	Human permanent molars and premolars/ enamel	4 hours/ hourly	no	Room	No	20 ml	Immersion	No	Profilometry	*In vitro* Subsurface group	No	Orange pH 3.74 Coke pH 2.43
Parry et al. 2001 (Parry et al., 2001)	Various	Freshly extracted teeth/ enamel (n=8)	5 min X6 Total 30 min/day Total 1 day	No	37 ˚C	Yes	1 ml	Immersion	No	Spectro-photometry	*In vitro*	No	NA
Larsen et al 2002 (Larsen and Richards, 2002)	Various	Human molars (n=2)	48 hrs	No	NA	Yes	500 ml	Immersion	No	Micro-radiographs Atomic absorption spectroscopy	*In vitro*	No	Coke pH 2.54

Author	Acid source of concern	Substrate (Sample size)	Acidic exposure time	Artificial saliva	Temp.	Stirring / agitation	Vol.	Exposure method	Dem. Rem. Cycling (per day)	Erosion assessment	Condition/ special features	Abrasion	pH
Attin et al 2003(Attin et al., 2003)	Citric acid 1%	Bovine incisors (n=12)	Total 15 mins/day Total = 1 day	According to (Klimek et al., 1982)	37 ˚C	No	See flow rates	Rinsing	((Dem. 1 min /Rem. 1 min) X 5 then Rem. 8 hours) X 3 Acid and saliva flow rate 3.25 and 1.1 ml/min (to simulate overnight rest period)	Microhardness. Laser profilometry.	*In vitro* First artificial mouth	No	pH 2.21
Barbour et al 2003(Barbour et al., 2003)	Citric acid	Human molars/ enamel (n=8)	120, 300 sec	No	Room 23.6 ˚C	Yes	50 ml	Immersion	No	Nano-indentation	*In vitro*	No	pH 3.3
Hammadeh and Rees 2003 (Hammadeh and Rees, 2003)	Coca cola Orange juice	Human permanent premolars & molars/ enamel (n=10)	1,2,3,4 hours	No	Room		20 ml	Immersion	No	Profilometry	*In vitro* Ultrasonic bath to remove softened layer	No	pH coke 2.43 orange juice 3.74
Hunter et al 2003(Hunter et al., 2003)	Coca cola diet	Primary and impacted third molars (n=6)	60 min x 4 Total 4 hours	No	37 ˚C	Yes	200 ml Repl. 60 min	Immersion	No	Profilometry	*In vitro*	No	No
Lupi-Pegurier et al. 2003 (Lupi-Pegurier et al., 2003)	Coca cola Wine	Human teeth/ enamel (n=30)	10,30,90,120 sec		37 ˚C		10 ml	Immersion	No	Microhardness SEM	*In vitro*	No	pH wine 3.9
Mahoney et al 2003 (Mahoney et al., 2003)	Orange juice Fanta	Primary teeth / enamel and dentin	10 min	No	Room	No	100 ml	Immersion	No	ultra-micro-indentation system “hardness”	*In vitro*	No	Orange pH 3.8 Fanta pH 2.72
Phelan et al 2003(Phelan and Rees, 2003)	Herbal teas Orange juice	Human third molars/ enamel (n=5	1 hour	No	37 ˚C	Yes	250 ml	Immersion	No	Profilometry	*In vitro*	No	Orange juice pH 3.7
Lippert et al 2004(Lippert et al., 2004)	Various	Primary molars and permanent premolars/ enamel (n=9)	1,1,1,1,1 min Total 5 mins/day Total 1 day	No	NA	No	60 µl	Dripping (one drop)	No	Atomic force microscopy nano-indentation	*In vitro*	No	pH 2.66
Von Fraunhofer and Rogers 2004 (von Fraunhofer and Rogers, 2004)	Various	Human molars and premolars/ enamel (n=2)	14 days	No	Room	No	5 ml Repl. 24 hrs	Immersion	No	Weight loss	*In vitro*	No	pH 2.48

Author	Acid source of concern	Substrate (Sample size)	Acidic exposure time	Artificial saliva	Temp.	Stirring / agitation	Vol.	Exposure method	Dem. Rem. Cycling (per day)	Erosion assessment	Condition/ special features	Abrasion	pH
Willerhausen et al 2004(Willershausen and Schulz-Dobrick, 2004)	Various	Human third molars (n=6)	6 hours	No	37 ˚C	No	NA	Immersion	No	Electron probe micro-analyser (Ca and P)	*In vitro*	No	pH 2.3
Attin et al 2005(Attin et al., 2005)	Various	Bovine incisors / enamel (n=12)	Total 15 mins/day Total 1 day	According to Klimek et al. 1982	37 ˚C	No	See flow rates	Rinsing	((Dem. 1 min /Rem. 1 min) X 5 then Rem. 8 hours) X 3 Acid flow rate 3.25 ml/min Saliva flow rate 1.1 ml/min (to simulate overnight rest period)	Profiolmetry	*In vitro*	No	Coke pH 2.53. Sprite pH 2.69.
Barbour et al 2005(Barbour et al., 2005)	Citric acid solution	Human molars / enamel	30, 60, 120, 300, 600 sec	No	Room	Yes	50 ml	Immersion	No	-A Digital Instruments Multimode Nanoscope IIIa -atomic force microscope to measure hardness	*In vitro*	No	pH 3.3
Hooper et al. 2005 (Hooper et al., 2005)	Sport drinks	Surgically removed third molars/ enamel (n=6)	1 hour X4 Total 4 hrs	No	NA	Yes	200 ml Repl. 1 hr	Immersion	No	Profilometry	*In vitro*	No	pH 3.17-3.81
Jensdottir et al 2005(Jensdottir et al., 2005)	Various	Human molars (n=2)	24 hrs 72 hrs	No	NA	Yes	10 ml	Immersion	No	Weight loss	*In vitro*	No	Coke pH 2.59
Ramalingam et al 2005(Ramalingam et al., 2005)	Sport drinks	Human enamel (n=5)	30 min	No	37 ˚C	Yes	50 ml	Immersion	No	SEM Profilometer	*In vitro*	No	pH 2.7
Rees et al 2005 (Rees et al., 2005b)	Sport drinks Orange juice	Enamel	1 hour	No	NA	No	NA	Immersion	No	Profilometry	*In vitro*	No	Sports: pH 3.16-3.70 Orange: pH 3.68

Author	Acid source of concern	Substrate (Sample size)	Acidic exposure time	Artificial saliva	Temp.	Stirring / agitation	Vol.	Exposure method	Dem. Rem. Cycling (per day)	Erosion assessment	Condition/ special features	Abrasion	pH
Seow and Thong 2005(Seow and Thong, 2005)	Various	Human premolars extracted for ortho reasons / enamel (n=3)	5,15,30 min	None or Natural saliva (50% v/v)	NA	No	NA	Immersion	No	Microhardness Degree of etching	*In vitro*	No	pH 2.3
Shellis et al 2005(Shellis et al., 2005)	Citric acid solution versus rebina toothkind	Un-erupted third molar teeth/ enamel (n=6)	20 min or 7 min 46 sec or 4 min 15 sec	No	Room 20-23 ˚C	No	26, 67, 126, ml/min	Rinsing	No	SEM and profilometry	*In vitro*	No	pH Citric acid 3.2 Ribena 3.8
Van Eygen et al. 2005(Van Eygen et al., 2005)	Coca cola	Human incisors (n=12)	20,40, 60 min/day Or 20 min/day Total 7 days	According to Zero et al 1996	NA	No	NA	Immersion	Dem. Rem. Cycle (Dem. 20 min Rem. 1 hour) x1 or x2 or x3 Or (Dem. 1 min Rem. 3 min) x20	Microhardness	*In vitro*	No	NA
Barbour et al 2006(Barbour et al., 2006)	Ribena toothkind versus regular juice	Human molars / Enamel	Nano-indentation: 5 min Profilometry: 30 min	No	4,25,50, 75 ˚C	No	Nano: 50 ml Prof.: 750 ml	Immersion	No	Atomic force microscopy, nano-indentation and optical profilometry	*In vitro*	No	Yes
Bizhang et al. 2006(Bizhang et al., 2006)	Coca cola	Bovine enamel (n=30)	1 hour/day Total 2 weeks	According to (Ten Cate and Arends, 1977)	NA	No	NA	Immersion	Dem. Rem. Cycles (Dem. 1 hour Rem. Overnight) x14	Micro-radiography	*In vitro*	No	No
Devlin et al. 2006(Devlin et al., 2006)	Coca cola	Human teeth/ enamel (n=10)	1,2,3,15 hours	No	NA	No	0.3 ml	Immersion	No	Microhardness	*In vitro*	No	pH 2.48
Hemingway et al 2006(Hemingway et al., 2006)	Various	Human Permanent molars / Enamel	10 mins X6 Total 60 mins/day Total 1 day	No	36 ˚C	Yes	500 ml	Immersion	No	Optical profilometry	*In vitro*	Y/N	Yes
Jensdottir et al 2006(Jensdottir et al., 2006a)	Various	Hydroxy-apatite	3 min 30 min	No	NA	No	50 ml	Immersion	No	Titratable acidity values	*In vitro*	No	Cola drinks pH 2.7
Rees et al 2006 (Rees et al., 2006)	Low acid orange juice vs orange juice	Enamel (n=5)	1 hour	No	37 ˚C	Yes	250 ml	Immersion	No	Profilometry	*In vitro*	No	pH 3.88

Author	Acid source of concern	Substrate (Sample size)	Acidic exposure time	Artificial saliva	Temp.	Stirring / agitation	Vol.	Exposure method	Dem. Rem. Cycling (per day)	Erosion assessment	Condition/ special features	Abrasion	pH
Wongkhantee et al 2006(Wongkhantee et al., 2006)	Various	human premolars / enamel and dentin ±fillings	Total 100 sec/day Total 1 day	Artificial saliva + mucin	Room	No	32.5 ml	Immersion	Dem. Rem. cycles (Dem. 5 sec Rem. Artificial saliva) X10	Microhardness	*In vitro*	No	pH 2.74
Chunmuang et al 2007(Chunmuang et al., 2007)	Orange juice	Human third molars / enamel (n=6)	Total 20 min/day Total 14 days	According to (McKnight-Hanes and Whitford, 1992)	Room 25 ˚C	Yes	20 ml	Immersion	Dem. Rem. Cycles (Dem. 5 min Rem. Art. saliva) x4 Rem. (overnight)	Profilometry Microhardness	*In vitro*	No	pH 3.26
De Carvalho Sales-Peres et al. 2007 (de Carvalho Sales-Peres et al., 2007)	Coca cola Coca cola light	Bovine incisors/ enamel (n=20)	Total 40 min/day Total 1 day	Artificial saliva	37 ˚C	Yes	15 ml	Immersion	Dem. Rem. Cycles 24 hrs Artificial saliva (Dem. 10 min Rem. 60 min) x4	Microhardness	*In vitro*	No	Coke pH 2.9 Coke light pH 3.2
Hooper et al. 2007(Hooper et al., 2007b)	Orange juice	Human third molars / enamel (n=6)	20 mins/day 7 days 15 days	No	35 ˚C	Yes	NA	Immersion	No	Prifolmetry	*In vitro*	No	pH 3.8
Hove et al. 2007(Hove et al., 2007)	0.01 M HCl	Human molars / enamel (n=12)	Natural saliva (2 hrs) Dem. 2,2,2,2 mins Total 8 mins/day Total 1 day	Natural saliva (pellicle)	23 ˚C	Yes	500 ml	Immersion	No	Interferometry	*In vitro* ±Pellicle	No	pH 2.2
Kato et al. 2007(Kato et al., 2007)	Coca cola	Bovine incisors/ enamel (n=24)	Total 40 min/day Total 1 day	According to (de Mello Vieira et al., 2005)	37 ˚C	Yes	25 ml	Immersion	Dem. Rem. Cycles (Dem. 10 min Rem. 60 min) X4	Microhardness Profilometry	*In vitro*	No	pH 2.1
Kitchens and Owens 2007(Kitchens and Owens, 2007)	Various	Human molars / enamel (n=2)	Total 24 hrs/day Total 14 days	No	37 ˚C	No	Repl. 24 hrs	Immersion	No	Profilometer (surface roughness)	*In vitro*	No	pH 2.49
Owens et al 2007(Owens and Kitchens, 2007)	Various	Human molars	14 days	No	37 ˚C	No	NA	Immersion	No	Scanning electron and light microscopy	*In vitro*	No	pH 2.49
Magalhaes et al. 2007 (Magalhães et al., 2007)	Coca cola	Bovine teeth/ enamel (n=15)	Total 10 min/day Total 4 days	Artificial saliva	NA	No	30 ml	Immersion	Dem. Rem. Cycles Dem. 10 min Rem. 50 min Rem. Overnight 18 hrs	Profilometry Microhardness	*In vitro*	No	Yes
Rees et sl. 2007 (Rees et al., 2007)	Various	Human third molars/ enamel (n=5)	1 hour	No	NA	Yes	250 ml	Immersion	No	Profilometry	*In vitro*	No	pH 2.64-3.68

Author	Acid source of concern	Substrate (Sample size)	Acidic exposure time	Artificial saliva	Temp.	Stirring / agitation	Vol.	Exposure method	Dem. Rem. Cycling (per day)	Erosion assessment	Condition/ special features	Abrasion	pH
Chuenarrom and Benjakul 2008(Chuenarrom and Benjakul, 2008)	Coca cola Orange juice	Human third molars/ enamel (n=6)	15,30,60,120,180 min	No	37 ˚C	Yes	30 ml	Immersion	No	Profilometry Measuring microscope	*In vitro*	No	Coke pH 2.38 Orange juice pH 3.67
Ehlen et al. 2008 (Ehlen et al., 2008)	Various	Human premolars and molars/ enamel and dentin (n=4)	25 hrs	No	NA	No	250 ml Repl. 5hrs	Immersion	No	A polarized light microscope and image pro plus	*In vitro*	No	Coke pH 2.65
Francisconi et al. 2008 (Francisconi et al., 2008)	Coca cola	Bovine incisors/ enamel	Total 15 min/day Total 5 days	According to Vieira et al. 2005	Room	Yes	30 ml	Immersion	Dem. Rem. Cycles (Dem. 5 min Rem. Hrs in between) x3	Microhardness Profilometry	*In vitro*	No	pH 2.6
Hara et al 2008(Hara et al., 2008)	Citric acid 1%	Human molars/ Enamel and root dentin (n=8)/(n=4)	Total 15 mins/day Total 3 days	Artificial saliva AS + mucin Human saliva Deionized W.	NA	Yes	15 ml	Immersion	(Dem. 5 mins Rem. 30 mins) X3	Profilometry	*In vitro*	Y/N	Citric acid pH 3.75
Hara et al 2008(Hara and Zero, 2008)	Various	Bovine incisors/ Enamel (n=10)	0, 5, 10, 30, 60, 120 mins	No	NA	No	30 ml Repl. 60 mins	Immersion	No	Microhardness Optical profilometry	*In vitro*	No	Coke pH 2.45
Hunter et al 2008(Hunter et al., 2008)	Fruit drinks	Human teeth/ enamel (n=5)	1 hour	No	37 ˚C	Yes	250 ml	Immersion	No	Profilometry	*In vitro*	No	Yes
Jager et al 2008(Jager et al., 2008)	Various	Bovine incisors/ enamel (n=10)	3,6,9,15,30 mins Total 63 mins/day Total 1 day	No	21 ˚C	Yes	500 ml or 1 ml	Immersion	No	Profilometry Atomic absorption spectroscopy for Ca, spectrophotometric method for Pi	*In vitro*	No	Coke pH 2.47
Low and Alhuthali 2008 (Low and Alhuthali, 2008)	Various	Human molars and premolars/ enamel (n=1)	7 days	No	NA	Y/N	200 ml	Immersion	No	Weight loss	*In vitro*	No	No
Machado et al. 2008 (Machado et al., 2008)	Sprite Orange juice	Human impacted third molars/ enamel (n=10)	30 min/day Total 5 weeks	No	Room	No	NA	Immersion	No	Profilometry Nano-indentation hardness	*In vitro*	No	Sprite pH 2.69 Orange juice 3.46

Author	Acid source of concern	Substrate (Sample size)	Acidic exposure time	Artificial saliva	Temp.	Stirring / agitation	Vol.	Exposure method	Dem. Rem. Cycling (per day)	Erosion assessment	Condition/ special features	Abrasion	pH
Magalhaes et al 2008(Magalhães et al., 2008a)	Sprite	Bovine incisors / Enamel (n=12)	Total 6 min/day Total 5 days	According to Klimek et al. [1982]	37 °C	No	See flow rates	Rinsing	Artificial mouth concept Dem. Rem. cycles (Dem. 1 min Rem. 59 min) X6 then Rem. overnight Acid flow rate: 3ml/min Saliva flow rate 1.1 ml/min	Profilometry SEM	*In vitro*	No	pH 2.6
Tantbirojn (Tantbirojn et al., 2008)	Coca cola	Bovine incisors	2 min x4 Total 8 min/day Total 1 day +2 days artificial saliva replenishment	No or Modified from (Mukai et al., 2001) 0.4 ml/min	Room	No	6 ml	Immersion	No	Microhardness	*In vitro*	No	pH 2.7
Willershausen et al 2008(Willershausen et al., 2008)	Apple juice	Primary teeth and surgically removed impacted third molars / enamel	24 hrs	No	37 °C	No	NA	Immersion	No	Colorimetrically "Ca release" / Profilometry	*In vitro*	No	pH 3.5
Ablal et al 2009(Ablal et al., 2009)	Alco-pops	Bovine incisors/ enamel (n=6)	Art. Saliva (2 hrs) 20 min, 1 hr, 24 hrs	Artificial saliva	Room	Yes	20 ml	Immersion	No	Quantitative Laser Fluorescence, Profilometry and Transverse Microradiography	*In vitro*	No	Orange juice pH 3.73
Cochrane et al 2009(Cochrane et al., 2009)	Various	Human molars (n=3)	30 mins (surface loss). 24 hrs (Calcium loss)	No	19 ˚C	No	40 ml	Immersion	No	Profilometry (non-contact)	*In vitro*	No	Coke pH 2.39 De-gassed pH 2.86
Hanning et al 2009(Hannig et al., 2009)	Various	Bovine incisors / enamel (n=12)	20 secs	No	NA	Y/N	NA	Immersion	No	Microhardness	*In vitro*	No	Sprite light pH 2.82 Coke light pH 2.85

Author	Acid source of concern	Substrate (Sample size)	Acidic exposure time	Artificial saliva	Temp.	Stirring / agitation	Vol.	Exposure method	Dem. Rem. Cycling (per day)	Erosion assessment	Condition/ special features	Abrasion	pH
Kato et al 2009(Kato et al., 2009)	Coca cola	Bovine incisors/ enamel (n=20)	Total 60 min/day Total 1 day	According to Vieira et al. 2005	37 ˚C	Yes	30 ml	Immersion	Artificial saliva (12 hrs) for superficial hydration Dem. Rem. Cycles (Dem. 10 min Rem. 60 min) X6	Profilometry	*In vitro*	No	No
Magalhaes et al 2009 (Magalhaes et al., 2009)	Citric acid based soft drink Sprite zero	Bovine teeth/ enamel (n=10)	Total 6 min/day Total 1 day	According to Vieira et al. 2005	NA	No	30 ml	Immersion	Dem. Rem. cycles (Dem. 1 min Rem. 59 min) X6 then Rem. 18 hrs	Profilometry	*In vitro*	No	pH 2.96
Murakami et al 2009 (Murakami et al., 2009)	Coca cola	human third molars and primary molars (n=10)	Total 30 min/day Total 7 days	Supersaturated artificial saliva	Room	Yes	15 ml	Immersion	Dem. Rem. cycles (Dem. 5 min Rem. 30 min) X6	Microhardness	*In vitro*	No	pH 2.3
Panich and Poolthong 2009 (Panich and Poolthong, 2009)	Coca cola	Labial surfaces of extracted incisors/ enamel (n=10)	Total 100 sec/day Total 1 day	According to Amaechi et al. 1999	Room 25 ˚C	No	32.5 ml	Immersion	Dem. Rem. Cycles (Dem. 5 sec Rem. 5 sec) X10 Rem. 6 hrs (Dem. 5 sec Rem. 5 sec) X10 Rem. 6 hrs Extra: Rem 6 hrs	Microhardness	*In vitro*	No	pH 2.7
Poggio et al. 2009 (Poggio et al., 2009)	Coca cola	Human incisors (n=10)	2 min X4 Total 8 min/day Total 1 day	Artificial saliva	Room	No	6 ml	Immersion	No	Atomic force microscopy	*In vitro*	No	pH 2.44
Ren et al 2009(Ren et al., 2009)	Orange juice	Human third molars/ enamel (n=15)	Total 100 min/day Total 1 day	According to (Lennon et al., 2006)	35 ˚C	Yes	20 ml	Immersion	Dem. Rem. cycles (Dem. 20 min Rem. 10 min) x5	Profilometry and Focus variation 3D scanning microscopy	*In vitro*	No	pH 3.8
Syed and Chadwick 2009 (Syed and Chadwick, 2009)	Various	Human molar teeth (n=5)	60 min	No	NA	Yes	500 ml	Immersion	No	Microhardness Profilometry	*In vitro*	No	pH 2.83
Wagoner et al 2009 (Wagoner et al., 2009)	Candies (slurries) ± artificial saliva	Human premolars and molars (n=5)	25 hours	According to (Al-Helal et al., 2003)	Room	Yes	250 ml	Immersion	No	Profilometry	*In vitro*	No	pH 2.47
Wiegand et al 2009(Wiegand et al., 2009)	Fluoride solutions	Bovine incisors/ enamel (n=12)	Total 9 min/day Total 3 days	Natural saliva Artificial saliva according to Klimek et al. 1982 0.5 ml/min	NA	No	3 ml/min	Rinsing	Dem. Rem. cycles “artificial mouth” natural saliva (salivary pellicle) (Dem. 90 sec Rem.1 hour) x6	Profilometry	*In vitro* Salivary pellicle by human saliva	No	pH 2.6

Author	Acid source of concern	Substrate (Sample size)	Acidic exposure time	Artificial saliva	Temp.	Stirring / agitation	Vol.	Exposure method	Dem. Rem. Cycling (per day)	Erosion assessment	Condition/ special features	Abrasion	pH
Willershausen et al 2009 (Willershausen et al., 2009)	Apple juice	Human molars	6 hrs	No	NA	No	NA	Immersion	No	Electron-probe microanalyser	*In vitro*	No	NA
Al-Jobair 2010(Al-Jobair, 2010)	Coca cola	Bovine incisors/ enamel (n=10)	(Total 6 min/day Total 3 days) x3	Artificial saliva	Room 25 ˚C	No	33 ml	Immersion	Dem. Rem. Cycles ((Dem. 2 min Rem. 6 hrs) x3 Rem. 18 hours) x3	Microhardness	*In vitro*	No	pH 2.6
Beyer et al 2010(Beyer et al., 2010)	Citric acid	Surgically removed impacted human third molars (n=3)	30,60,90,120 sec	No	Room	Yes	30 ml	Immersion	No	Atomic force microscope Nanohardness	*In vitro*	No	pH 2.3, 3.3, 4
Bueno et al 2010(Bueno et al., 2010)	Coca cola	Bovine incisors / enamel (n=20)	Total 60 mins/day Total 1 day	According to Vieira et al. 2005	Rem 37 ˚C	Yes	30 ml	Immersion	Artificial Saliva 12 hrs (to allow superficial hydration) then: Dem. Rem. cycles (Dem. 10 mins Rem. 60 mins) X6	Profilometry	*In vitro*	No	pH 2.6
Kato et al 2010(Kato et al., 2010b)	Coca cola	Bovine incisors/ enamel (n=4)	Total 40 mins/day Total 1 day	Artificial saliva	37 ˚C	Yes	30 ml	Immersion	Dem. Rem. cycles (Dem.10 mins Rem. 1 hour) X4	Profilometry	*In vitro*	No	pH 2.6
Lodi et al 2010(Lodi et al., 2010)	Milk beverages	Bovine incisor teeth / enamel (n=10)	Total 20 min/day Total 1 day	Artificial saliva	NA	No	15 ml	Immersion	Dem. Rem. cycles 24 hrs (artificial saliva) (Dem. 5 min Rem. 60 min) x4	Microhardness Profilometry	*In vitro*	No	Yes
Magalhaes et al 2010(Magalhães et al., 2010)	Sprite zero	Bovine roots/ dentin (n=10)	Total 6 min/day Total 5 days	According to Vieira et al. 2005	25 ˚C	No	30 ml	Rinsing	Dem. Rem. cycles (Dem. 90 sec Rem. 2 hrs) X 4 Rem. overnight	Profilometry	*In vitro*	No	pH 2.6
Manton et al 2010(Manton et al., 2010)	Various	Human teeth (n=3)	30 min	No	37 ˚C	No	50 ml	Immersion	No	Profilometry	*In vitro*	No	pH 2.2-2.4
Moretto et al 2010(Moretto et al., 2010)	Sprite	Bovine teeth/ enamel	Total 20 min/day Total 7 days	According to Vieira et al. 2005	Room	No	10 ml	Immersion	Dem. Rem. cycles (Dem. 5 min Rem. 2 hrs) X4	Profilometry Microhardness	*In vitro*	Y/N	pH 2.8
Murrell et al. 2010 (Murrell et al., 2010)	Various	Human molars and premolars/ enamel (n=5)	Total 25 hrs	No	Room	Yes	250 ml Repl. 5 hrs	Immersion	No	Polarized light microscopy Image pro plus system	*In vitro*	No	Coke pH UK 2.38 US 2.38 Sprite pH UK 2.85 US 2.82

Author	Acid source of concern	Substrate (Sample size)	Acidic exposure time	Artificial saliva	Temp.	Stirring / agitation	Vol.	Exposure method	Dem. Rem. Cycling (per day)	Erosion assessment	Condition/ special features	Abrasion	pH
Poggio et al. 2010 (Poggio et al., 2010b)	Various	Human central incisors (n=6)	Total 20 min/day Total 14 days	Artificial saliva	NA	No	NA	Immersion	Dem. Rem. cycles (Dem. 10 min Rem. Artificial saliva)x2	Atomic force microscopy	*In vitro*	No	NA
Poggio et al 2010 (Poggio et al., 2010a)	Coca cola	Human central incisors	2 min X4 Total 8 min/day	No	Room	No	6 ml	Immersion	No	Atomic force microscopy	*In vitro*	No	pH 2.44
Setarehnejad et al. 2010(Setarehnejad et al., 2010)	Citrate buffer 0.1 M	Crystalline hydroxyl-apatite	5 min then 5 min	No	NA	Yes	1 ml	Immersion	No	Atomic absorption spectroscopy	*In vitro*	No	pH 2.5, 3.5, 4.5
Shellis et al. 2010(Shellis et al., 2010)	Citric acid 0.3%	Human molars/ enamel, dentin, HA	30 min	No	Room	Yes	NA	Immersion	No	Atomic absorption spectroscopy	*In vitro*	No	pH 3.2
Souza et al. 2010(Souza et al., 2010)	Coca cola	Bovine incisors/ enamel (n=12)	Total 6 min/day Total 5 days Total 10 days	According to Klimek et al. 1982	25 ˚C	No	30 ml	Immersion	Dem. Rem. cycles (Dem. 90 sec Rem. 2 hrs) X4	Profilometry	*In vitro*	No	pH 2.3
Torres et al. 2010 (Torres et al., 2010)	Various	Human primary incisors/ enamel (n=15)	Total 15 min/day Total 60 days	Artificial saliva	NA	Yes	75 ml	Immersion	Dem. Rem. Cycle Artificial saliva (24hrs) (Dem. 5 min Rem. 4 hrs) x3 Rem. overnight	Microhardness	*In vitro* Different depths: 0-200 µm	No	pH 2.35
Barbosa et al. 2011(Barbosa et al., 2011)	Various	Bovine incisors/ dentin (n=20)	Total 40 mins/day Total 1 day	According to Vieira et al. 2005	Dem. 25 °C Rem. 37 °C	Yes	30 ml	Immersion	Dem.- Rem. cycles (Dem. 10 mins (30 ml) Rem. 60 mins (30 ml)) X 4	Profilometry	*In vitro*	No	Coke pH 2.6
Benjakul et al. 2011 (Benjakul and Chuenarrom, 2011)	Various	Human third molars / enamel (n=8)	60 mins	No	37 °C	Yes	NA	Immersion	No	Profilometry	*In vitro* Prediction equation	No	Pepsi pH 2.61
Braga et al. 2011(Braga et al., 2011)	Gastric and orange juice	Surgically removed impacted third molars (n=10)	Total 20 mins/day Total 14 days	Yes	24 °C	Yes	3 ml	Immersion	Dem. Rem. cycles (Dem. 5 mins Rem. 3 hrs) X4	Atomic emission spectroscopy. Fourier transform Raman spectroscopy	*In vitro*	No	pH Orange juice 3.7 Gastric juice 1.6
Haghgoo et al. 2011(Haghgoo et al., 2011)	Lemon soft beer	Surgically removed impacted third molars (n=18)	5 mins	No	NA	No	40 ml	Immersion	No	Microhardness	*In vitro*	No	pH 4

Author	Acid source of concern	Substrate (Sample size)	Acidic exposure time	Artificial saliva	Temp.	Stirring / agitation	Vol.	Exposure method	Dem. Rem. Cycling (per day)	Erosion assessment	Condition/ special features	Abrasion	pH
Hemingway et al. 2011(Hemingway et al., 2011)	Various Citric acid	Human molars (n=8)	Nat. Sal. (2 hrs) Dem. 10 mins Total 10 mins Total 1 day	No Natural human saliva	36 ˚C	Yes	100 ml	Immersion	No	Profilometry	*In vitro* *In vitro* pellicle	No	Citric acid pH 2.8, 3.2, 3.8
Nirmala and Subba Reddy 2011(Nirmala and Subba Reddy, 2011)	Various	Human premolars / tooth surface (n=2)	15, 24 hrs	No	NA	No	NA	Immersion	No	Polarised light microscopy	*In vitro*	No	pH 3.75
Scaramucci et al. 2011 (Scaramucci et al., 2011)	Orange juice	Human enamel and dentin (n=10) Bovine (n=5)	Total 30 min/day Total 5 days	Artificial saliva + mucin	Room	No	10 ml	Immersion	Dem. Rem. Cycles (Dem. 5 min Rem. 60 min) x6 Rem. overnight	Profilometry Microhardness	*In vitro*	No	pH 3.83
Vieira et al. 2011(Vieira et al., 2011)	Sprite	Bovine incisors / enamel (n=6)	Total 12 min/day Total 4 day	Orthana artificial saliva	NA	No	25 ml	Immersion	Dem. Rem cycles (Dem. 2min X6 Rem. 1.5 hrs)	Profilometry	*In vitro*	No	pH 2.81
Wang et al. 2011(Wang et al., 2011)	Orange juice	Human molars/ enamel	Total 6 min/day Total 4 days	Stimulated natural saliva Mucin-artificial saliva	30 ˚C	Yes	25 ml	Immersion	Dem. Rem. cycles Nat. stim. sal. (2 hrs) (Dem. 3 min Rem. Natural saliva 4 hrs) X2 Artificial saliva (overnight 15 hrs)	Microhardness	*In vitro*	No	pH 3.6
Wegehaupt et al. 2011(Wegehaupt et al., 2011)	Orange juice	Bovine incisors/ enamel (n=12)	120 sec X20 and X40	Artificial saliva (limited application)	NA	No	200 ml	Immersion	No	Profilometry	*In vitro*	Yes	pH 3.96
Cochrane et al. 2012(Cochrane et al., 2012)	Various	Human molars/ enamel (n=10)	30 min Total 1 day	No	Room	Yes	50 ml	Immersion	No	Microhardness Profilometry Ion Chromatography	*In vitro*	No	pH 2.45
Jager et al. 2012 (Jager et al., 2012)	Coca cola (Degassed)	Bovine incisors/ enamel (n=5)	3,6,9,15,30 min	No	NA	Yes	1 ml	Immersion	No	Atomic absorption spectroscopy (Loss of calcium)	*In vitro*	No	pH coke 2.47 sprite 2.81
Lussi et al. 2012(Lussi et al., 2012b)	Various	Human Premolars / enamel (n = 5)	Nat. sal. (3 hrs) 2 min or 4 min	Natural saliva	30 ˚C	Yes	60 ml	Immersion	No	Microhardness	*In vitro*	No	Coke pH 2.45

Author	Acid source of concern	Substrate (Sample size)	Acidic exposure time	Artificial saliva	Temp.	Stirring / agitation	Vol.	Exposure method	Dem. Rem. Cycling (per day)	Erosion assessment	Condition/ special features	Abrasion	pH
Passos et al. 2013 (Passos et al., 2013)	Coca cola	Human third molars/ Enamel and dentin (n=10)	Total 3 min/day Total 5 days	Artificial saliva	Room	Yes	5 ml	Immersion	Dem. Rem. Cycles (Dem. 60 sec Rem. 60 min) x3 Rem. overnight	Microhardness Profilometry FVM	*In vitro*	Y/N	No
Poggio et al. 2013 (Poggio et al., 2013)	Coca cola	Human incisors/ enamel and dentin (n=10)	2 min x4	Artificial saliva	Room	No	6 ml	Immersion	After experiment: Artificial saliva	Profilometry (roughness)	*In vitro*	No	pH 2.44
Aykut-Yetkiner et al. 2014 (Aykut-Yetkiner et al., 2014)	Coca cola Orange juice Sprite	Bovine incisors/ enamel (n=18)	10 min	No	20 ˚C	No	30 ml See flow rate	Rinsing 3ml/min	No	Profilometry	*In vitro*	No	pH coke 2.44 orange 3.72 sprite 2.65
Owens et al. 2014(Owens et al.)	Various	Extracted permanent teeth/ enamel (n=5)	Total 24 hrs/day Total 10 days	No	37 ˚C	No	NA Repl. daily	Immersion	No	Weight loss	*In vitro*	No	pH 2.49
Barac et al 2015(Barac et al., 2015)	Various	Human impacted third molars/ enamel	Total ½ , 1, 2 min/day Total 15, 30, 60 min (after 10 days)	Natural saliva	Room	Yes	50 ml	Immersion	Dem. Rem. cycles (Dem. ½ , 1, 2 min) Rem. Natural slaiva) X3	Profilometry	*In vitro*	No	Coke pH 2.67
Rezvani et al. 2015 (Rezvani et al., 2015)	Coca cola	Human premolars/ enamel	Dem. 8 min Rem. 10 min	Artificial saliva (Kin Hirdat spray)	NA	No	40 ml Repl. 2 min	Immersion	No	Microhardness	*In vitro*	No	pH 2.7
Xavier et al. 2015 (Xavier et al., 2015)	Coca cola Sprite	Human deciduous incisors and permanent premolars (n=10)	15,60 min/day Total 1 day	According to Zero et al. 1996	NA	Yes	10 mmol/l per mg of enamel	Immersion	Dem. Rem. Cycles (Dem. 5 or 20 min Rem. 5 min) x3	Spectro-photometry Microhardness	*In vitro*	No	Coke pH 2.58 Sprite pH 2.98

Author	Acid source of concern	Substrate (Sample size)	Acidic exposure time	Artificial saliva	Temp.	Stirring / agitation	Vol.	Exposure method	Dem. Rem. Cycling (per day)	Erosion assessment	Condition/ special features	Abrasion	pH
Rugg-Gunn et al. 1998 (Rugg-Gunn et al., 1998)	Various	Bovine incisors	*In situ* 15 min X4 Total 60 min/day Total 6 days	Natural saliva	Room	NA	NA	Immersion	*In situ*	Profilometry	*In situ*	Yes	pH 3.1
Fushida and Cury 1999 (Fushida and Cury, 1999)	Coca cola	Bovine incisors/ enamel and dentin	[(Dem. 10 sec Rem. 5 sec) X4]x1 []x2 (8hrs interval) []x4 (4hrs interval) []x8 (2hrs interval) Total 1 day	Natural saliva	NA	NA	50 ml	Natural drinking	*In situ*	Microhardness	*In situ* Acquired pellicle	No	pH 2.29
Hughes et al 2002(Hughes et al., 2002)	Orange juice	Un-erupted third molars / enamel samples	*In situ* 10 min X4 Total 10 days	Natural saliva	NA	NA	250 ml	Natural drinking	*In situ*	Profilometry	*In situ*	No	pH 3.3
Hara et al 2003(Hara et al., 2003b)	Sprite light	Bovine incisors roots / dentin	*In situ* 90 sec X2 Total 4 days (3 day erosion)	Natural saliva	NA	NA	NA	Immersion	*In situ*	Profilometer	*In situ* Salivary pellicle	Y/N	No
Hooper et al 2003(Hooper et al., 2003)	Orange juice	Extracted third molars / Enamel and dentin	*In situ* 10 min x4 Total 10 days	Natural saliva	NA	NA	250 ml	Natural drinking	*In situ*	Profilometer	*In situ*	Y/N	No
Hunter et al 2003 (Hunter et al., 2003)	Coca cola diet	Primary and permanent impacted third molars	*In situ* (10 min 25 ml/min) x4 Total 15 days	Natural saliva	NA	NA	250 ml	Natural drinking	*In situ*	Profilometry	*In situ*	No	No
West et al 2003(West et al., 2003)	Soft drinks	Enamel	*In situ* 10 min X4 Total 20 days	Natural saliva	NA	No	250 ml 25 ml/min	Natural drinking	*In situ*	Profilometry	*In situ*	No	pH 3.14 Tango Diet
Attin et al 2004(Attin et al., 2004)	Sprite light	Surgically removed impacted third molars/ Dentin	*In situ* 90 sec x2 Total 21 days	Natural saliva	NA	NA	50 ml	Immersion	*In situ*	Profilometry	*In situ* salivary pellicle	Yes	Sprite light pH 2.9
Hooper et al 2004(Hooper et al., 2004)	Sport drink	Impacted third molars / enamel	10 min x4 15 day	Natural saliva	NA	No	250 ml	Natural drinking	*In situ*	Profilometry	*In situ*	No	Sports drink pH 3.15
west et al 2004 (West et al., 2004)	Various + a modified drink	Surgically removed third molars / enamel	*In situ* 10 min X4 Total 15 days	Natural saliva	NA	No	250 m 25 ml/min	Natural drinking	*In situ*	Profilometry "surfometry"	*in situ*	No	Robinson's special R pH 3.6

Author	Acid source of concern	Substrate (Sample size)	Acidic exposure time	Artificial saliva	Temp.	Stirring / agitation	Vol.	Exposure method	Dem. Rem. Cycling (per day)	Erosion assessment	Condition/ special features	Abrasion	pH
Hooper et al 2005(Hooper et al., 2005)	Sport drinks	Surgically removed third molars/ enamel	35 ml/min 10 min 5 min rest 26 ml/min 25 min 5 min rest 50 ml/min 10 min 5 min Total 10 days	Natural saliva	NA	No	350ml in 10 650ml in 25 500ml in 10 1.5L total	Natural drinking	*In situ*	Profilometry	*In situ*	No	pH 3.17-3.81
Venables et al 2005(Venables et al., 2005)	Sport drinks	Human third molar teeth / enamel	Dem. 5 min (200 ml) x4 Dem. 10 min (400 ml) x4	Natural saliva	NA	No	(50 ml sip) 200 ml, 400 ml	Natural drinking 50 ml/75 sec	*In situ*	Profilometry	*In situ*	No	pH 3.16 3.81
Hara et al 2006(Hara et al., 2006)	Orange juice	Bovine incisors / Enamel and dentin (n=12)	*In situ* (Zero or 12 hrs for pellicle) (15 sec X 40) Total 10,20,30 min	Natural saliva	NA	No	(10 ml sip) 400, 800, 1200 ml	Natural drinking	*In situ*	Microhardness for enamel and EOM for dentin	*In situ* Salivary pellicle	No	pH 3.8
Rios et al. 2006 (Rios et al., 2006a)	Coca cola	Bovine incisors enamel	*In situ* 10 min X4 Total 40 min/day Total 5 days	Natural saliva	NA	NA	150 ml	Immersion	*In situ*	Profilometry Microhardness	*In situ* Salivary pellicle	Y/N	pH 2.8
Rios et al 2006 (Rios et al., 2006b)	Coca cola	Human third molars and bovine incisors/ Enamel (n=6)	*In situ* 5 min X4 Total 20 min/day Total 7 days	-Natural saliva -Natural stimulated saliva (30 min/day)	NA	No	150 ml	Immersion	*In situ*	Microhardness Profilometry	*In situ* Salivary pellicle	Y/N	No
Hooper et al 2007 (Hooper et al., 2007a)	Modified and unmodified soft drinks	Surgically removed third molars/ Enamel	10 min X4 Total 10 days	Natural saliva	NA	No	250 ml	Natural drinking	*In situ*	Profilometry	*In situ*	No	pH 3.4
Hooper et al. 2007(Hooper et al., 2007b)	Orange juice	Human third molars/ enamel	10 mins X4 Total 40 mins/day Total 5 days	Natural saliva	NA	NA	250 ml	Natural drinking 25ml/min	*In situ*	Profilometry	*In situ*	No	pH 3.8
Vieira et al.2007 (Vieira et al., 2007)	Sprite	Human molars and premolars/ enamel	*In situ* 5 min X3 Total 15 min/day Total 15 days	Natural saliva	NA	Yes	NA	Immersion	*In situ*	Profilometry	*In situ*	Y/N	pH 2.81
Honorio et al 2008(Honório et al., 2008)	Coca Cola	Impacted third molars/ enamel (n=2)	*In situ* (24hrs) 5 mins X3 Total 15 min/day Total 14 days	Natural saliva	NA	No	150 ml	Immersion	No	Microhardness Profilometry	*In situ* ±Plaque	No	pH 2.6
Sales-Peres et al 2007(Sales-Peres et al., 2007)	Coca cola	Human enamel and dentin	*In situ* 5 min X4 Total 20 min/day Total 5 days	Natural saliva	NA	No	150 ml	Immersion	*In situ*	Microhardness Profilometry	*In situ*	Y/N	No

Author	Acid source of concern	Substrate (Sample size)	Acidic exposure time	Artificial saliva	Temp.	Stirring / agitation	Vol.	Exposure method	Dem. Rem. Cycling (per day)	Erosion assessment	Condition/ special features	Abrasion	pH
Magalhaes et al 2008 (Magalhães et al., 2008b)	Coca Cola	Surgically removed Impacted third molars/ Enamel (n=3)	*In situ* (12 hrs) 5 min X4 Total 20 min/day Total 7 days	Natural saliva	Room	No	150 ml	Immersion	*In situ*	Profilometry Microhardness	*In situ* Salivary pellicle	No	pH 2.5
Rios et al 2008(Rios et al., 2008b)	Coca cola	Bovine incisors and human third molars/ enamel	*In situ* 5 min X4 Total 20 min/day Total 7 days	Natural saliva (+stimulated)	NA	No	150 ml	Immersion	*In situ*	SEM	*In situ* Salivary pellicle	Yes	No
Rios et al 2008(Rios et al., 2008a)	Coca cola	Bovine invisors/ enamel + fillings (n=4)	*In situ* 5 min X3 Total 15 min/day Total 7 days	Natural saliva	Room	No	150 ml	Immersion	*In situ*	Profilometry Microhardness	*In situ*	No	pH 2.6
Wiegand et al 2008 (Wiegand et al., 2008a)	Acid	Enamel	*In situ* 40 sec X3 Total 14 days	Natural saliva	NA	No	NA	Immersion	*In situ*	Profilometry	*In situ*	Yes	No
Hanning et al 2009(Hannig et al., 2009)	Various	Bovine incisors / enamel	20 secs	Natural Saliva	NA	No	200 ml	Natural drinking	*In situ* 120 mins Dem. 20 secs or *In situ* 120 mins Dem. 20 secs Rem. *In situ* 120 mins	Microhardness TEM	*In situ* Salivary pellicle	No	Sprite light pH 2.82 Coke light pH 2.85
Kato et al 2009(Kato et al., 2009)	Coca Cola	Bovine incisors / dentin	*In situ* (12 hrs) 5 min X4 Total 20 mins/day Total 5 days	Natural saliva	Room	No	150 ml	Immersion	No	Microhardness Profilometry	*In situ* Salivary pellicle	Y/N	pH 2.6
Rios et al 2009(Rios et al., 2009)	Coca cola "regular vs diet"	Surgically removed impacted human third molars/ enamel	*In situ* 5 min X3 Total 14 days	Natural saliva Stimulated natural saliva	NA	No	150 ml	Immersion	*In situ*	Microhardness Profilometry	*In situ* Salivary pellicle	No	Yes
Domiciano et al 2010(Domiciano et al., 2010)	Sprite diet	Bovine incisors / Dentin	Total 6 mins/day Total 3 days	No Natural Saliva	NA	No	NA	Immersion	Dem. (90 secs X4) Rem. *In situ*	Microhardness	*In situ* Salivary pellicle	No	Sprite diet pH 2.84
Kato et al. 2010 (Kato et al., 2010a)	Coca cola	Bovine incisors/ dentin	*In situ* 5 min x4 Total 20 min/day Total 5 days	Natural saliva	Room	NA	150 ml	Immersion	*In situ*	Profilometry	*In situ*	No	pH 2.6

Author	Acid source of concern	Substrate (Sample size)	Acidic exposure time	Artificial saliva	Temp.	Stirring / agitation	Vol.	Exposure method	Dem. Rem. Cycling (per day)	Erosion assessment	Condition/ special features	Abrasion	pH
Srinivasan et al. 2010 (Srinivasan et al., 2010)	Coca cola	Human third molars/ enamel	8 min	Natural saliva	*In vitro* Room	No	*In vitro* 5 ml	Immersion	Dem. In vitro Rem. In situ (2 days)	Microhardness	*In situ*	No	pH 2.3
Turssi et al 2010(Turssi et al., 2010)	Orange juice	Bovine incisors and human third molars / enamel-dentin	*In situ* 10 min X4 Total 40 min/day Total 10 days	Natural saliva	Room	No	250 ml	Natural drinking	*In situ*	Microhardness	*In situ*	No	pH 3.47
Wiegand et al. 2010 (Wiegand et al., 2010)	Sprite	Bovine incisors/ enamel and dentin (n=2)	*In situ* 90 sec X4 Total 6 min/day Total 3 days	Natural saliva	NA	No	100 ml	Immersion	*In situ*	Profilometry	*In situ* Salivary pellicle	Y/N	NA
Ren et al. 2011(Ren et al., 2011)	Orange juice	Human third molars / enamel	*In situ* 10 min X4 Total 40 min/day Total 5 days	Natural saliva	Room	No	250 ml	Immersion	*In situ*	Focus variation 3D vertical scanning microscope	*In situ* Acquired pellicle	No	pH 3.8
Wegehaupt et al. 2012 (Wegehaupt et al., 2012)	Sprite light	Bovine lower incisors	*In situ* Total 2 min/4hrs Total 4 hrs	Natural saliva	NA	NA	2 ml	Immersion	*In situ*	Microhardness	*In situ*	No	NA

Table 2.4. Summary of **results** (i.e. hardness and surface loss) for studies assessing erosive beverages that were published in the past 2 decades. Surface loss values were all converted to per hour values to allow for comparison. The application or addition of any modifying or preventive measurement was ignored; in such cases, the control group and/or the positive control were included only. Whenever possible, when a regime included abrasion, the control group without abrasion was taken into consideration only.

Study	Hardness (percentage)	Profilometry (surface loss)	Comments, Substrate & Beverage type
Rugg-Gunn et al. (1998)		12.84 ± 2.23 µm Protocol: *In situ* 15 min X4 Total 60 min/day Total 6 days Calculations: 2.14 µm/hour	- Bovine enamel - Diet
Amaechi et al. (1999a)		12 hrs Human permanent (20˚C): 59 µm 12 hrs Human deciduous (20˚C): 77 µm 12 hrs Bovine (20˚C): 100 µm Protocol: Total 30 min/day Total 24 days Calculations: 4.92 µm/hour (permanent)	- Human enamel - Orange juice Lesion depth and mineral loss increase as temp. increases. Protocol: Total 30 min/day Total 24 days
Fushida and Cury (1999)	1 cup: 81.3% 2 cups: 79.1% 4 cups: 75.5% 8 cups: 72.0% Recovery: 89.2%, 87.6%, 84.0%, 82.3% respectively. Protocol: *In situ* [(Dem. 10 sec Rem. 5 sec) X4] x1 or x2 x4 x8 Total 1 day		- Bovine enamel - Regular
Larsen and Nyvad (1999)		0.15-0.5 mm (24 hrs) 0.75-1.8 mm (1 week) Protocol: 24 hrs or 1 week Calculations: up to ≈10.7 µm/hour	- Human enamel - Regular
Maupome et al. (1999)	3 day: 75.4%, 59.5%, 54.1% 5 day: 66.8%, 53.7, 44.6% Protocol: 5,25,50 min/day Total 8 days		- Regular In each day from 1 to 8 hardness was assessed. Day 3 and 5 only were chosen.
Hughes et al. (2000)		3.93-7.47 µm Protocol: 10 min x3 7.86-14.94 µm/hour	- Human enamel - Citric acid
Lussi et al. (2000)	Coke 62.3% Sprite 55.1% Orange juice 92.9% Protocol: 3 min		- Human enamel - Regular-Degassed
Hammadeh and Rees (2001)		2.2-8.8 µm/hour	- Human enamel - Regular

Study	Hardness (percentage)	Profilometry (surface loss)	Comments, Substrate & Beverage type
Parry et al. (2001)			- Human enamel - Regular 6.352 µg of enamel (P)(phosphorus) dissolved Protocol: 5 min X6 Total 30 min/day Total 1 day
Hughes et al. (2002)		2 day 0.19±0.25 5 day 0.53±0.60 10 day 2.03±2.26 µm Protocol: *In situ* 10 min X4 Total 10 days Calculations: 0.14-0.30 µm/hour	- Human enamel - Orange juice
Hughes et al. (2002)		224 ± 24 µm (mixed with artificial saliva) Protocol: 25 hours Calculations: 8.96 µm/hour	Candy slurry
Larsen and Richards (2002)		613 µm Protocol: 48 hrs Calculations: 12.77 µm/hour	- Human enamel - Regular Calcium loss mmol/l: 2 min: 0.14, 10 min: 0.28, 60 min: 0.46
Attin et al. (2003)	36% Protocol: 1% citric acid total 15 mins/day (total 1 day) Dem. Rem. Cycling	0.82 µm Protocol: 1% citric acid total 15 mins/day (total 1 day) Dem. Rem. Cycling Calculations: 3.28 µm/hour	- Bovine enamel - Citric acid The group of (citric acid + phosphate) had 15% lower hardness value compared with the (citric acid + calcium, phosphate and fluoride) but the surface loss was comparable for both (around 15 µm).
Barbour et al. (2003)	≈5.3-19.6% Protocol: 120, 300 sec		- Human enamel - Citric acid
Hammadeh and Rees (2003)		Surface: Coke: 20.6-24.1 /ultrasonic+: 21.7-27.5 µm Orange: 12.8-15.5 /ultrasonic+: 13.2-16.9 µm Subsurface: Coke: 33.0-34.5 /ultrasonic+: 36.8-37.2 µm Orange: 10.6-16.0 /ultrasonic+: 10.7-16.1 µm Protocol: 4 hrs	- Human enamel - Regular + Orange juice Calculations: (no ultrasonic) Coke: surface 4.4-6.2 µm/hour Subsurface: 8.0-10.8 µm/hour Orange: surface 1.5-3.9 µm/hour Subsurface: 2.5-4.0 µm/hour
Hara et al. (2003b)		1.62 µm (erosion only) Protocol: *In situ* 90 sec X2 Total 4 days (3 day erosion) Calculations: 10.8 µm/hour	- Bovine Dentin - Sprite light

Study	Hardness (percentage)	Profilometry (surface loss)	Comments, Substrate & Beverage type
Hooper et al. (2003)		5 day 1.91 µm 10 day 2.83 (erosion only) Protocol: *In situ* 10 min x4. Total 10 days Calculations: 0.42-0.57 µm/hour	- Human enamel - Orange juice
Hunter et al. (2003)		*In vitro*: Diet coke 1 hr 3.57 2 hr 6.7 3 hr 7.99 4 hr 12.25 µm *In situ*: 15 day 1.18 ± 1.54 µm Protocol: *In vitro*: 60 min x 4. Total 4 hours *In situ*:10 min 25 ml/min. Total 15 days Calculations: *in vitro* 2.66-3.57 µm/hour *In situ* 15 day 0.47 µm/hour	- Human enamel - Diet *In vitro* and *in situ* experiments yielded contradicted results (high SD *in situ*)
Lupi-Pegurier et al. (2003)	88.5%, 78.5%, 70.9%, 63.8% respectively. Protocol: 10,30,90,120 sec		- Human enamel - Regular
Mahoney et al. (2003)	Orange juice: enamel 98% dentin 73% Fanta: enamel 53% dentin 71% Protocol: 10 min		- Human enamel - Orange juice
Phelan and Rees (2003)		3.3 ± 0.05 µm (hour) Protocol: 1 hour	- Human enamel - Orange juice + Herbal teas
West et al. (2003)		Day 2: 0.54 µm. Day 5: 1.18µm. Day 10: 2.0 µm. Day 15: 3.19 µm. Day 20: 4.92 µm Protocol: *In situ* 10 min X4 Total 20 days Calculations: 0.3-0.4 µm/hour	- Tango diet
Attin et al. (2004)		12.6 ± 6.7 µm (no abrasion) Protocol: *In situ* 90 sec x2. Total 21 days Calculations: 24 µm/hour-	- Human Dentin - Sprite light
Hooper et al. (2004)		15 day: 3.91 µm Protocol: 10 min x4 15 day Calculations: 0.39 µm/hour	- Human enamel - Sport drink
Lippert et al. (2004)	1 min: ≈72.2% 5 min: ≈20.6% Protocol: 1,1,1,1,1 min Total 5 mins/day Total 1 day		- Human enamel - Regular
von Fraunhofer and Rogers (2004)			- Human enamel - Regular Weight loss ≈2.8 mg/cm^2 (1.4%) Protocol: 14 days

Study	Hardness (percentage)	Profilometry (surface loss)	Comments, Substrate & Beverage type
West et al. (2004)		5 µm Protocol: *In situ* 10 min X4 Total 15 days Calculations: 0.5 µm/hour	- Human enamel - Soft drink + modifications
Willershausen and Schulz-Dobrick (2004)			- Human enamel - Regular Total mineral loss 14.5% (coca cola) in the depth of 10 µm Loss up to the depth of 30µm (4%) A stable ratio of Ca and P will dissolve throughout the erosive process. Protocol: 6 hours
Attin et al. (2005)		Coca Cola 0.19 ± 0.03 µm (standard error of mean) Sprite 1.59 ± 0.23 µm Protocol: 15 mins/day (total 1 day) Dem. Rem. C. Calculations: 0.76 µm/hour (coke) 6.36 µm/hour (sprite)	- Bovine enamel - Regular + Sprite
Barbour et al. (2005)	74%, 57.7%, 39.9%, 22.1%, 18.3% respectively Protocol: 30, 60, 120, 300, 600 sec		- Human enamel - Citric acid
Hooper et al. (2005)		*In situ*: Gatorade 4.08 µm (10 day) 1.84 µm (5 day) *In vitro*: Gatorade 17.44 µm (1 hour) Protocol: *In situ*: 35 ml/min 10 min 5 min rest 26 ml/min 25 min 5 min rest 50 ml/min 10 min 5 min Total 10 days *In vitro*: 1 hour X4 Total 4 hrs Calculations: *in situ* 0.49-0.54 µm/hour *In vitro* 17.44 µm/hour	- Human enamel - Sports drink
Jensdottir et al. (2005)			- Human enamel - Regular Calcium lost: 2.48 mmol/l Weight loss: 0.7 % Protocol: 24 hrs
Ramalingam et al. (2005)		3.87 µm Protocol: 30 min Calculations: 7.74 µm/hour	- Human enamel - Sports drink

Study	Hardness (percentage)	Profilometry (surface loss)	Comments, Substrate & Beverage type
Rees et al. (2005b)		Sports: 1.18-5.36 µm Orange: 3.68 µm Protocol: 1 hour	- Human enamel - Sports drinks and orange juice
Seow and Thong (2005)	No saliva: 76% (Natural saliva 50% v/v): 100% Protocol: 30 min		- Human enamel - Regular After adding 50% v/v nat. saliva to coke: pH rose to 5.2 (surface enamel)
Shellis et al. (2005)		4.87-14.25 µm (citric acid) Protocol: 20 min or 14.61-42.75 µm/hour	- Human enamel - Citric acid or Ribena
Van Eygen et al. (2005)	3 day: 68.4%, 62.8%, 68.1% respectively 7 day: 82.3%, 85.4%, 58.3% respectively or 3 day: 83.8% 7 day: 85.9% Protocol: 20,40, 60 min/day (20 min)x1,x2,x3 Or 20 min/day (1 min)x20 Total 7 days		- Human enamel - Regular Underlined: Hardness increased due to enamel being washed away? Or re-hardened by rem? The fact that hardness loss is proportional to acidic exposure period has been disputed; theoretically correct but practically enamel loss might render this technique questionable in terms of assessing erosion *in vitro*
Venables et al. (2005)		4.23 µm Protocol: Dem. 5 min (200 ml) x4 Dem. 10 min (400 ml) x4	- Human enamel - Sport drink
Barbour et al. (2006)	4 ˚C: 87.2% 25 ˚C: 76.1% Protocol: 5 min	4 ˚C: NA 25 ˚C: NA Protocol: 30 min	- Human enamel - Robinson's and Ribena
Bizhang et al. (2006)		13.7 µm Protocol: 1 hour/day Total 2 weeks Calculations: 0.98 µm/hour	- Bovine enamel - Regular Mineral loss: 581.85 vol% µm
Devlin et al. (2006)	92.6%, 93.25%, 85.7%, 80.3% respectively 1,2,3,15 hours		- Human enamel - Regular
Hara et al. (2006)	Enamel: No pellicle: 65.32%, 49.52%, 29.06% With pellicle: 50.88%, 39.49%, 26.44% Protocol: *In situ* (Zero or 12 hrs for pellicle) (15 sec X 40) Total 10,20,30 min	Dentin: (30 min) No pellicle: 18.55 µm With pellicle: 18.02 µm	- Bovine enamel - Orange juice

Study	Hardness (percentage)	Profilometry (surface loss)	Comments, Substrate & Beverage type
Hemingway et al. (2006)		≈2.5 – 35 µm per hour Protocol: 10 mins X6 Total 60 mins/day Total 1 day	- Human enamel - Various soft drinks (Group with No abrasion only)
Rees et al. (2006)		5.23 ± 0.46 µm Protocol: 1 hour	- Orange juice
Rios et al. (2006b)	14.9% Protocol: *In situ* 5 min X4 Total 20 min/day Total 7 days	6.4 ± 3.0 µm Protocol: *In situ* 5 min X4 Total 20 min/day Total 7 days Calculations: 2.74 µm/hour	- Human enamel - Regular
Rios et al. (2006a)	8.39% (erosion only) Protocol: *In situ* 10 min X4 Total 40 min/day Total 5 days	2.77 ± 1.21 µm Protocol: *In situ* 10 min X4 Total 40 min/day Total 5 days Calculations: 0.83 µm/hour	- Bovine enamel - Regular
Wongkhantee et al. (2006)	63.3% Protocol: Total 100 sec/day Total 1 day		- Human enamel - Regular The acidic exposure protocol was simulated from a subject consuming a 325 ml can of beverage
Chunmuang et al. (2007)	1 day 69.6%, 3 day 56.9%, 7 day 30.8%, 14 day 13.7% Protocol: Total 20 min/day Total 14 days	1 day 2.62, 3 day 7.38, 7 day 15.14, 14 day 26.08 µm Protocol: Total 20 min/day Total 14 days Calculations: 5.59-7.86 µm/hour	- Human enamel - Orange juice
de Carvalho Sales-Peres et al. (2007)	Coke 22.7% coke light 27.4% Protocol: Total 40 min/day Total 1 day		- Bovine enamel - Regular
Hooper et al. (2007a)		5 day 2.92 ± 3.67 µm 10 day 6.04 ± 6.32 µm Protocol: 10 min X4 Total 10 days Calculations: 0.88-0.95 µm/hour	- Human enamel - Soft drinks
Hooper et al. (2007b)		Day 5: 1.397 µm Day 10: 2.424 µm Day 15: 3.233 µm Protocol: 10 mins X4 Total 40 mins/day Total 5, 10, 15 days	- Human enamel - Orange juice *In situ*

Study	Hardness (percentage)	Profilometry (surface loss)	Comments, Substrate & Beverage type
Hooper et al. (2007b)		Day 7: 29.61 µm Day 15: 55.97 µm Protocol: 20 mins/day 7 days 15 days Calculations: 11.2 µm/hour	- Human enamel - Orange juice
Hove et al. (2007)		No pellicle (8 mins): 12 µm Pellicle (8 mins): 11 µm Protocol: Natural saliva (2 hrs) Dem. 2,2,2,2 mins Total 8 mins/day Total 1 day	- Human enamel - HCl
Kato et al. (2007)	23.3% Protocol: Total 40 min/day Total 1 day	3.5 ± 0.2 µm Protocol: Total 40 min/day Total 1 day Calculations: 5.25 µm/hour	- Bovine enamel - Regular
Kitchens and Owens (2007)			- Human enamel - Regular Surface roughness increased by 118% Protocol: Total 24 hrs/day Total 14 days
Magalhães et al. (2007)	2 day: 12.04% / with varnish 11.72% 4 day: 5.85% / with varnish 7.96 % Protocol: Total 10 min/day Total 4 days	2 day: 3.43 ± 1.13 µm 4 day: 7.31 ± 0.53 µm Protocol: Total 10 min/day Total 4 days Calculations: 10.29-10.96 µm/hour	- Bovine enamel - Regular - ± Varnish
Rees et al. (2007)		1.18-6.86 µm Protocol: 1 hour	- Human enamel - Various
Vieira et al. (2007)		Day 5: 13.49 ± 5.80 µm Day 10: 23.93 ± 9.16 µm Day 15: 37.81 ± 11.89 µm Protocol: *In situ* 5 min X3. Total 15 min/day. Total 15 days Calculations: 10.8-13.49 µm/hour + Varnish 1.55–4.39 µm/hour	- Human enamel - Sprite - ± Varnish
Sales-Peres et al (2007)	19.2 % Protocol: *In situ* 5 min X4 Total 20 min/day Total 5 days Calculations: 5.58 µm/hour	9.3 ± 6.1 µm Protocol: *In situ* 5 min X4 Total 20 min/day Total 5 days Calculations: 5.58 µm/hour	- Human enamel - Regular

Study	Hardness (percentage)	Profilometry (surface loss)	Comments, Substrate & Beverage type
Chuenarrom and Benjakul (2008)		Coke: 0.71,1.49,2.73,6.73,8.29 µm respectively (profilometry) Protocol: 15,30,60,120,180 min Calculations: coke 2.73-3.02 µm/hour Orange juice 0.75-0.85 µm/hour	- Human enamel - Regular + Orange juice
Ehlen et al. (2008)		92 µm Protocol: 25 hrs Calculations: 3.68 µm/hour	- Human enamel - Regular
Francisconi et al. (2008)	32.6% - 35.9% Protocol: Total 15 min/day Total 5 days	2.18-2.55 µm Protocol: Total 15 min/day Total 5 days Calculations: 1.74-2.04 / hour	- Bovine enamel - Regular
Hara et al. (2008)		AS: 0.56µm AS+M: 0.6µm NatS: 1.08µm DIW: 1.58µm Protocol: (Dem. 5 mins Rem. 30 mins) X3 Total 3 days Calculations: 0.75, 0.8, 1.44, 2.11 µm/hour respectively	- Human enamel - Citric acid The group of No abrasion
Hara and Zero (2008)	≈35% after ½ hour ≈20% after 1 hour ≈10% after 2 hours	≈1 mm after ½ hour ≈2 mm after 1 hour ≈3 mm after 2 hours Protocol: 120 minutes Calculations: 2 mm / hour	- Bovine enamel - Regular
Honório et al. (2008)	13% (erosion only) 86.5% (erosion + plaque) Protocol: *In situ* (24hrs) 5 mins X3 Total 15 min/day Total 14 days	4.82 ± 1.78 µm (erosion only) 0.14 ± 0.04 µm (erosion + plaque) Protocol: *In situ* (24hrs) 5 mins X3 Total 15 min/day Total 14 days Calculations: 1.38 µm/hour (erosion only)	- Human enamel - Regular
Hunter et al. (2008)		2.45-7.03 µm Protocol: 1 hour	- Human enamel - Fruit drinks
Jager et al. (2008)		1 ml: 2.08 ± 0.58 µm 500 ml: 8.04 ± 3.62 µm Protocol: 3,6,9,15,30 mins Total 63 mins/day Total 1 day Calculations: 7.66 µm/hour (500 ml)	- Bovine enamel - Various 3 min: Ca 0.15 P 0.15 6 min: Ca 0.26 P 0.26 9 min: Ca 0.32 P 0.33 15 min: Ca 0.42 P 0.61 30 min: Ca 0.61 P 0.87

Study	Hardness (percentage)	Profilometry (surface loss)	Comments, Substrate & Beverage type
Low and Alhuthali (2008)			- Human enamel - Diet Weight loss ≈ 3% (No agitation) Protocol: 7 days
Machado et al. (2008)	27.7% Protocol: 30 min/day Total 5 weeks		- Humane enamel - Sprite + Orange juice Surface roughness increased from 5.3 to 6.86 µm
Magalhães et al. (2008a)		Total 5 days: 3.94 µm (first 3 days): each day 0.92 µm Protocol: (Dem. 1 min Rem. 59 min) X6 then Rem. overnight Acid flow rate: 3ml/min Saliva flow rate 1.1 ml/min Calculations: 7.88 µm/hour (based on total) 9.2 µm/hour (based on 1st 3 days) + Varnish 1.73 µm/hour	- Bovine enamel - Sprite - ± Varnish
Magalhães et al. (2008b)	10.37% Protocol: *In situ* (12 hrs) 5 min X4 Total 20 min/day Total 7 days	3.63 ± 1.54 µm Protocol: *In situ* (12 hrs) 5 min X4 Total 20 min/day Total 7 days Calculations: 1.56 µm/hour	- Human enamel - Regular
Rios et al. (2008a)	Control group mean (n=4): 32.22% Protocol: *In situ* 5 min X3 Total 15 min/day Total 7 days	Control group mean (n=4): 3.04 µm Protocol: *In situ* 5 min X3 Total 15 min/day Total 7 days 1.74-2.43 µm/hour	- Bovine enamel - Regular
Tantbirojn et al. (2008)	4 min: ≈83% 8 min: ≈69% From 4 to 8 min: ≈83% Protocol: 2 min x4 Total 8 min/day. Total 1 day +2 days artificial saliva replenishment		- Bovine enamel - Regular The scope of the study was to assess remineralisation rather than the erosive process.
Wiegand et al. (2008a)		2.3 ± 1.0 µm (abrasion before erosion group) Protocol: *In situ* 40 sec X3 Total 14 days Calculations: 4.9 µm/hour	

Study	Hardness (percentage)	Profilometry (surface loss)	Comments, Substrate & Beverage type
Willershausen et al. (2008)			- Human enamel - Apple juice Ca release permanent: 0.41± 0.085 mg/20mm^2 Primary 0.61 ± 0.035 mg/20mm^2 Protocol: 24 hrs
Ablal et al. (2009)		60 min: 12.7 µm Comment: Art. Saliva (2 hrs) 20 min, 60 min, 24 hrs	- Bovine enamel - Alcopops
Cochrane et al. (2009)		7.04 ± 0.29 µm Protocol: 30 mins Calculations: 14.08 µm/hour	- Human enamel - Regular + Sprite Calcium loss (24 hrs): Coke 10.89 Sprite 4.97 µmol/mm^2
Hannig et al. (2009)	*In vitro* (coke light group) ≈94% No agitation ≈91.5% with agitation *In situ* (coke light group) ≈92% 120mins Rem. *in situ* ≈93% 240mins Rem. *In situ*		- Bovine enamel - Light
Kato et al. (2009)		0.98 ± 0.13 µm Protocol: *In situ* (12 hrs) 5 min X4 Total 20 mins/day. Total 5 days	- Bovine Dentin - Regular
Kato et al. (2009)		1.014 ± 0.033 µm Protocol: Total 60 min/day Total 1 day Calculations: 1.014 µm/hour	- Bovine enamel - Regular
Magalhaes et al. (2009)		0.79 ± 0.21 µm Protocol: (Dem. 1 min Rem. 59 min) X6 then Rem. 18 hrs Total 6 min/day. Total 1 day Calculations: 7.9 µm/hour	- Bovine enamel - Sprite zero
Murakami et al. (2009)	48 hrs: 51.4% / Varnish 68.3% 7 days: 56.9% / Varnish 72.6% Protocol: Total 30 min/day Total 7 days		- Human enamel - Regular - ± Varnish
Panich and Poolthong (2009)	87.67% Extra Rem. 89.89% Protocol: Total 100 sec/day Total 1 day		- Human enamel - Regular Labial surfaces of incisors

Study	Hardness (percentage)	Profilometry (surface loss)	Comments, Substrate & Beverage type
Poggio et al. (2009)		0.5 ± 0.15 µm Protocol: 2 min X4 Total 8 min/day. Total 1 day Calculations: 3.75 µm/hour	- Human enamel - Regular
Ren et al. (2009)		5.43-5.5 µm Protocol: Total 100 min/day Total 1 day Calculations: 3.26-3.3 µm/hour	- Human enamel - Orange juice
Rios et al. (2009)	21.5% Protocol: *In situ* 5 min X3 Total 15 min/day Total 14 days	3.1 ± 1.0 µm Protocol: *In situ* 5 min X3 Total 15 min/day. Total 14 days Calculations: 0.88 µm/hour	- Human enamel - Regular + Diet
Syed and Chadwick (2009)	Coke 39.1% Sprite 52.7% Protocol: 60 min	Coke 4.47 ± 2.74 µm Sprite: 2.37 ± 1.75 Protocol: 60 min	- Human enamel - Regular + Sprite
Wiegand et al. (2009)		3.0 ± 1.4 µm Protocol: Total 9 min/day Total 3 days Calculations: 20 µm/hour	- Bovine enamel - Fluoride free acidic placebo pH 3.9
Willershausen et al. (2009)			- Human enamel - Apple juice Mineral loss: 13% Loss up to the depth of 30 µm Protocol: 6 hrs
Al-Jobair (2010)	Cycle 1: 56.1% Cycle 2: 57.6% Cycle 3: 58.6% Protocol: (Total 6 min/day Total 3 days) x3		- Bovine enamel - Regular Comparable percentages even after repetition for 3 times.
Beyer et al. (2010)	35%, 16.7%, 9.8% respectively)pH 2.3 only Protocol: 30,60,120 sec		- Human enamel - Citric acid
Bueno et al. (2010)		Coca cola 0.94 ± 1.1 µm Protocol: Dem. Rem. cycles (Dem. 10 mins Rem. 60 mins) X6 Calculations: 0.94 µm/hour	- Bovine enamel - Regular
Domiciano et al. (2010)	76.8% Protocol: Dem. (90 secs X4) Rem. *In situ* Total 3 days		- Bovine Dentin - Sprite diet +Z250 filling

Study	Hardness (percentage)	Profilometry (surface loss)	Comments, Substrate & Beverage type
Kato et al. (2010b)		≈2.9 µm Protocol: Dem. Rem. cycles (Dem.10 mins Rem. 1 hour) X4 Calculations: 4.35 µm/hour	- Bovine enamel - Regular
Kato et al. (2010a)		1.77 ± 0.35 µm Protocol: In situ 5 min x4 Total 20 min/day Total 5 days Calculations: 1.06 µm/hour	- Bovine dentin - Regular
Lodi et al. (2010)	59-71% Protocol: Total 20 min/day Total 1 day	0.17 µm Protocol: Total 20 min/day Total 1 day Calculations: 0.51 µm/hour	- Bovine enamel - Milk beverages
Magalhães et al. (2010)		3.25 ± 0.5 µm Protocol: Total 6 min/day Total 5 days Calculations: 6.5 µm/hour	- Bovine Dentin - Sprite zero
Manton et al. (2010)		11.45 µm Protocol: 30 min Calculations: 22.9 µm/hour	- Human enamel - Regular
Moretto et al. (2010)	17.7% Protocol: Total 20 min/day Total 7 days	3.36 ± 0.23 µm Protocol: Total 20 min/day Total 7 days Calculations: 1.44 µm/hour	- Bovine enamel - Sprite
Murrell et al. (2010)		Coke: UK 148 ± 28 µm US 179 ± 22 µm Sprite: UK 143 ± 38 US 88 ± 30 Protocol: 25 hrs Calculations: Coke UK 5.92 µm US 7.16 µm Sprite UK 5.72µm US 3.52 µm	- Human enamel - Regular + sprite
Poggio et al. (2010a)		0.50 ± 0.15 µm Protocol: 2 min X4 Total 8 min/day Calculations: 3.75 µm/hour	- Human enamel - Regular
Shellis et al. (2010)			- Human enamel - Citric acid Estimated mineral loss after 30 min: 276 nmol mm^{-2} of hydroxyapatite calculations: after 60 min: 552 nmol mm^{-2} of hydroxyapatite Hydroxyapatite: Ca 39.9% (w/w) P 18.5% (w/w)

Study	Hardness (percentage)	Profilometry (surface loss)	Comments, Substrate & Beverage type
Souza et al. (2010)		5 day: 4.7 ± 0.38 µm 10 day: 7.19 ± 0.57 µm Protocol: Total 6 min/day Total 5 days Total 10 days Calculations: 7.19-9.4 µm/hour	- Bovine enamel - Regular
Srinivasan et al. (2010)	76.2% Protocol: 8 min		- Human enamel - Regular
Torres et al. (2010)	7,15,30,45,60 days 97.65%, 91.67%, 91.71%, 84.94%, 79.92% respectively. Protocol: Total 15 min/day. Total 60 days Calculations: 0.33% drop per 15 min 1.32% / hour		- Human Primary teeth enamel - Regular Day 15: hardness drop Up to day 30: non-significant hardness change Hardness loss comparable for different depths up to 150µm from surface
Turssi et al. (2010)	62.3% Protocol: *In situ* 10 min X4 Total 40 min/day Total 10 days		- Bovine enamel - Orange juice
Wiegand et al. (2010)		0.88 µm Protocol: *In situ* 90 sec X4 Total 6 min/day Total 3 days Calculations: 2.93 µm/hour	- Bovine enamel - Sprite
Barbosa et al. (2011)		3.6 ± 1.1 µm Protocol: 40 minutes/day (total 1 day) Calculations: 5.4 µm/hour	- Bovine Dentin - Regular
Benjakul and Chuenarrom (2011)		3.05 ± 0.74 µm/hour Calculations: 3.05 µm/hour	- Human enamel - Pepsi
Braga et al. (2011)			- Human enamel - Gastric + Orange juice 14 days Calcium loss orange: 7.07 ± 1.44 mg/l Calcium loss gastric: 12.74 ± 3.33 mg/l
Haghgoo et al. (2011)	92.5% Protocol: 5 mins		- Humane enamel - Lemon soft drink
Hemingway et al. (2011)		≈1.5-4.5 µm Protocol: Nat. Sal. (2 hrs) Dem. 10 mins Total 10 mins. Total 1 day Calculations: 9-27 µm/hour	- Human enamel - Various soft drinks

Study	Hardness (percentage)	Profilometry (surface loss)	Comments, Substrate & Beverage type
Ren et al. (2011)		5 day: ≈7.2 µm 10 day: ≈11.9 µm 15 day: ≈18 µm Protocol: *In situ* 10 min X4 Total 40 min/day Total 5 days Calculations: ≈1.8-2.16 µm/hour	- Human enamel - Orange juice
Scaramucci et al. (2011)	Human enamel ≈35.8% Protocol: Total 30 min/day Total 5 days	Human enamel 0.49 µm dentin 5.92 µm Bovine enamel 1.36 µm Protocol: Total 30 min/day Total 5 days Calculations: Human enamel 0.2 µm/hour Human dentin 2.37 µm/hour Bovine enamel 0.54 µm/hour	- Human enamel - Orange juice
Vieira et al. (2011)		12 min: 1.28 ± 0.67 µm 48 min: 3.72 ± 0.75 µm Protocol: Total 12 min/day Total 4 day Calculations: 4.65-6.4 µm/hour	- Bovine enamel - Sprite
Wang et al. (2011)	2x2 min: 43.5 % (after rem. 55%) then decreased down to 11.5% after the fourth day. Protocol: Total 6 min/day Total 4 days		- Human enamel - Orange juice
Wegehaupt et al. (2011)		20 cycles: 0.605 µm 40 cycles: 1.375 µm Protocol: 120 sec X20 and X40 Calculations: 0.9-1.03 µm/hour	- Bovine enamel - Orange juice
Cochrane et al. (2012)	48.9% Protocol: 30 min Total 1 day	3.22 µm Protocol: 30 min Total 1 day Calculations: 6.44 µm/hour	- Human enamel - Regular Protocol: 30 min Total 1 day
Jager et al. (2012)		Alternative measurement of surface loss	- Bovine enamel - Regular-Degassed Enamel loss 0.04 µm per minute Protocol: 3,6,9,15,30 min Calculations: 2.4 µm/hour
Lussi et al. (2012b)	2 min: Regular 69.0% / Citreous 62.4% 4 min: Regular 39.5% / Citreous 39.1 % Protocol: 2 or 4 min		- Human enamel - Regular + Sprite

Study	Hardness (percentage)	Profilometry (surface loss)	Comments, Substrate & Beverage type
Wegehaupt et al. (2012)	After Dem. 74.3% After Rem. 79.8% Protocol: *In situ* Dem. 2 min Rem. 4 hrs Total 4 hrs		- Bovine enamel - Sprite light Regardless of the remineralisation attempts utilised; baseline hardness values could not be achieved (in contrast to our results)
Passos et al. (2013)	Enamel (erosion only) 46.6% Enamel (erosion+abrasion) 75.1% Protocol: Total 3 min/day Total 5 days	Enamel (erosion only) 0.36-0.89 µm Dentin (erosion only) 1.36-3.85 µm Protocol: Total 3 min/day Total 5 days Calculations: enamel 1.44-3.56 µm/hour Dentin 5.44-15.4 µm/hour	- Human enamel - Regular Abrasion resulted in a harder surface owing to the removal of the softened layer
Aykut-Yetkiner et al. (2014)		Coke 5.60 ± 1.04 µm Sprite 5.49 ± 0.94 Orange 1.35 ± 0.4 µm Protocol: 10 min Calculations: 33.6, 32.9, 8.1 µm/hour respectively	- Bovine enamel - Regular + Sprite + Orange juice System of pumps, tubing and channels
Owens et al. (2014)			- Human enamel - Regular 27.19% weight loss Protocol: Total 24 hrs/day Total 10 days Calculations: ≈1% / hour
Barac et al. (2015)		15 min: 1.49 ± 0.08 µm 30 min: 1.63 ± 0.05 µm 60 min: 1.82 ± 0.01 µm Protocol: Total ½ , 1, 2 min/day Total 15, 30, 60 min (after 10 days) Calculations: 1.82 µm/hour	- Human enamel - Regular
Rezvani et al. (2015)	65.4% Rem. 82.4% Protocol: Dem. 8 min Rem. 10 min		- Human enamel - Regular
Xavier et al. (2015)	Coke: 5x3 group: permanent 45% primary 38% 20x3 group: permanent 30% primary 31% Sprite: 5x3 group: permanent 44% primary 41% 20x3 group: permanent 27% primary 31% Protocol: 15,60 min/day Total 1 day		- Human enamel - Regular + Sprite 5x3 group: coke Ca loss 38.3 mmol/l P loss 35.2 mmol/l 20x3 group: Ca 43 mmol/l P 52.3 mmol/l Protocol: 15,60 min/day Total 1 day

2.5.2 Erosion testing: methods and regimes

Based upon the foregoing summary tables (Tables 2-3 and 2-4) this section examines the methods currently available to the researcher to test the potential erosive effects of foods and beverages. These may be classified as *in vitro, in situ* and *in vivo* methods.

Current validated methods are unable to accurately measure an eroded tooth surface *in vivo* owing to the lack of highly accurate tools for measuring tooth tissue loss *in vivo*. Theoretically speaking, such a procedure implies the need for long duration studies. In any such study there is the challenge of controlling the extent of exposed tooth structure and preventing other factors from affecting the target area (i.e.: abrasion)(West et al., 2011b).

In vitro experiments are very convenient for their setting and time period can be customised. A standardized experimental protocol can be made, to examine one variable at a time and new variables can be introduced at any time. Furthermore, the ability to accurately measure an eroded tooth surface *in vitro* using precise devices and techniques means that many researchers favour this approach over *in vivo* experimental settings. However, clinical conditions with all of their associated biological parameters are far from being accurately simulated (West et al., 2011b).

Moreover, *in vitro* experiments can provide invaluable information that can be later utilised by researchers to fine-tune clinical experiments. They also facilitate the assessment of infinite sets of different variables, trends and protocols. In view of the large number of confounding variables that influence the process of dental erosion; one to several variables can be engaged in such experiments while others may be muted to more fully understand the exact role each variable plays in dental erosion. Additionally, compared to *in situ* and *in vivo* experiments, *in vitro* systems can test a

large number of specimens either at once or in batches for they can easily be reproduced (West et al., 2011b).

In situ experiments combine the advantages of *in vitro* and *in vivo* models; they not only allow controllable erosion scenarios, but also expose the samples to the oral environment (i.e.: flow of saliva, salivary pellicle formation and oral care habits)(West et al., 2011b) On the other hand, *in situ* models are still incapable of simulating the process of salivary buffering during normal drinking. It is also considered non-legitimate to form a salivary pellicle *ex vivo,* as an attempt to simulate the enamel/saliva interaction and so clinically relevant simulations are possible using *in situ* studies (Young and Tenuta, 2011) for they do in part take into account the salivary pellicle.

In situ experiments potentially overcome some of the ethical problems that may be encountered under *in vivo* studies. These relate to directly exposing test subjects to excessive acidic attacks. This can be done by indirectly exposing tooth specimens to acidic attacks *ex vivo* then returning the specimens back in the mouth via a removable appliance to benefit from the natural remineralisation process that usually occurs in the oral cavity. Another approach would be allowing test subjects to consume an amount of an erosive beverage that is equal to the average consumption of beverages in the population which will, most of the times, be ethically acceptable.

In general, *in situ* erosion experiments involve short acidic exposure times and repeated consumption patterns of offensive beverages. Many such experiments have been carried out and are summarized in Table 2-3.

The process of dental erosion, once initiated, will affect the most superficial tooth surface layer resulting in its partial dissolution of its ionic constituents via the so called early-stage surface softening (Young and Tenuta, 2011). Initial erosive attack leads to surface softening rather than surface loss.

When the structure is exposed to an erosive beverage for a period long enough to cause surface loss, the remaining enamel framework has capacity to regain its hardness but not its original structure provided that ideal remineralisation conditions are provided (Lussi et al., 2012b). In other words, for the rate of remineralisation to catch up with rate of destruction, quick acidic clearance is essential which is deemed difficult in the case of a prolonged and continuous exposure of tooth structure to erosive attacks.

Throughout the literature, a broad range of different experimental regimes have been adopted to assess dental erosion. This creates a serious problem both in interpreting these results as a whole and when comparing different results. In general, a lack of a guided standardisation among different *in vitro* and *in situ* experiments render them non comparable as a result of different experimental variables (West et al., 2011b). Utilisation of different dental erosion regimes has led to a broad variation in results owing to the different volumes, periods of exposure, flow rates and methods of acidic exposure.

At all times, in normal physiological conditions, a thin salivary film covers the surfaces of teeth as a result of the continuous production of saliva by the salivary glands. This keeps the oral cavity “bathed” in saliva. After swallowing, a residual volume of saliva will be left in the mouth. This residual film has been estimated to have a volume of approximately 1 ml (Young and Tenuta, 2011). When an erosive beverage is consumed,

the beverage to saliva ratio is not evenly distributed throughout the oral cavity. In other words, the volume of the acid containing vehicle far exceeds the amount of saliva present and this state lasts at least for the duration of retention of the erosive beverage in the mouth. This period of time is usually short in duration and once elapsed a mixture of beverage/saliva solution will remain until full clearance occurs via the aid of the stimulated saliva (Young and Tenuta, 2011). It should therefore be borne in mind that beverage-to-saliva and solution-to-substrate ratios need to be carefully assessed and piloted in order not to produce arbitrary or artificial results that do not reflect clinical conditions (Shellis et al., 2011).

With time, the superficial enamel becomes more mature and its exposure time-span to fluoride is therefore increased which will, in turn, affect the erosive attack outcome as fluoride substituted apatite is more erosion resistant. Enamel specimen preparation usually requires teeth to be sectioned and polished to obtain measurable flat enamel surfaces. This procedure renders enamel more prone to acidic attacks owing to the fact that removing the superficial layer eliminates the relatively stubborn and variable part of enamel that contains fluoride substituted apatite. This factor should be taken into consideration when interpreting erosion studies for it tends to accelerate the process of erosion but reduces the experimental time required in which to observe an erosive effect (Lussi et al., 2011). This procedure will probably lessen fluoride content differences among tooth specimens regardless of their age potentially reducing inter specimen variation in relation to this (Lussi et al., 2011). The same accelerated acid attack response can also be seen when using bovine enamel specimens instead of human enamel ones owing to the difference in their composition and morphology. Bovine enamel has more porosities than human enamel which results in an increased

susceptibility to acids for their presence prolongs acid contact time (Young and Tenuta, 2011).

Generally, *in vitro* and *in situ* experiments utilise either citric acid or the erosive beverages themselves to test for erosion caused by daily food and drink consumption. Some have also used hydrochloric acid to test for erosion caused by exposure to intrinsic factors (i.e. acidic reflux and vomiting). The use of soft drinks compared to a custom made acidic solution however, offers the advantage of more realistic experimental setting (Shellis et al., 2011).

An acidic attack caused by an erosive beverage after a single sip of drink will cause the pH to drop for no longer than 2 minutes, while consuming a whole can will presumably result in a more prolonged pH drop. Therefore, for a realistic scenario to be replicated *in vitro*, a balanced regime should be adopted where acidic exposure is neither unjustifiably limited nor prolonged (Young and Tenuta, 2011).

It is generally accepted that, for *in vitro* erosion experiments acidic exposures longer than 10 minutes will result in the loss of tooth structure in depth while shorter periods tend to only soften the superficial enamel layer (Hara and Zero, 2008). Another group of researchers have stated that acidic exposure times less than 3 minutes will most probably result in softening of the superficial layer up to a depth of 0.5 μm, while longer exposure periods of up to 2 hours can lead to, apart from structure loss, surface softening ranging from 2 to 4 μm in depth (Wiegand and Attin, 2011). It is worth mentioning that surface softening and enamel loss are both considered as two continuous interconnected processes that cannot be treated separately (Young and Tenuta, 2011). This is an important point to recognize when interpreting the results of any such experiment.

Attractive though it may be at first glance, the mere action of immersing tooth specimens in acidic solutions is too simple to be considered as a realistic erosion simulation. This approach has also resulted in considerable differences among the experimental regimes in the literature. The fact that there is a high number of inconsistent results is correlated with the broad variation in experimental parameters such as time of acidic exposure, temperature, volume and flow rate of offensive solution. This necessitates the development of strict guidelines to govern the aforementioned parameters so as to yield comparable, reproducible and consistent results (Young and Tenuta, 2011).

In an attempt to convert *in vitro* acidic exposure times (in specimen-immersion models) to real-life ones; the average daily consumption of carbonated drinks has been calculated to be 710 ml and the time required to clear the drink from the oral cavity by saliva was reported to be 20 seconds (von Fraunhofer and Rogers, 2004). Therefore, enamel was calculated to have a total exposure to acids of 25 hours per year. In other words, one hour of acidic exposure under *in vitro* conditions is equivalent to two weeks of normal beverage consumption in real-time (Kitchens and Owens, 2007, von Fraunhofer and Rogers, 2004).

It has been claimed that erosion models that adopt demineralisation/remineralisation cycles, utilising artificial saliva, can better reflect what actually happens in the oral cavity especially in models with controlled flow of artificial saliva and the erosive acidic solution (open systems) (Attin et al., 2003). In such systems specimens can be alternately rinsed with artificial saliva and the erosive solution. This, to a certain degree, is claimed to more closely simulate enamel/beverage interaction (Young and Tenuta, 2011).

Several studies have utilised erosion regimes with alternating erosion/abrasion cycles as an attempt to simulate the dual effect of acidic attacks and tooth brushing (table 2-3). Such an approach has the tendency to exaggerate clinical conditions in order to obtain measurable results (Benjakul and Chuenarrom, 2011). It is recommended therefore to tailor the duration and frequency of erosion based upon realistic clinical conditions especially when real-life scenarios are to be simulated (Wiegand and Attin, 2011). It is also advisable to disperse the erosive attacks over several successive days rather than condensing them all into a single period of time. This reduces the possibility of having random effects of the experimental conditions on a single day (Wiegand and Attin, 2011)

Most of the *in vitro* models reviewed exposed the enamel specimens to a predetermined quantity of erosive beverage for a period of time sufficient to yield a measurable effect. This often does not reflect real-life consumption rates and behaviour.

It is believed, up to the moment of writing this review, that modifying biological and physiological parameters such as dilution of the acidic solution by artificial saliva and simulating the protective effect the acquired pellicle provides against erosion (Wiegand and Attin, 2011) cannot be simulated adequately under *in vitro* conditions. It is also believed that programming *in vitro* models with human drinking behaviour parameters is difficult to achieve (Ehlen et al., 2008).

Several recommendations have been made to more realistically simulate real-life scenarios. One of these is to keep *in vitro* acidic exposure times to about 2 minutes or lower depending on the objective (Wiegand and Attin, 2011). This recommendation is based upon the calculations of Millward et al. (1997) who concluded that it takes the

oral cavity around 2 minutes to dilute, clear and buffer an erosive acid and therefore raise the pH back to a non-critical level (Millward et al., 1997). It is however worth mentioning that a single episode of acidic attack *in vitro* is supposed to represent what is considered to be the equivalent to 1 sip or bolus of an offensive element (i.e. food or drink). This 2-minute period limit can be legitimate especially if acidic exposure was performed without the interaction of saliva (Wiegand and Attin, 2011).

The total time period of acidic exposure varied, in the *in vitro* erosion experiments reviewed, between tens of seconds up to 40 min per cycle bearing in mind that usually several cycles were run in a single day. Complete immersion of specimens was also most frequently adopted for time periods ranging from 1 min up to several days (Wiegand and Attin, 2011). Carbonated drinks start losing their carbon dioxide content immediately after the seal of their container is removed. So whether it is an opened can or a poured glass of beverage; the pH gradually increases due to degassing (Larsen and Nyvad, 1999). This needs to be addressed in *in vitro* experiments that simulate beverage consumption.

Temperature has a proportional (approximately linearly) relationship with the degree of erosion; the greater the temperature the more erosive the acidic solution becomes (Barbour et al., 2006) thus lower tooth structure hardness values and greater enamel apatite loss is seen in those models that utilized high temperatures such as 37 °C. The acidity level of carbonated beverages is reported to increase (pH to decrease) with increasing temperature (Manton et al., 2010, Amaechi et al., 1999a, West et al., 2000). This could be attributed to greater chemical reactivity for it would be expected that a higher temperature would actually result in less erosion due to the fact that more CO_2 would be lost from the drink as a consequence of degassing.

A pilot study to determine intraoral beverage temperature, conducted on 4 subjects, concluded that the beverage temperature rose from 4 ˚C to 19 ˚C after instructing test subjects to rinse the beverage in their mouth for 15 seconds (Cochrane et al., 2009). To offer guidance for future erosion research; Shellis et al. (2011) suggested the adoption of a controlled body temperature (37 ˚C), mouth temperature (36 ˚C) or room temperature (25 ˚C) in erosion testing regimes via incubators or water baths (Shellis et al., 2011). The same group of researchers also recommended that the pattern of cycling to be adopted in acidic attack simulation models should reflect real-life dietary habits. Parameters to be taken into account included, the time period of acidic attacks, their frequency and the precise chronological order and timing of introducing different variables into the testing regime (Shellis et al., 2011).

Agitation and stirring is usually associated with more erosion compared to acidic exposures with static beverage to enamel contact (Barbour et al., 2003). This procedure is undertaken to simulate beverage swishing in the oral cavity. The use of a pump-assisted erosion model has the potential of more reliably reflect the kinematic behaviour of imbibed beverages compared to the rather more primitive “stirring” action under static specimen immersion protocols (Shellis et al., 2011).

The discrepancy of between-subject variables in dental erosion models could be due to variations in saliva, salivary pellicle formation and properties, and oral soft tissue surroundings (West et al., 2011b). Jensdottir et al (2006) added salivary proteins isolated from a pool of human saliva to his model (Jensdottir et al., 2006a). A system of pumps has been advocated to mimic salivary flow and to regulate the exposure of samples to the erosive beverages (Magalhães et al., 2008b). Honorio et al. (2008) added the plaque accumulation factor by incorporating meshes into the intraoral

appliances *in situ.* Bacteria might be added to a proper environment for it to create a biofilm hence rendering the model susceptible to plaque accumulation (Honório et al., 2008). In turn, this might lead to adding specific proteins to artificial saliva regulated by pumps along with adding bacteria to facilitate plaque accumulation and therefore potentially approach a more realistic regime.

For the models to be more clinically relevant in the future, validation of such models should be undertaken. This could be achieved by weighing the acidic attack outcomes observed in such models against predicted real-life erosion values (Young and Tenuta, 2011).

2.5.3 Comparison between Natural and Artificial Saliva

In view of the problems of collection and utilisation of natural saliva in erosion research there is an urgent need for a chemically representative artificial saliva (Shellis et al., 2011). This must react with enamel and the acidic challenge in a manner similar to the way natural saliva would react under the same circumstances. While this may in fact be essentially impossible to achieve, it does not lessen the implicit requirement that the approximation be made as good as is feasible, and that this is a demonstrable characteristic.

The properties of human saliva cannot be precisely duplicated owing to its unstable and inconsistent nature. As a general rule, if a natural substance has no definitive composition; a simulation that mimics the original substances chemical and physical characteristics is impossible. This limitation renders natural saliva itself impractical for use under *in vitro* conditions hence the need for an artificial saliva recipe that is able to simulate most, if not all, of the characteristics of natural saliva. Apart from some commercially available products, there has been a wide range of saliva preparation

attempts throughout the literature but most of them are quite arbitrary, with neither justification nor reference to an authority being given (Leung and Darvell, 1997).

Ideally, an artificial saliva recipe must contain “typical” amounts of the major ionic constituents of human saliva. Darvell prepared a recipe based on data extracted from reported human saliva analysis (Darvell, 1978). This was originally utilised in dental materials corrosion studies, and was improved later to accommodate experiments involving the calcium phosphate system in the oral cavity (Leung and Darvell, 1991).

There are some aspects that are however overlooked in saliva preparation attempts;

I. Firstly, bicarbonate in artificial saliva serves not only as a buffer but also as a complexing agent. In addition, carbon dioxide is volatile, therefore, experimental settings that are not equipped with carbon dioxide preservation and/or compensation measurements are most probably deemed to fail in simulating natural saliva for carbon dioxide loss when exposed to air is the main cause of the undesirable rise in pH hours after the recipe preparation is complete. Such an effect was minimized by replenishing the working solutions of artificial saliva several times during the experiment and by using tight-seal containers to prevent carbon dioxide from escaping the formula (Darvell, 1978). This would be rather impossible with regimens that are adopting specimen immersion techniques in assessing dental erosion.

II. Secondly, in human saliva, calcium appears to be present in high concentrations and in a supersaturated state with respect to hydroxyapatite. Yet, a considerable portion of calcium is complexed with proteins present in natural saliva, so caution should be taken when deciding on the exact calcium

concentration to be used for a certain recipe that is protein-deprived for total calcium concentration cannot reflect what actually happens in the absence of proteins (Leung and Darvell, 1997, Leung and Darvell, 1991).

III. Thirdly, the viscosity of natural saliva cannot be duplicated without difficulty for it is practically burdensome to maintain the desirable chemical composition while simulating the viscosity at the same time (Darvell, 1978). .

IV. Finally, compositional differences between stimulated and unstimulated saliva, with the latter being mostly overlooked, should be taken into consideration particularly when dietary dental erosion is under investigation due to the involvement of foods and drinks that are associated with salivary stimulation at the most critical points of time that govern the process of erosion.

In general, the use of artificial saliva in laboratory experiments aims primarily to standardise the testing procedures and conditions. Therefore, an easy to prepare, stable, and reproducible alternative formula of what is believed to be, to a certain degree, representative of natural saliva is highly desirable. Previous attempts of pooling human whole saliva from numerous subjects yielded sub-ideal results relative to the clinical conditions owing to the fact that the presence of microorganisms, denaturation of proteins and chemical differences rendered their results unpredictable (Darvell, 1978).

Artificial saliva however has proved to be effective in facilitating the remineralisation process (Attin et al., 2000, Attin et al., 1998, Klimek et al., 1982). It can be readily prepared in the amounts required by *in vitro* experiments. It is considered to have a

long shelf life and a consistent composition which renders its standardization feasible. In contrast, human saliva cannot be mass produced nor easily collected. It is considered unstable under *in vitro* conditions where it readily gets altered and degraded. Natural saliva is also highly variable in terms of subject to subject or even intra-subject consistency which further complicates its use under *in vitro* conditions (Wiegand and Attin, 2011, Attin et al., 2003). In certain erosion models that utilise pumps, natural saliva can be problematic for its tendency to block tubing and connections (Attin et al., 2003). In addition, the use of natural saliva raises the problem of cross-infection which necessitates its disposal in special containers not to mention the burden of granting an ethical approval for such use (Shellis et al., 2011).

Interestingly, mucin deprived artificial saliva is capable of reducing enamel surface loss *in vitro* when compared with natural human saliva and mucin-containing artificial saliva. This difference has been shown to be statistically significant (Hara et al., 2008). Saliva contains a huge number of proteins other than mucin, some of which is believed to play a role in the process of erosion (Magalhães et al., 2009). As a consequence, the addition of mucin alone to artificial saliva will have its own limitations which might affect the outcome of laboratory erosion (Hara et al., 2008). With reference to natural human saliva, the deterioration of its compositional stability with time along with the difficulties associated with its collection, storage and introduction into erosion models have all resulted in shedding more light on the importance of utilising artificial saliva for the purposes of *in vitro* erosion testing (Hara et al., 2008, Magalhães et al., 2009).

2.5.4 Quantitative methods for the measurement of dental erosion

From the review of the literature the following measurement techniques have been employed:

I. Profilometry

Throughout the literature, dental tissue loss has been quantified using several techniques. One of the most reliable techniques is profilometry. This technique measures the amount of loss relative to a non-affected reference area (West et al., 2011b, Schlüter et al., 2011).

The assessment of tooth tissue loss as a result of erosion using profilometry has been proved both suitable and reliable as a method to evaluate the extent of the erosive lesion extent (Attin et al., 2005, Hughes et al., 2002, Bartlett et al., 1997). However, flat specimens are preferred for optimum sensitivity and accuracy of measurement (Schlüter et al., 2011). Erosive and abrasive tissue loss can also be quantified using this method either singly or in combination. Yet, measuring the degree of softening is unfeasible (Schlüter et al., 2011). Thus, only advanced stages of erosion are usually assessed using this method (Barbour and Rees, 2004).

This method is commonly used to determine tooth structure loss in erosion studies under *in vitro* and *in situ* conditions with an accuracy of 0.3 to 0.5 µm for perfectly smooth and flat sample surfaces (Attin, 2006). It might be of concern to some, that the stylus might produce scratches along its pathway on the tooth specimen but this should not be a problem, since all groups will be affected and no biased results will be yielded (Barbour and Rees, 2004). It is suggested that *in vitro* assessment of erosion

depth yields results that are 10-fold those obtained via clinical studies using comparable methodologies (Hughes et al., 1999).

Generally, when erosion is to be assessed, the values obtained via profilometry will reflect the cause-effect image adequately; yet, not to the full extent required. The process of erosion does not only lead to tooth structure loss but also softens the superficial surface. Therefore, in order to accurately quantify the erosive effect, quantification of both surface structure loss and subsurface ionic integrity is essential. Profilometry, for instance, can reliably measure the amount of surface lost after an erosive episode but it won't provide any insights regarding the state of the subsurface layer. To overcome this, chemical analysis and/or hardness testing can be adjunctively used along with profilometry (Jager et al., 2008).

II. Microradigraphy

This technique directly measures the mineral content of dental substrates by recording a penetrating beam of monochromatic X-rays. Analysis of X-ray absorbance yields two parameters; surface loss depth and relative mineral loss percentage (Arends and Ten Bosch, 1992, Barbour and Rees, 2004). However, the microradigraphy technique is rather time consuming and destructive (Arends and Ten Bosch, 1992)

III. Atomic force microscopy

This technique uses a sharp tip attached to a flexi-cantilever that probes the specimen surface tracking its features. This with the help of a reflected diode laser beam can build up a map for the tracked specimen's surface. Although this technique is considered time consuming, its conservativeness and high accuracy favour it over other microscopy techniques (Barbour and Rees, 2004).

IV. Surface mapping

Chadwick et al. (1997) developed a system for assessing dental erosion via a surface-mapping device, which utilised a computerized probe which scans electroconductive replicas in order to create surface maps that can be subsequently compared with baseline maps via its associated shape matching software (Chadwick et al., 1997, Mitchell and Chadwick, 1998)

V. Hardness testing

The assessment of tooth tissue loss as a result of erosion using hardness testing has been proved both suitable and reliable as a method to evaluate the erosive lesion extent (Barbosa et al., 2011, Curzon and Hefferren, 2001). The degree of loss of hardness "softening" can be measured by assessing how resistant is a substrate to the penetrating diamond indenter. The indenter can be a Knoop, Vickers or Berkovich (nano-indentation) (Schlüter et al., 2011). It is noteworthy that hardness measurements cannot quantify the amount of surface loss in advanced dental erosion cases, therefore they are mainly used to assess the degree of softening (Schlüter et al., 2011).

It is known that enamel specimens will give higher hardness values if they were allowed to dry out after acidic exposures; hence the tendency to measure hardness values while specimens are kept moist (Staines et al., 1981). Enamel hardness values usually have an increased standard deviation owing to the differences in the degree of mineralisation of enamel from site to site and throughout its thickness. This will also be reflected on the rate of erosion; yielding values with higher standard deviations in different specimens and even within the same specimen (Devlin et al., 2006).

Indentations produced using micro-indenters usually yield indentation depths ranging from a few micrometres to tens of micrometres, while the ones produced by nano-indenters have much lesser depths of a few hundred nanometres (typically 200 nm) (Barbour and Rees, 2004). The hardness value however does not only reflect the material directly associated with the indenter's tip. In other words, the physical characteristics of areas as far as 10 times the dimensions of the indentation from the spot under investigation can also affect the hardness value. Therefore, micro-indenters rather than nano-ones are able to reflect the state of the intact layer that is underneath the softened one which ranges typically from 2 to 5 μm (a value determined by ultrasonication)(Hughes et al., 2002). This leaves nano-indentation as the technique of choice for short term, very brief, acidic attacks where early stages of enamel softening are to be assessed (Barbour and Rees, 2004).

Up to the moment of writing this review, none of the microindentation techniques used for assessing hardness value changes after an erosive attack was capable of producing an instant image of the area in contact with the indenter's tip. On the other hand, some nanoindentations systems are able to do so by means of scanning across the specimen surface line by line, producing an image that can be later assessed by the operator. This image can become handy when testing irregular or rough specimens allowing for identifying artefacts or cracks (Barbour and Rees, 2004).

Micro- and nano-hardness techniques require the specimens to be flat. This can be achieved by polishing the surface of the substrate prior to the erosive attack. Such a procedure will affect the end hardness value owing to the fact that the superficial layer of enamel contains considerably higher concentrations of fluoride and lower concentrations of carbonate and magnesium relative to deeper layers of enamel. Thus,

a deeper layer is usually more susceptible to erosion compared to a more superficial layer (Barbour and Rees, 2004)

VI. Chemical analysis

Other adjunctive means of assessing the extent of dental erosion include chemical analysis of the dissolved minerals. This approach can be very informative in terms of understanding the behaviour of calcium and phosphate ions during the process of dental erosion. Such a method is applicable for long term measurements but caution should be employed when saliva is present for its ions will interfere with the analysis and therefore yield arbitrary results. In addition, microradiography, quantitative light-induced fluorescence and optical coherence tomography have been utilised successfully for the purpose of erosion quantification (Schlüter et al., 2011, Barbour and Rees, 2004).

Qualitative and semi-quantitative methods have been also utilised to assess ultrastructural morphological changes which occur in dental tissues as a result of the erosive process. These methods include transmitted light microscopy, confocal laser scanning microscopy, transmission electron microscopy, scanning electron microscopy, scanning probe microscopy, scanning tunnelling microscopy and secondary ion mass spectroscopy (Schlüter et al., 2011). Overall, all current methods have limitations, it is therefore recommended to combine different methods in order to fulfil the requirements of dental erosion research.

2.5.5 Conclusion

In sum, 136 research papers assessing erosive beverages and foods were reviewed from which 103 papers were under *in vitro* conditions and 33 *in situ* (Table 2-3). Among *in vitro* papers, the author found 38 papers that utilised the Demineralisation-Remineralisation cycles out of which only 5 papers introduced the erosive substance to dental substrates by rinsing (i.e. artificial mouth concept) rather than immersion (Attin et al., 2003; Attin et al., 2005; Magalhaes et al., 2008a; Wiegand et al., 2009; Magalhaes et al., 2010). Experimental settings of these 5 papers varied in terms of daily exposure time periods, total number of days, temperature and erosive substance type while on the other hand all of them had one common characteristic and that was they all utilised bovine teeth as the dental substrate under investigation. Daily exposure time periods varied from 6 to 15 min/day; total number of days also varied from 1 to 5 days; and finally the temperature ranged from 25 to 37 °C. Clearly, there is no one consensus among researchers on which experimental settings to use while conducting an in vitro study assessing dental erosion whatsoever.

2.6 Dental substrates

In developing a new erosion testing regime it is important that a review of potential test substrates and their method of acquisition is undertaken.

2.6.1 Human teeth

Apart from the noticeable reduction in dental extractions in the developed countries which in turn have significantly depleted extracted human teeth reservoirs available for dental research, other factors have complicated the availability of extracted teeth for dental research further (West et al., 2011b).

Carious lesions, cracks and other defects are considered major impediments to using extracted teeth from routine extractions as substrates for erosion. Sound teeth could potentially be obtained either from extractions for orthodontic treatment, or from surgical removal of impacted third molars. Impacted third molars are often preferred because of their lack of oral exposure, age of the patient (usually third decade of age) and a lack of any physiological changes to the tooth structure.(West et al., 2011b)

Unless collection of teeth is attentively steered by the researcher it will be almost impossible to control the source in terms of ethnic groups, patients' age groups and storage media. Not accounting for these will eventually create discrepancies among the same group of experiments.

In many instances, flat enamel blocks have been created from the extracted teeth rather than keeping the natural enamel curves, thickness and topographical features in order for the samples to fit into specific tests or become part of a device-sensor assembly amenable to such tests as profilometry (West et al., 2011b).

In the UK as a result of public inquiries into post-mortem organ retention scandals at both the Bristol Royal Infirmary and the Royal Liverpool Children's Hospital (Alder Hey); the law on the removal, storage and use of human organs and tissues was reviewed including both the deceased and the living. Consequently, the Human Tissue Act (HTA) 2004 was enacted on the 1st of September 2006 in the UK.

The human tissue act gave birth to the Human Tissue Authority to regulate activities relating to the removal, storage, use and disposal of human tissues. Generally, for research purposes, the Human Tissue Act categorized teeth and saliva as "relevant material" upon which regulation through licensing and appropriate consent applies, subject to specific exceptions. Any human tissue that consists of/or contains cells is considered relevant material. The storage and use of relevant material requires consent and in addition its storage also requires a license issued by the Human Tissue Authority (Human Tissue Authority, 2014).

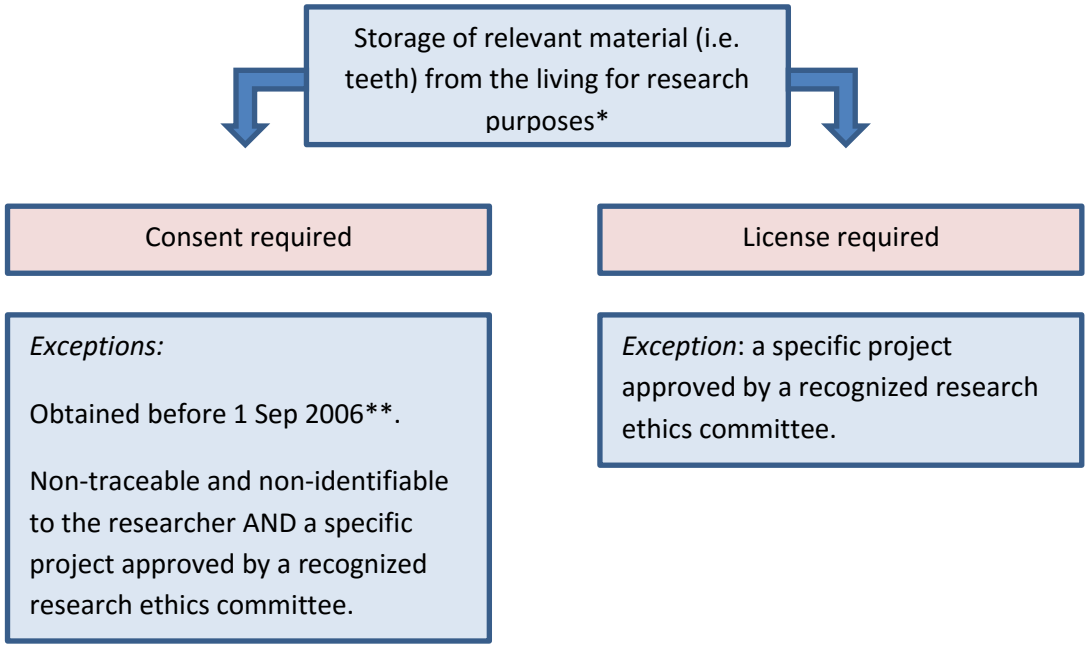
At the time of legislation the outlines of the Human Tissue Act relating to the deceased were agreeable; the inclusion of the living was however widely controversial. When the Human Tissue Act outlines were first published, it was considered as a superfluous response to the main issues of the organ retention scandals (Forsyth and Woof, 2006).

The Act suggested the adoption of the following definition of research in order to embrace all of what falls within the Human Tissue Act's remit: "*a study which addresses clearly defined questions, aims and objectives in order to discover and interpret new information or reach new understanding of the structure, function and disorders of the human body. Research attempts to derive new knowledge and includes studies that aim to generate hypotheses, as well as studies that aim to test them or develop practical applications or new knowledge*" (Human Tissue Authority, 2014).

Relevant material obtained for education, training, clinical audit or diagnostic archiving do not need to be stored under a Human Tissue Act license providing all the samples are not to be involved in research. If part or all of the samples are to be used for research purposes; the batch must be stored on Human Tissue Act-licensed premises. For the avoidance of doubt the application of decision algorithms is recommended (figures 2-2 and 2-3) (Human Tissue Authority, 2014).

As regards universities, a university based ethics committee cannot replace the role of a recognised research ethics committee. Thus, even if the research was approved by a university ethics committee, consent remains to be required for relevant materials to be used in a research project. The same conditions apply where the researcher is unlikely to come into possession of information that can trace back or identify the subject from whom the tissue was obtained (Human Tissue Authority, 2014).

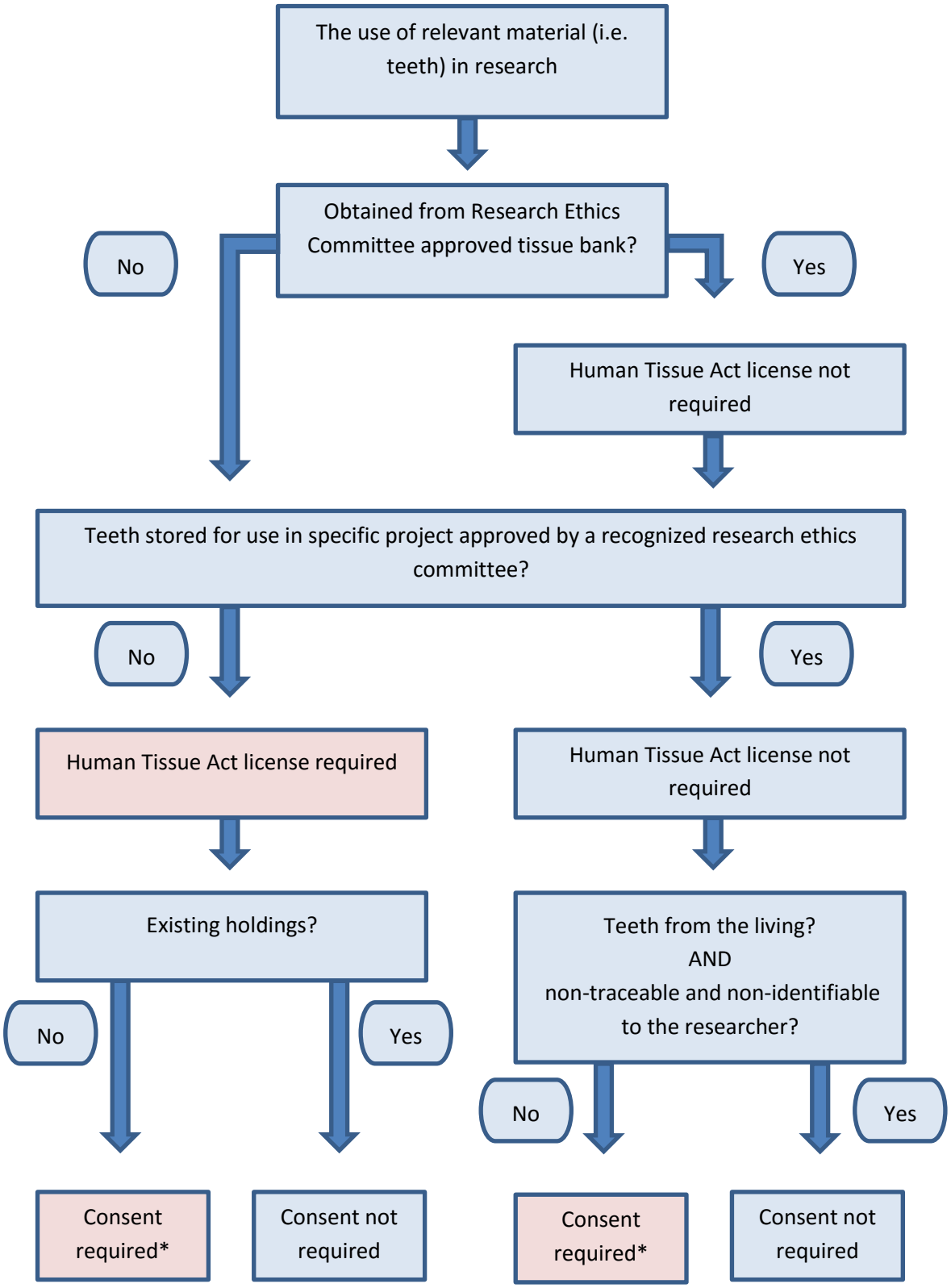
Figure 2-2. A flow chart describing licensing and consent requirements for storage of relevant material from the living for research purposes. Based and modified from Human Tissue Authority - Code of practice 9.



*Relevant material from the living includes all tissues taken while the person was alive; this categorization persists after their death.

**The consent requirements of the Human Tissue Act are not retrospective. It is not legally necessary to obtain consent to store or use a material from a living that was already existing by the time Human Tissue Act came into force (i.e. 1 Sep 2006).

Figure 2-3. A flow chart describing the link between ethical approval and the licensing and consent exceptions. Based and modified from Human Tissue Authority - Code of practice 9.



*The Human Tissue Act requires that consent must be obtained for the removal, storage and use of relevant material for certain purposes including research.

Research ethics committees can approve banks that in turn can provide relevant material (e.g. teeth) to researchers; the researchers upon receiving the material are not required to store it under Human Tissue Act license regulations during the period of their research project especially if the research project fulfils the requirements of the Human Tissue Act criteria. Upon completion of the project, researches are obliged to transfer the material back to the bank or to an alternative Human Tissue Act-licensed establishment or dispose of the material appropriately. It is worth mentioning that a researcher can alternatively apply for their own Human Tissue Act license or apply for another project-specific approval by the research ethics committee if further research is to be undertaken that does not fall under the scope of the previous approval (Human Tissue Authority, 2014).

An example of relevance to this work, was given by the Human tissue Authority (2014):

"*a dental teaching hospital establishes a bank of human teeth to carry out research into tooth erosion, wear and hypersensitivity; and control of dental plaque and staining. The teeth will be donated with consent from the donor after routine dental extraction. The hospital obtains a storage licence from the HTA as well as ethical approval as a research tissue bank. An individual researcher receiving teeth from the bank does not need to make further applications for project specific ethical approval or for an HTA licence, provided the research project falls within the research aims, material disposal terms, and terms of donor consent specified in the hospital's research tissue bank ethics approval. In this way, valuable human tissue for research is controlled and made more accessible to a number of research projects*." (Human Tissue Authority, 2014).

Clearly, the Human Tissue Act 2004 will have its implications on dentistry; in view of the aforementioned points. Ethical concerns have been raised regarding the feasibility of collecting teeth in the future for dental research (Forsyth and Woof, 2006). The exact impact of the act on the quality and quantity of dental research undertaken after 2006 in the UK remains to be assessed and determined.

In general, the Human Tissue Act did not affect dentists who collected teeth for education, training or clinical audit. On the other hand, researchers in dental schools and hospitals needed to develop their own procedures of collection of extracted teeth to comply with the consent provisions of the HTA. In addition, teeth and saliva can only be obtained and used by holders of Human Tissue Act licenses.

However, institutions that possess a Human Tissue Act license can establish their own tissue banks into which relevant material can be stored provided appropriate consent is obtained. Consequently, teeth can be collected for several projects granted with a generic approval where no project-specific ethics committee approval is required. In addition, it's not necessary to obtain consent for research if the identity of the living can remain anonymous provided the research was approved by a Research Ethics Committee (Forsyth and Woof, 2006).

All in all, the strict regulatory nature of tissue banking protocols aggravates the administrative load of research teams and postgraduate students. The impact of this upon tooth availability for research has not been quantified.

It is pertinent however to ask 'Has the human tissue act had an impact upon UK dental research?' - The Scopus search engine was used to retrieve published dental research conducted in United Kingdom institutions utilising human teeth under *in vitro*

conditions over the period from 1980 to 2014. This utilised the search strategy ["in vitro" human teeth dent*; For 1980-2014; Limited to UK; Limited to Dentistry]. Figure 2-4 gives the curve of number of outputs versus time of publication.

Three periods of time can be identified. In the first period (i.e. 1980-1995) a shy but steady number of documents were published each year until a burst of the number of documents was evident in 1996 demarcating the beginning of the second period (i.e. 1996-2006). It is worth mentioning here that the Human Tissue Act came into force on the 1st of September, 2006. In the third period (i.e. 2007-2014), there has been a noticeable decline in the number of publications from 254 documents, published over a period of 8 years prior to the HTA, to 165 documents published over the same period of time after the HTA was introduced (a drop of more than 35 %)(tables 2-5 and 2-6). Interestingly, the number of published document in 2014 has gone down to its lowest since 1995. Moreover, the total number of publications in 2013 and 2014 combined is lower than the yearly mean number of publications in any single year over the period from 1996 to 2006 (table 2-7).

Figure 2-4. The number of documents published on yearly basis over the period from 1980 to 2014.

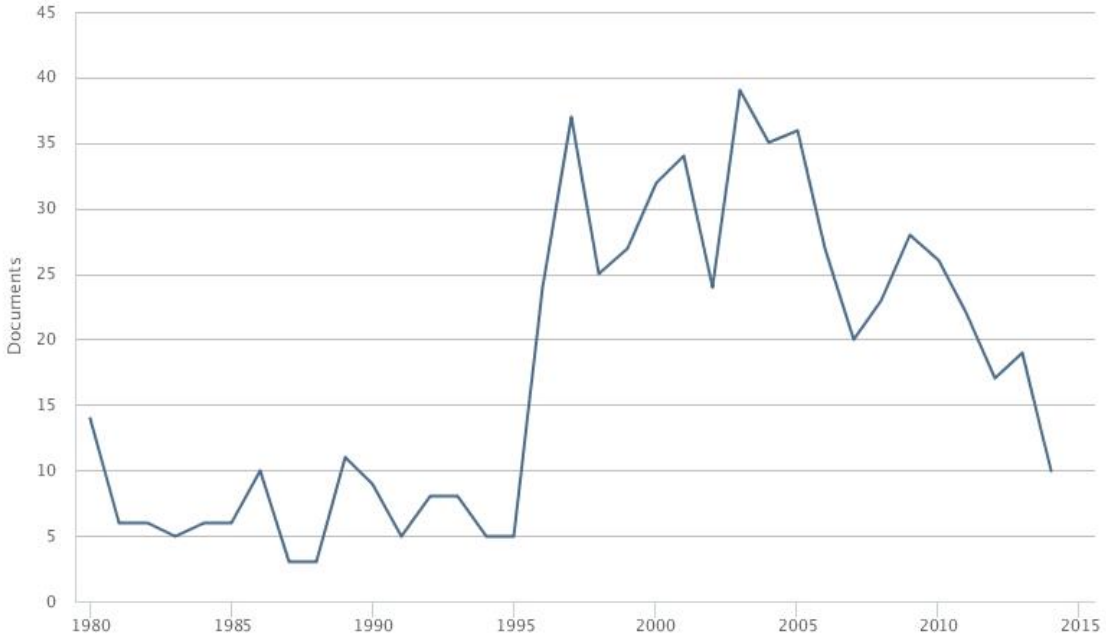


Table 2-5. The number of research papers published by UK organizations using human teeth during both pre- and post-HTA 2006 over an 8 year-period.

	8 years pre-HTA	8 years post-HTA
Range	**1999-2006**	**2007-2014**
Documents	254	165

Table 2-6. The mean number of research papers using human teeth published during pre-HTA 2006 over a 10-year period and post-HTA 2006 over an 8 year period.

	pre-HTA	post-HTA
Range	**1996-2006**	**2007-2015**
Mean # of documents per year	30.9	20.6

Table 2-7. The number of research papers using human teeth published each year from 1980-2014.

Year	Number of Documents
2014	10
2013	19
2012	17
2011	22
2010	26
2009	28
2008	23
2007	20
2006	27
2005	36
2004	35
2003	39
2002	24
2001	34
2000	32
1999	27
1998	25
1997	37
1996	24
Total	615

The HTA came into force 1 Sep 2006 →

2.6.2 Dental Substrates: Other concerns

In the past many *in situ* experiments concerning dental erosion were conducted. These typically involved a subject wearing a dental appliance that contained either human or bovine dental hard tissue from self, another human donor or animal. The ability to remove the sample from the mouth simplified measurements of its characteristics pre- and post-exposure. More recently ethical issues together with worries of infection hazards, especially when dealing with human and bovine teeth with regard to prions, have led to fastidious precautions regarding infection control procedures (Hara et al., 2003a; West et al., 2011). Therefore, alternative substrates have been proposed for use in dental research (Yassen et al., 2011).

Permanent enamel is composed of approximately 85% fluor- hydroxy-apatite crystals organised in the classical appearance form of prisms, which are in turn large in size, uniform in shape and regularly distributed. The remainder 15% volume comprises of water and organic matter (Risnes, 1998, Braly et al., 2007). With such a composition of minerals and structural organization, finding a comparable alternative cannot be achieved without difficulty.

Recently, the search for an alternative to human teeth for dental research has intensified due to the concerns raised about ethical implications of collecting human teeth in view of the Human Tissue Act 2004. Yet, from a clinical point of view, human teeth are considered to be the most suitable substrate if dental hard tissue is to be examined (Human Tissue Authority, 2014).

2.6.3 Tried Alternatives to Human Dental Tissue

Bovine, primate, equine, swine, ovine and shark teeth are all examples of non-human sources of substrates for *in vitro* and *in situ* experiments (Edmunds et al., 1988, Poole et al., 1981, Lopes et al., 2006, Takagi et al., 2000). The first of these being the most broadly utilised substitute for human teeth in dental erosion studies (Yassen et al., 2011). Bovine teeth are said to be more easily collected than human teeth (Mellberg, 1992a); both the quantity and quality of teeth are said to be more predictable in bovine tooth collections compared with classic human tooth pools (Mellberg, 1992b). In the UK however there are concerns that the dental pulp of bovine teeth may be a reservoir of Creutzfeldt–Jakob disease (CJD) and so obtaining such teeth has become more difficult (Hara et al., 2003a, Yassen et al., 2011).

Bovine teeth have thicker enamel prism crystallites, increased enamel porosity and lower fluoride concentration compared to human teeth (Mellberg, 1992b). Human enamel possesses greater hardness and is less erosion susceptible compared to bovine enamel (Rios et al., 2006b). On the other hand, bovine teeth are still considered a good alternative to human teeth (Laurance-Young et al., 2011), although several *in vitro* studies suggest the results obtained in investigations using them may differ from those obtained from human teeth due to enamel structural differences (West et al., 2011b, Turssi et al., 2010).

Under *in vitro* or *in situ* conditions, human tooth structure characteristics and behaviour can differ from their bovine counterparts. For instance, human dentine hardness changes after acidic beverage intake were significantly different compared with the change in bovine dentine *in situ* while human and bovine enamel hardness changes were comparable (Turssi et al., 2010). On the other hand, bovine enamel

structure was lost twice as fast compared to human enamel when exposed to orange juice *in vitro* (Amaechi et al., 1999a). Nevertheless, as concerns dental erosion, bovine teeth can serve as an acceptable alternative, at least under *in vitro* conditions even though it might not completely mimic the real-life scenario (Wegehaupt et al., 2008).

Interestingly, hydroxyapatite powder used as a substrate in several erosion studies has yielded promising results as an alternative to human teeth. (Setarehnejad et al., 2010, Brown et al., 2007, Jensdottir et al., 2006b, Jensdottir et al., 2006a, Caglar et al., 2006). The use of such substrate is applicable only for in vitro experiments that are, almost entirely, exploratory in nature (Shellis et al., 2011). It should however be borne in mind that pure hydroxyapatite, owing to its perfect crystal structure is relatively erosion resistant compared to biological apatite where the crystal lattice displays imperfections due to ion substitutions that render it less resistant to acid attack.

2.6.4 Untried alternatives to human dental tissue - the Ostrich eggshell

The Ostrich (Struthio camelus) is the largest bird on earth; it is a member of *ratitae*, known as running birds, which includes ostrich, kiwi, emu, rhea and cassowary. Roughly, there are more than 2 million ostrich birds of which one third inhabiting Africa. The majority of the ostrich population is farmed; yet, a satisfactory number of ostriches still live in the wild with no danger of species extinction (Cooper et al., 2009). Ostriches have been farmed for over a century and currently they are being raised commercially. Due to the increased demand for Ostrich meat and the resultant expansive growth of ostrich farms and its related industries, many farms have adopted mass production techniques for ostriches along with their eggs.

In such farms one million eggs are laid annually in South Africa of which 25% do not hatch (Sales et al., 1996). The modern artificial incubation techniques used in mass production result in significant value for infertile eggs, low hatchability and embryonic mortality (Cooper, 2001). Such eggs are of no value for human consumption. Such Ostrich eggshells may be readily purchased from shops or even via online e-shops and have found popularity among craftsmen and artists.

The ostrich (Struthio camelus) egg is the largest among all avian species with a shell thickness of about 2 mm (range from 1.6 to 2.2 mm). The geometrical and physical properties of Ostrich eggs are detailed in table 2-8. The eggshell is a natural biocomposite comprising an organic and mineral matrix. The mineral matrix constitutes more than 97% of an Ostrich eggshell's composition of which 97.4% is calcium carbonate, 1.9% magnesium phosphate and 0.7% tricalcium phosphate (Yadao et al., 2004). According to Szczerbinska & Wiercinska (2010), the ostrich eggshell contains 369.6 ± 12.73 mg/g Calcium and 0.21 ± 0.06 mg/g Phosphorus (Szczerbinska and Wiercinska, 2010).

Table 2-8. Mean values for the major geometrical and physical properties of Ostrich eggs (Cooper et al., 2009, Christensen et al., 1996, Szczerbinska and Wiercinska, 2010).

Geometrical properties	Cooper et al. 2009	Szczerbinska & Wiercinska 2010	Christensen et al. 1996
Weight	1.5 kg	1.519 ± 0.931 kg	1.470 ± 0.108 kg
Length	15.6 cm		16.0 ± 0.5 cm
Width	12.9 cm		12.7 ± 0.6 cm
Vertical circumference	45 cm		
Horizontal circumference	40 cm		
Internal volume	1350 ml		
Weight of albumen*	900 g	892.9 ± 56.2 g	
Weight of yolk*	317 g	330.9 ± 35.4 g	
Weight of voided shell*	296 g	295.7 ± 25.0 g	242.5 ± 38 g
Shell thickness	1.6-2.2 mm	2.13 ± 0.1 mm	1.9 ± 0.03 mm
Eggshell density			0.23 ± 0.01 g/cm3
Eggshell volume			105 ± 16 cm^3

* Weight values of albumen and yolk provided are for eggs weighing 1.5 kg.

The egg is characterized by its unique dense structure and hence has remarkable mechanical properties. It can withstand an applied force of up to 55 kg. Compared to other avian species, Ostrich eggshell lacks the cuticle layer or any shell accessory material which renders the outermost layer of it a continuous unit of substantial thickness and uniform structure (> 1800 µm) (figure 2-5). In addition, the vertical crystal layer is characterized by an amorphous crystalline structure with no evidence of porosities (Cooper et al., 2009, Cooper, 2001).

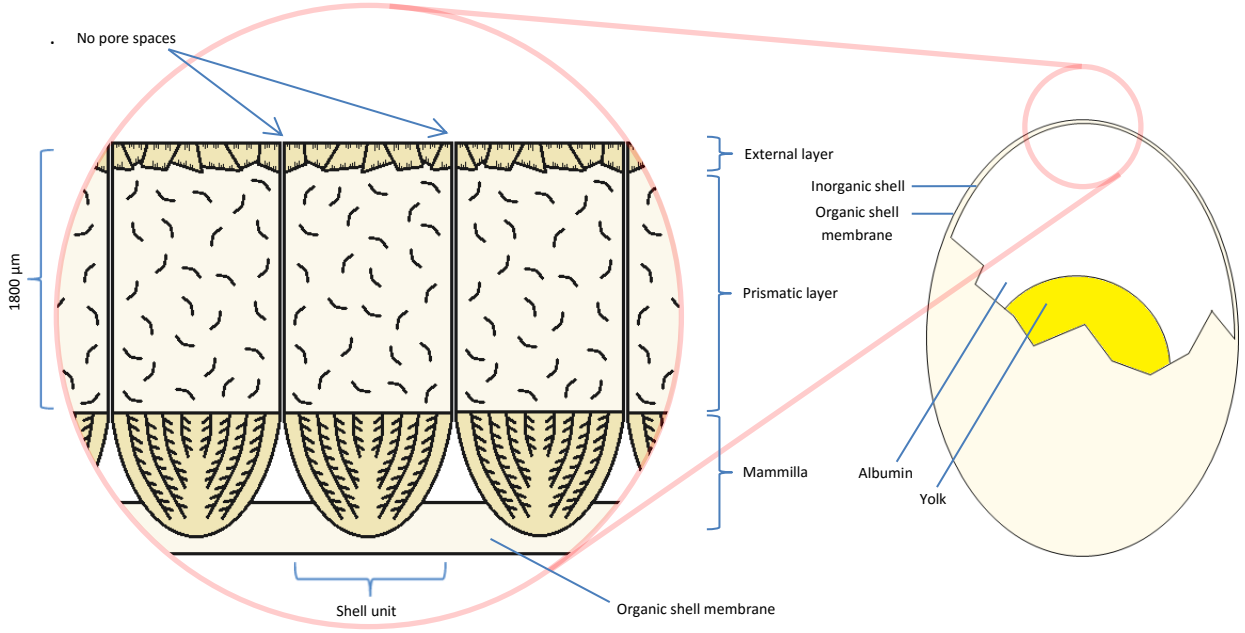
As a result of this and the fact that the egg shell can reach up to 2 mm in thickness, it has the potential to be considered as a favourable substrate upon which several surface tests can be conducted in dental research. From a laboratorial perspective, Ostrich eggshell is considered operator-friendly for it can readily and conveniently be cut to desired shape with a dental high-speed handpiece and a diamond bur (Yadao et al., 2004). One ostrich egg can yield up to 300 g of eggshell. Moreover, the structural configuration of the eggshell allows it to be sterilized by autoclaving without affecting its biological properties (Yadao et al., 2004).

Ostrich eggshell's potential of substituting bone in reconstructive surgeries has been investigated by several researchers (Dupoirieux et al., 2001, Dupoirieux et al., 1999, Dupoirieux et al., 1995, Yadao et al., 2004), as well as the reconstruction of cystic defects in the jaw (Baliga et al., 1998). The Ostrich eggshell biocompatibility along with its ability to facilitate the healing of cranial defects in rabbits was also assessed (Durmuş et al., 2003). The reasons that lead to such attempts can be attributed to the eggshell's close resemblance to mineralised bone matrix along with its ease of handling and convenient dimensions (Durmuş et al., 2008). However, Ostrich eggshell has not been implemented in dental research yet.

The organic matrix which constitutes 2% of the total eggshell weight contains proteins and proteoglycans that in turn can affect the rate of calcium carbonate crystal precipitation and morphology. Osteoponin, for instance, increases the osteoblastic activity and is able of binding to hydroxyapatite (Durmuş et al., 2008).

Given these facts, and increasing difficulties in acquiring human teeth, it seems that this tissue has potential for use as an alternative dental erosion testing substrate.

Figure 2-5. Illustration showing the basic components of the Ostrich eggshell, representing the organic cell membrane, mammilla, prismatic layer, and external layer



2.7 Literature Review Conclusions

From the foregoing it can be concluded that:

- Dental erosion is prevalent
- Consumption of carbonated soft drinks, though not in a cause and effect relationship with dental erosion, is considered to increase erosion risk significantly.
- Human saliva and acquired pellicle are major protective factors against dental erosion.
- Simulating and assessing dental erosion *in vitro* is challenging and should reflect normal behaviour and salivary function.
- There is a need to validate and source a viable alternative to human teeth as a testing substrate for dental erosion given difficulties in obtaining human tissue.

CHAPTER 3

3. Methodology

The work of this thesis comprised

- Ascertaining dentists' knowledge of the human tissue act.
- Informing the development of a realistic laboratory erosion testing regime.
- Building and validating an artificial mouth model.
- Applying the artificial mouth model in erosion testing in a range of situations to:
    - Evaluate a potential alternative erosion substrate to tooth substance
    - Evaluate the effects of different diets
    - Evaluate erosive prevention agents

3.1 The Human Tissue Act Questionnaire

Postal and online questionnaires along with their covering letters were designed for distribution to qualified dentists in the United Kingdom according to the principles of Dillman (1978) and Lumsden (2007). Questionnaire questions were carefully tailored and phrased in accordance with the objectives of this work. Before the final submission, both formats of the questionnaire, postal and online, were piloted upon and discussed with a convenience sample of relevant respondents (i.e. Dundee Dental Hospital staff members) to maximize the clarity of the questionnaire. The

questionnaire was designed to assess both the knowledge and understanding level of the participants.

Once a consensus on the questionnaire format was reached, a copy was sent to the scientific advisory officer of the East of Scotland Research Ethics Service and the R&D manager of the Tayside Medical Science Centre (TASC), with the proposed covering letters, to determine if ethical approval and/or NHS management Research and Development (R&D) permissions were required. The response received stated that the work proposed did not require ethical review, under the terms of the Governance Arrangements for Research Ethics Committees (GAfREC) in the UK nor NHS R&D approval (Appendix 1).

The questionnaire was constructed using an online survey service tool (Bristol Online Surveys) (Appendix 2) and the opportunity for anonymous return was given. It was distributed nationally by post within the UK to 500 UK registered dentists. Each package contained a printed questionnaire, a covering letter, a prepaid addressed return envelope and a URL link to the questionnaire allowing participants to return the questionnaires either in paper format by the provided pre-paid envelope or by accessing the web-link provided. Participants were directed to respond by only one method. Copies of both the questionnaire and covering letter of invitation are in appendix 2.

The 500 potential participants were randomly selected from the General Dental Council online registers directory (www.gdc-uk.org/Pages/SearchRegisters.aspx) using their registered address postcode. A randomized UK postcode database spreadsheet file was used to generate the required 500 random post codes by generating random numbers in the range of the row numbers of the spreadsheet. Randomisation was

carried out using the random number function in the spreadsheet package excel (Microsoft Excel 2010, Microsoft Ltd., Reading UK). The registers directory allowed for a search of the register based on the postcode via the “advanced search option”. Upon searching the directory, erased and suspended registrants were filtered out and only the 1st general dental practitioner from the list of results so generated was invited to participate. Where there was no entry the next randomly selected postcode was inputted until all 500 potential participants were identified.

The cover letter invited the potential participants to take part and stated that there would be no other contact should they elect not to respond. To ensure maximum confidentiality of all respondents no hidden codes were embedded in any part of the postal questionnaire. The option to track respondents who chose to respond via the online service was disabled. It was thus impossible to know who had participated and, for this reason, sending follow-up letters to non-respondents was not possible.

The questionnaire sought to assay, amongst general dental practitioners, their knowledge of the Human Tissue Act, as it related to the collection of teeth for both dental research and teaching.

To permit analysis of the responses a relational database was constructed using the computer programme Paradox (Paradox 3.5, Borland International, USA) platformed using an x86 emulator programme DOSBox (DOSBox version 0.74, Free Software Foundation, Inc.) for input of data from the completed questionnaires and interrogation. This was necessary for surprisingly, there are no longer readily programmable relational databases on the market. Statistical analysis of the responses was undertaken using Prism (GraphPad Prism 6, GraphPad Software Inc., San Diego, USA) and Excel (Microsoft Excel, 2010, Microsoft Ltd., Reading UK).

3.2 Informing a realistic laboratory erosion-testing regime

The observational component of this work sought to measure aspects of fizzy drink consumption in a social environment to inform the development of a laboratory testing regime.

Prior to the commencement of the study a copy of the experimental protocol was sent to the East of Scotland Research Ethics Service (EoSRES) to determine the need or otherwise for ethical approval. The reply received stated that no ethical approval was required for this work (Appendix 1).

Those who consented to participate in the study were asked to complete a pre-visit questionnaire that assessed their beliefs concerning their personal fizzy drink consumption and preferences (beverage choice, method of drinking, serving temperature, quantity and rate of drinking). It also served as a method of checking for any food or drink allergies that would impact adversely upon the smooth running of the experiment.

3.2.1 Pre-experimental questionnaire

An online questionnaire along with a covering letter was designed for distribution to those University of Dundee students who expressed a wish to participate in the study according to the guidelines of Lumsden (2007). Questionnaire questions were carefully tailored and phrased in accordance with the objectives of this work. Before the final submission, the questionnaire was piloted upon and discussed with a convenience sample of relevant respondents (i.e. University of Dundee postgraduate students) to maximize the clarity of the questionnaire. For intra-respondent reliability another

convenience sample of University of Dundee postgraduate students was given the questionnaire again within 2 weeks. The reliability of these responses was assessed by calculating the Kappa statistic.

The questionnaire was constructed using an online survey service tool (Bristol Online Surveys) (Appendix 3-1). Potential participants were sought from all University of Dundee students by the weekly email they receive advertising events in that institution (University of Dundee SOMiS Hermes-II email distribution system) (Appendix 3-2). This contained a link to the project that gave information on what it entailed. The purpose stated was to gather data to develop an artificial mouth. Students enrolled on dental courses were excluded from the study as it was felt by the researcher their knowledge of erosion may affect their dietary behaviour.

Upon receipt of the completed questionnaire an invitation was issued to attend one of a series of four “pizza and soft drink parties”. This title was chosen in an endeavour to foster a relaxed atmosphere in which to observe normal behaviour.

3.2.2 Pizza and Soft Drink Party: a mock run

Six participants (University of Dundee dental postgraduate students) were recruited to take part in a mock run of the Pizza and Soft Drink Party. This run allowed the researchers to adjust camcorders, rehearse measurement procedures and clarify their roles throughout the experiment. Data from this mock run was only used to fine tune the procedures used in the investigation.

3.2.3 Pizza and Soft Drink Party

On each occasion this was held in the same air conditioned room at a temperature of 24 ˚C (figure 3-1). Each participant was allocated to one of four observers and was issued with two graduated 60 ml measuring cups (Nutriculture, Skelmersdale, UK). Prior to the serving of food they were invited to select a drink from those on display. The choice of beverages available represented the previously declared preferences of those attending. All drinks were at a temperature of 4 ˚C having been refrigerated for at least 24 hours before the commencement of the experiment. All participants were asked to spit out, into the graduated cups, their first and second sips. The observers immediately measured the temperature of these, using a digital thermometer (Basetech BT-80, Conrad, Colchester, UK) allowing a period of 60 seconds for equilibrium to be reached before the reading was taken. A note was also made of the volumes of each sip.

Thereafter a standard selection of pizzas was served and supplies of drinks at 4 ˚C were made continuously available. Table 3-1 gives details of the available pizzas and beverages. Throughout the experiment a music video was played (Andre Rieu, Live in Italy) to foster a casual atmosphere and encourage social interaction amongst the participants. No time limit for the activity was imposed.

Figure 3-1.

(A) Venue setting. The choice of beverages and drinking accessories available represented the previously declared preferences of those attending.

(B) A casual atmosphere was achieved promoting spontaneous social interaction.

Table 3-1. The pizza and beverages served in this work. (108 packs of pizza in total)

Pizza type	Company
Ristorante Mozzarella	Dr. Oetker Ristorante, Bielefeld, Germany
Ristorante Pollo	Dr. Oetker Ristorante, Bielefeld, Germany
Ristorante Funghi	Dr. Oetker Ristorante, Bielefeld, Germany
Ristorante Vegetale	Dr. Oetker Ristorante, Bielefeld, Germany
Ristorante Spinace	Dr. Oetker Ristorante, Bielefeld, Germany
Beverage type	**Company**
Coca-cola	Coca-Cola Great Britain, London, UK
Coca-cola Diet	Coca-Cola Great Britain, London, UK
Coca-cola Zero	Coca-Cola Great Britain, London, UK
Sprite	Coca-Cola Great Britain, London, UK
Sprite Zero	Coca-Cola Great Britain, London, UK
Fanta	Coca-Cola Great Britain, London, UK
Fanta Zero	Coca-Cola Great Britain, London, UK
Schweppes	Coca-Cola Great Britain, London, UK
Dr. Pepper	Coca-Cola Great Britain, London, UK
Dr. Pepper Zero	Coca-Cola Great Britain, London, UK
Pepsi	Britvic Soft Drinks Ltd, London, UK
Pepsi Diet	Britvic Soft Drinks Ltd, London, UK
Pepsi Max	Britvic Soft Drinks Ltd, London, UK
7-UP	Britvic Soft Drinks Ltd, London, UK
Mountain Dew	Britvic Soft Drinks Ltd, London, UK
Irn Bru	A.G.Barr Public Limited Company, Cumbernauld, Scotland, UK
Irn Bru Sugar free	A.G.Barr Public Limited Company, Cumbernauld, Scotland, UK
Grapetiser	Coca-Cola Enterprises Limited, Middlesex, UK
Appletiser	Coca-Cola Enterprises Limited, Middlesex, UK

Upon completion of the party analysis of the retained opened beverage containers of the participants permitted the researchers to calculate the volume of drinks apparently consumed and adjust this downwards to the actual volume consumed by measuring any residual drink by means of a 250 ml measuring cylinder (MBL Volumetrics, SciLabware, Stoke-on-Trent, UK). The total consumed volume per subject (V_T) was calculated using the formula:

$$V_T = (CV_C) - (V_R + V_E)$$

V_T = Total consumed volume per subject
C = Number of containers opened for the subject
V_C = Container volume
V_R = Residual volume
V_E = Total expectorated volume

Throughout the experiment two camcorders with fish-eye lenses (3 in 1 lens, Olloclip, Huntington Beach, USA) mounted at opposite corners of the room, recorded video footage of the experiment from two different angles. This was subsequently analysed to yield for each participant sip count and the elapsed time period between first and last sip. Based on these observations for each subject a calculated sip volume (V_{cal}) and consumption rate (R) were calculated:

$$V_{Cal} = \frac{V_T}{S}$$

$$R = \frac{V_T}{t}$$

V_{Cal} = Calculated sip volume per subject
S = Sip count
R = Consumption rate
t = Time period from first sip until last sip

The values derived in this way were compared to the analogous measured value of expectorated volume in order to assess the usefulness of video observation in the context of this work.

The analysis of the video footage recorded 3 independent values for each subject, namely V_T, t and S, upon which all subsequent calculations were based. Consumption rate (R) and calculated sip volume (V_{Cal}) were individually calculated for each subject using the given formulas; subsequently, mean R and mean V_{Cal} were calculated for the whole population of subjects.

Analysis of variance of all values obtained in this study was undertaken, with post hoc student's t comparison to identify significant differences between the sexes, using commercial statistical software (Prism, Version 6, GraphPad Software Inc., San Diego California, USA).

3.2.4 Post-experimental questionnaire

A post-experimental survey was designed and distributed to attendees after the experiment had finished. It assayed whether or not the participants felt that they had behaved and performed in a way that reflects their normal behaviour. This questionnaire was previously piloted upon and discussed with the participants of the mock run to ensure maximum clarity. The questionnaire comprised of two questions (Appendix 3-3) and allowed the participants to rank their behaviour and performance during the experiment.

3.3 The artificial mouth model

This aspect of the work sought to design and build a model that had the potential to mimic the interaction of saliva and enamel during the process of consuming an erosive beverage. This last aspect was informed by the behaviour observed in the pizza and soft drink parties. The design aimed to allow the operator to gather data from customizable experimental diets. In this section, the design and build of the artificial mouth model along with its associated equipment and parameters is described. An operational manual for the artificial mouth is given in appendix 5.

The device permitted the operator to control several variables such as salivary kinematic behaviour, beverage flow rate and volume of consumption.

3.3.1 Design

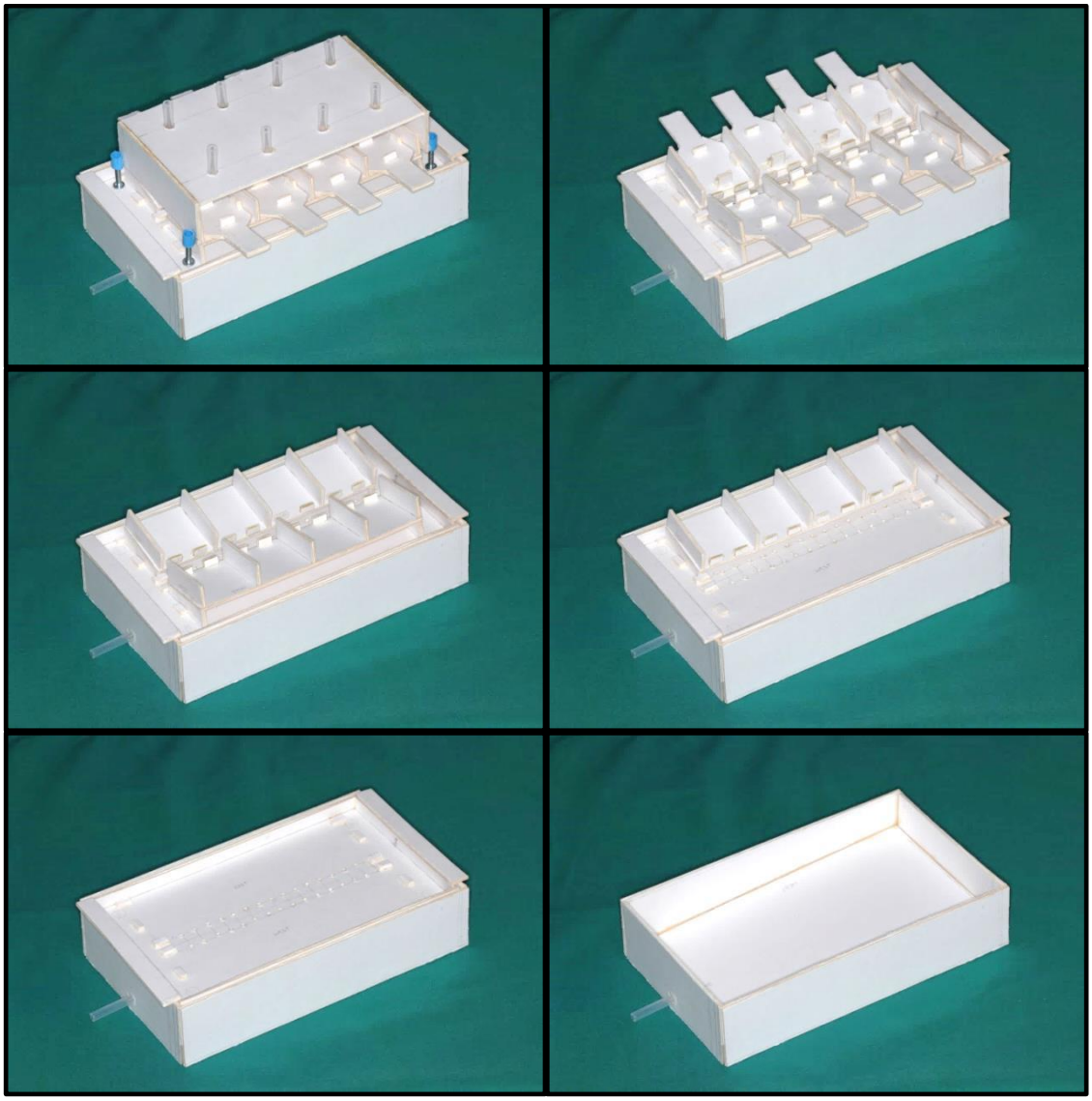
The prototype

Preliminary pencil sketches on grid paper were made that suggested the general shape, design, dimensions and the relationship among the different parts of the model. After constructive critical discussions these were modified and transferred to a cardboard mock-up (figure 3-2).

This was a rough approximation of the framework, dimensions, curves, slopes and tubing. It let the researcher explore the mechanics of operation, discover any impediments and refine the design.

In brief, 3 mm thick cardboard sheets were cut and glued to the desired configuration.

Figure 3-2. Saltus cardboard prototype



The prototype comprised five major components:

- Body: A reservoir that consisted of a sloping surface, drainage outlet and tray-holding slots.
- Tray: A base, onto which sample holder shelves sat, consisting of drainage holes, handles and shelf-holding slots
- Roof: This formed the Tubing/model interface and consisted of tubing inlets, a sample-set separator and four height-jacks.
- 2 sample holder shelves: Each shelf consisted of 4 sample cells with both shelf bases sloping towards the centre.
- 8 sample holders: Each consisted of a handle, an anti-slope step and sample-holding slots. Each holder was designed to receive 1 disc-shaped sample of 3 mm in thickness and 30 mm in diameter.

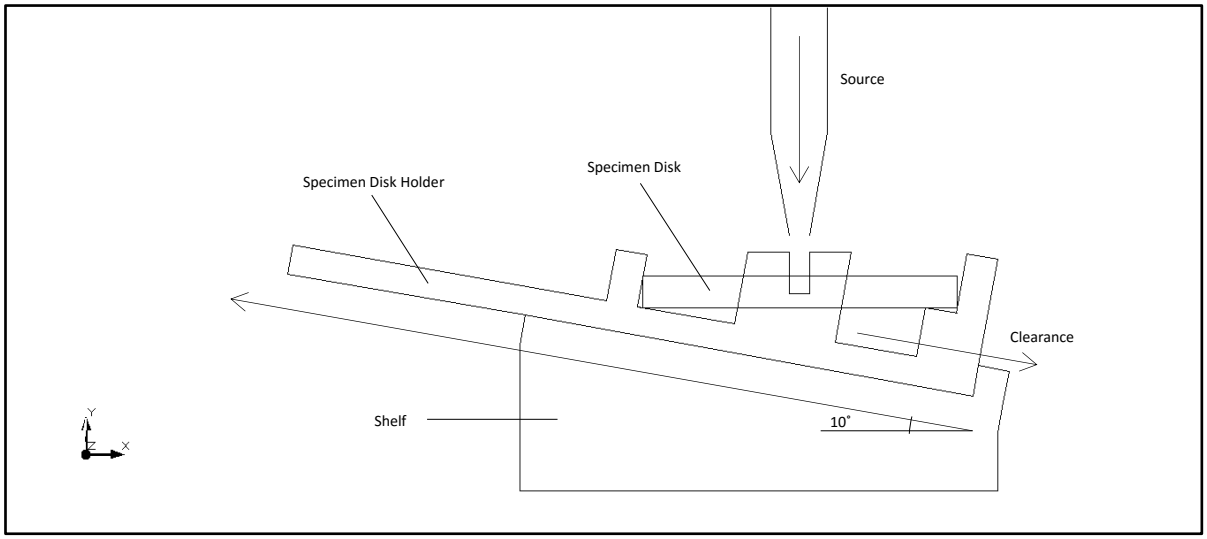
It was formed from A1 White Cardboard sheets of 3 mm thickness held together with white glue (Bostik Art, Bostik Ltd, Leicester, UK), and various lengths of tubing 2” long.

A computer-aided design and drafting software package (AutoCAD 2012, Autodesk Inc., San Rafael, California, US) was used to make drawings of the model’s component parts. These were refined and printed out to verify that construction was possible.

The completed AutoCAD drawings were transferred to a 3D creation suite (Blender™ 2.72, Stichting Blender Foundation, Amsterdam, The Netherlands) via another 3D modelling program, (SketchUp 2013, Trimble Navigation Limited, Sunnyvale, CA, US) where the building blocks previously created on AutoCAD were virtually assembled to produce a final 3D model. The Blender™ software was used to generate realistic model rendering. Its comprehensive array of modelling tools allowed modification of the

working skeleton and creation of accurate male/female slot assemblies to anchor components in 3D. A virtual model resulted and its potential to receive fluids was assessed by means of allowing Blender™ to generate a mock fluid flow throughout the system. This benefitted from its powerful fluid kinematic behaviour simulation ability via the Blender™ virtual simulation tool. Fluid simulations were carried out under a virtual temperature of 15 ˚C, a dynamic viscosity of 1.002 centipoise (cP) and a kinematic viscosity of 1.002 x 10^{-6} m^2s^{-1}. Such a simulation verified that the design, with its associated slopes, allowed for the desired fluid flow, circulation and collection.

Figure 3-3. AutoCAD drawing representing a single cell of a shelf in the model. Each cell was set so as to represent 1 human subject.



Appendix 4 lists all materials and equipment used in the fabrication of Saltus together with the software packages used in its design.

3.3.2 Build

This section describes the physical build of the artificial mouth model. This was named Saltus, the ancient name of a Roman Garrison City in Jordan, in keeping with the names of other robotic machines in the dental materials laboratory of Dundee University.

I. The skeleton

After the prototype's final design had been verified, the AutoCAD concept drawings were exported to a desktop computer which, in turn, was connected and synchronised with an ILS-III NM Intelligent Laser cutting system (Laser Tools and Technics Corp, Hsin Chu City, Taiwan). The cutting system's settings were adjusted to:

- Laser source: 100W
- Speed: 0.2"/sec
- Power: 50%
- Resolution (DPI): 1000
- Operating mode: Vector cutting
- Exhaust: 250 CFM air flow

Perspex® acrylic cast sheets were accurately placed onto the fully isolated engraving area of the device and the component parts of Saltus were then cut out under computer control. All the freshly cut building-blocks were allowed to rest for 30 minutes to ensure the full extraction of the resultant acrylic monomer odor before future assembly. All such components thereafter were wiped clean to ensure the complete removal of any residual acrylic monomer. This process was carried out under a fume hood using cotton pellets soaked with cellulose thinner (J Perkins Distribution Ltd, Lenham, Kent, UK). Thereafter, each component was checked for conformity, desired dimensions and surface finish before assembly. Any found to be substandard

were rejected and replacements made. The assembly relied upon both chemical and mechanical (basic and adjunctive) bonding techniques:

- Chemical: A two-component polymerisation cement (Tensol 70, Perspex Distribution Ltd, Blackburn, UK), suitable for cementing acrylic blocks together and insuring a fluid-tight seal, was used according to the manufacturer's instructions. Components A and B of this were mixed in a ratio of 20:1 and applied to the surfaces to be bonded. The cement was mixed in a plastic dispenser bottle and a modified 18G syringe needle tip was attached to the bottle's nozzle to maximize the reach of the cement into block-to-block junction micro-spaces. Application of the cement was repeated to all line junctions after 72 h of initial cementation. For each application 24 hours was allowed to reach set.

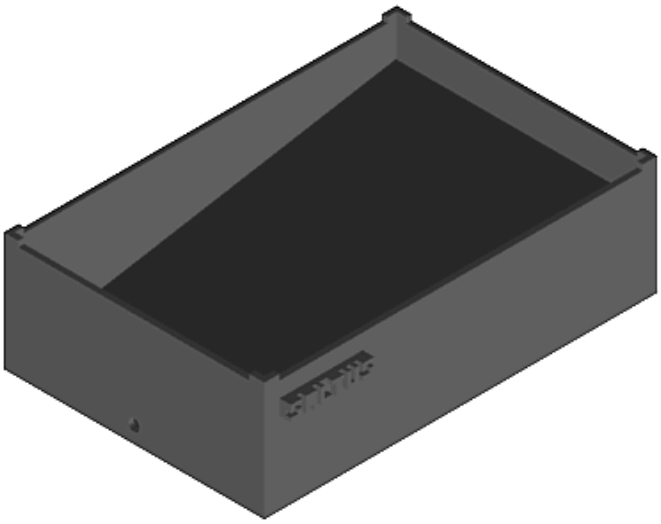
- Mechanical: Male and female slots to ensure precise unit placement and an increased bonding-surface area were machined into the components at manufacture. In addition, many components were stabilized in position using temporary scaffolds of Perspex and balsa wood throughout the protracted 24 h setting period of the cement.

In finalised form, Saltus consisted of;

- A body: A reservoir that consisted of a 10° sloping surface, drainage outlet and tray-holding slots (figure 3-4).

Figure 3-4. (A) Body: A reservoir that consists of a 10° sloping surface, drainage outlet and tray-holding slots. (B) The building blocks of the body before assembly.

A



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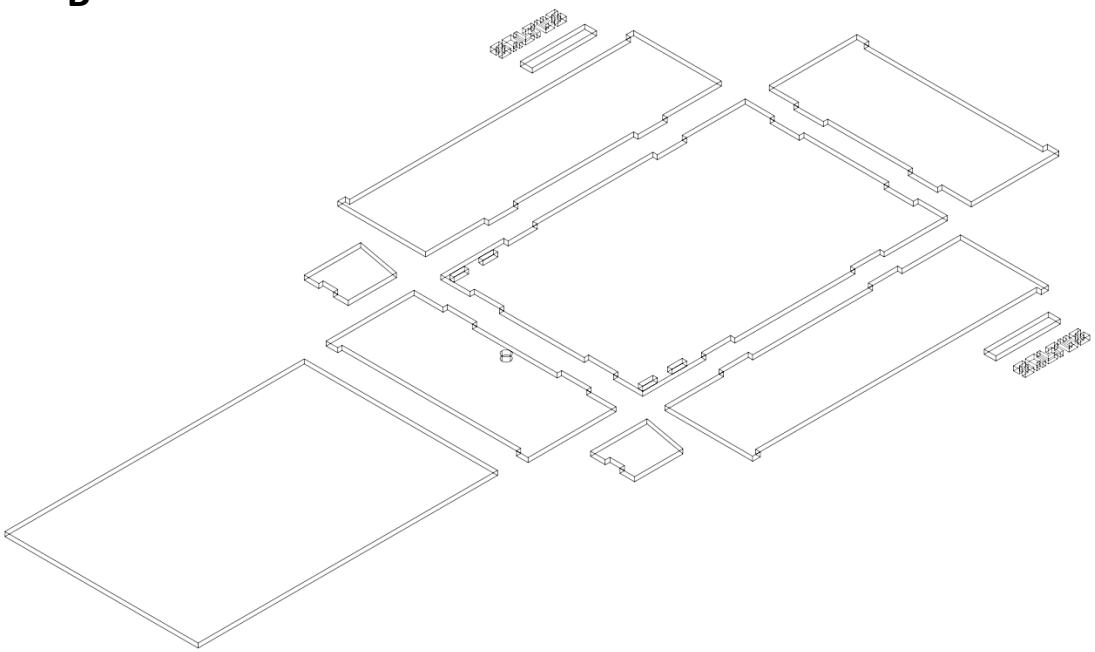
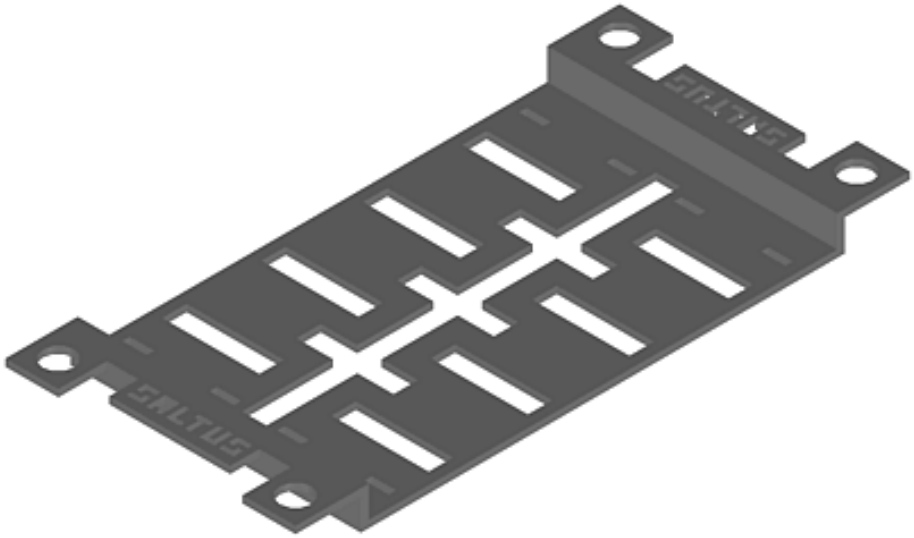


- A tray: A base onto which the sample holder shelves sat, consisting of drainage holes, handles and shelf-holding slots (figure 3-5).

Figure 3-5. (A) Tray: A base, onto which sample holder shelves sit, consists of drainage holes, handles and shelf-holding slots. (B) The building blocks of the tray before assembly.

A



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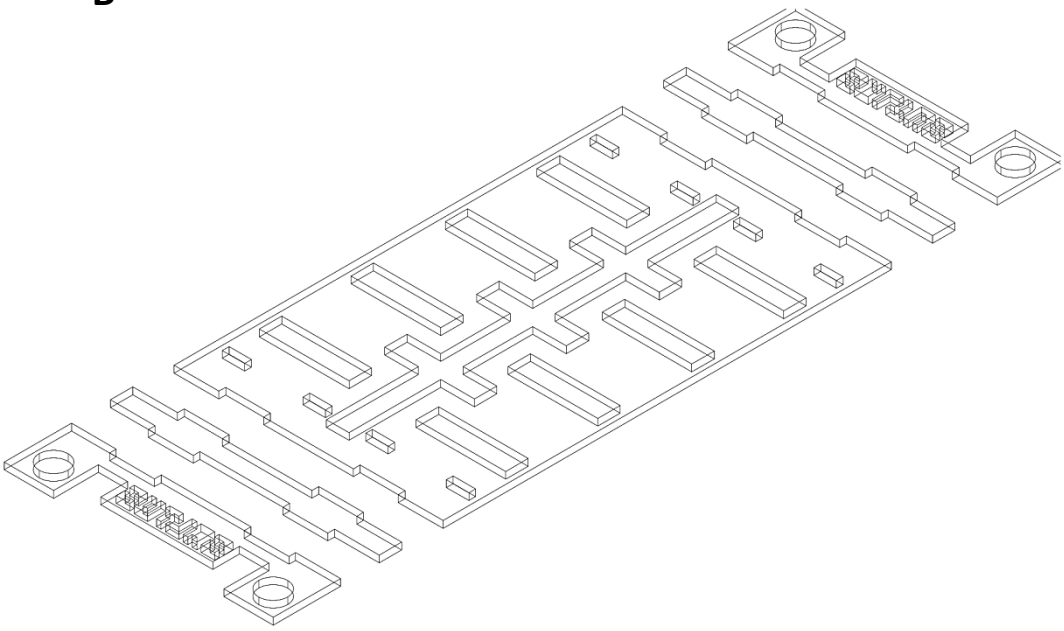
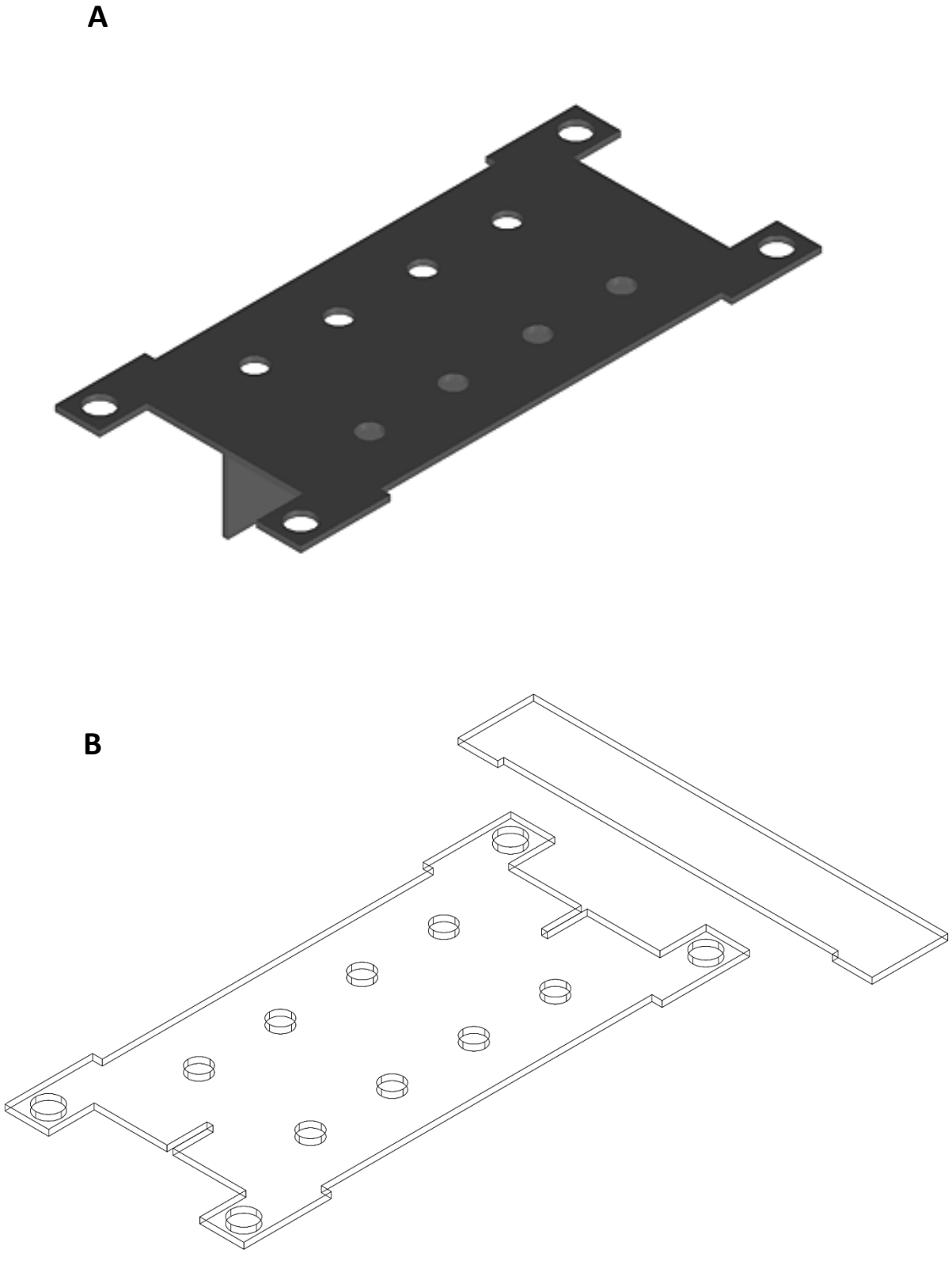


- A roof: Tubing/model interface that consisted of tubing inlets, sample-set separators and adjustable height pedicles (figure 3-6).

Figure 3-6. (A) Roof: Tubing/model interface that consisted of tubing inlets, sample-set separators and adjustable height pedicles. (B) The building blocks of the roof before assembly.

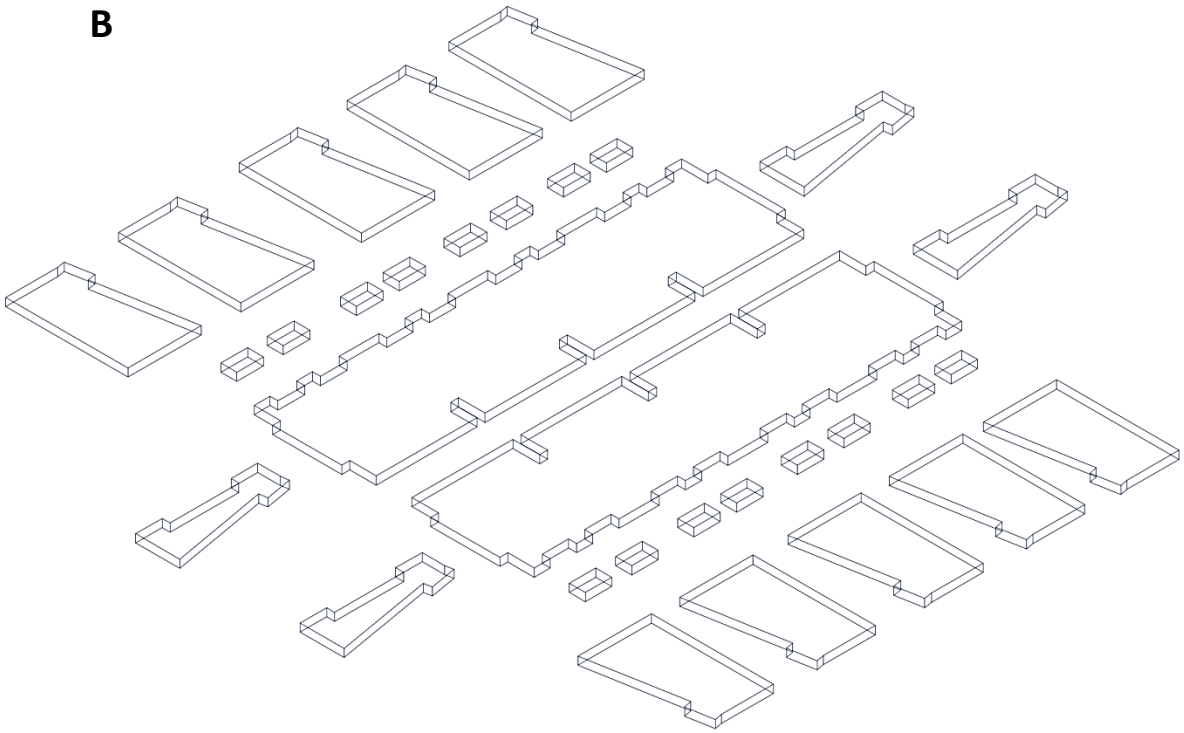


- 2 shelves: Each shelf consisted of 4 cells. The shelf base sloped towards the centre by 10° (figure 3-7).

Figure 3-7. (A) Shelves: Each shelf consists of 4 cells. The shelf base is sloping towards the centre by 10°. (B) The building blocks of the shelves before assembly.

A

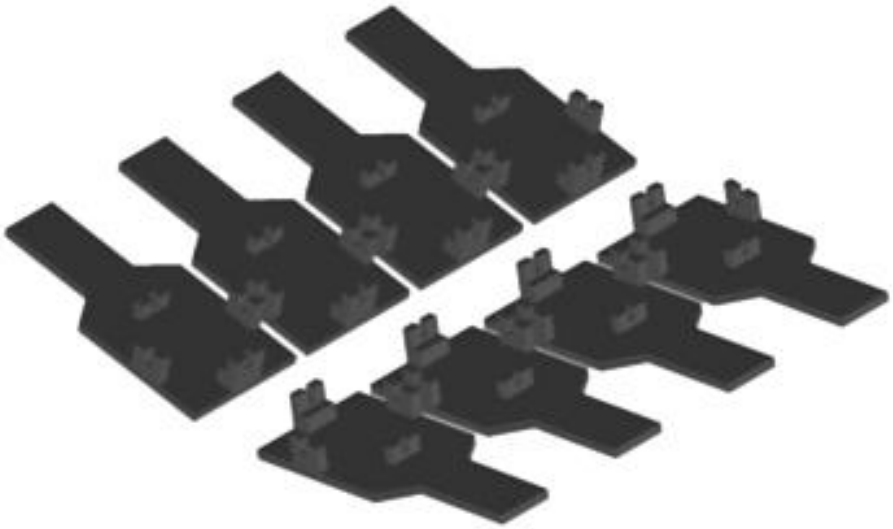
B



- 8 Specimen disk holders: The holder consisted of a handle, an anti-slope step and sample-holding slots. Each holder was designed to receive 1 specimen disk of 3 mm in thickness and 30 mm in diameter (figure 3-8).

Figure 3-8. (A) Specimen disk holders: The holder consists of a handle, an anti-slope step and sample-holding slots. Each holder is designed to receive 1 specimen disk of 3mm in thickness and 30mm in diameter. (B) The building blocks of the shelves before assembly.

A



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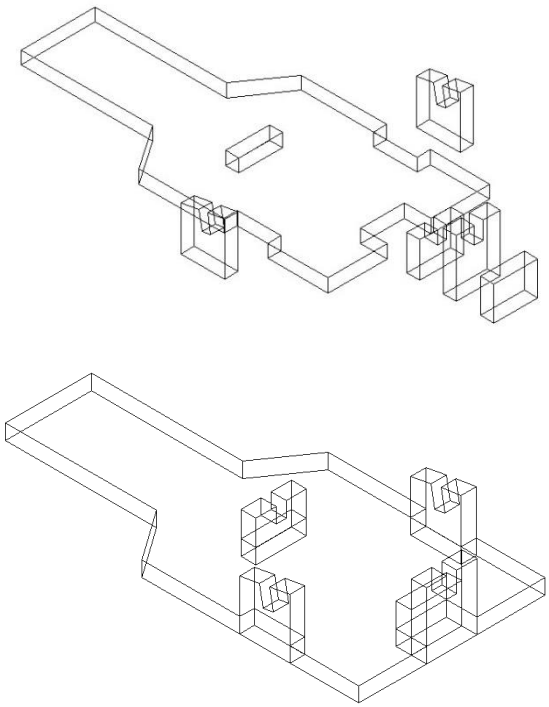
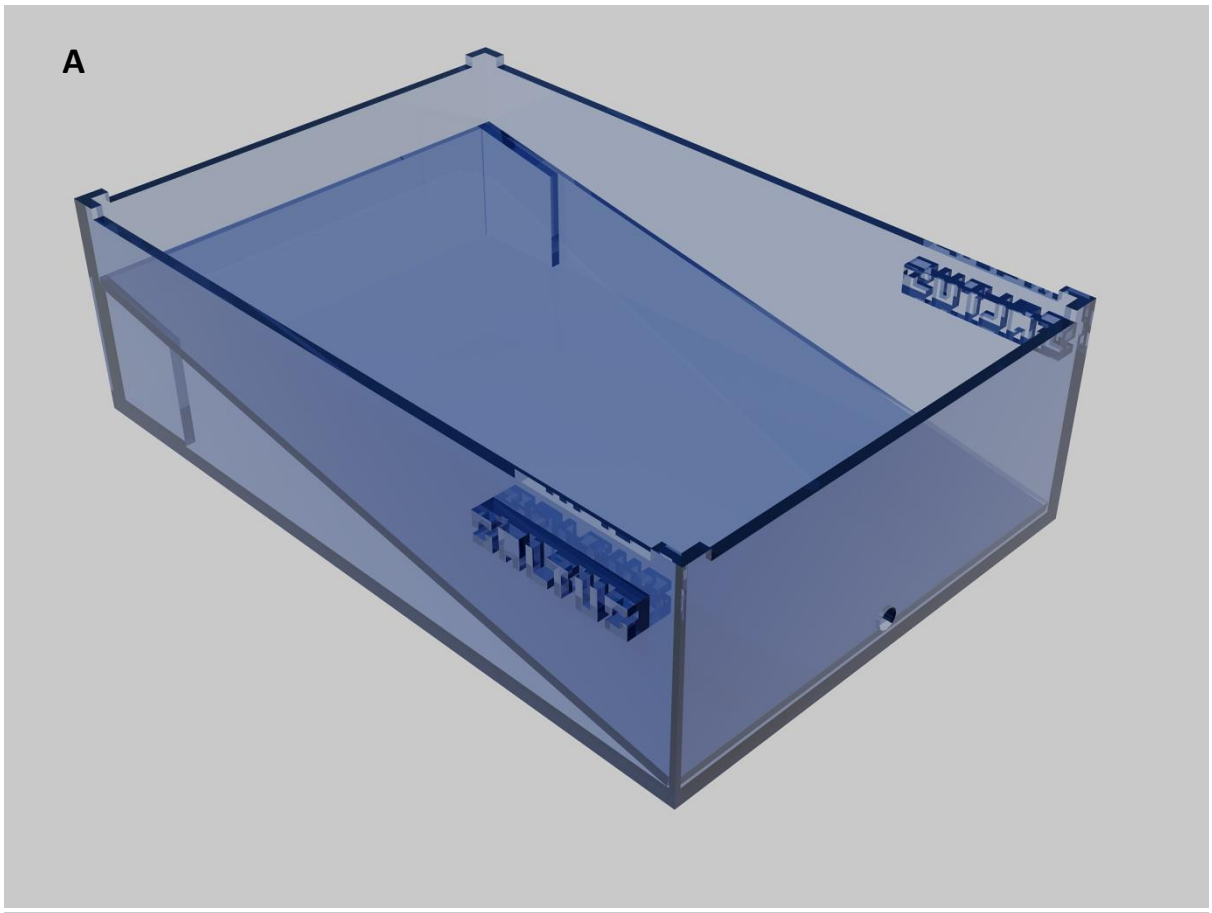


72 h after assembly and final cementation, all dimensions and angulations were checked and the fluid-tightness of the model was tested by filling the body to its maximum capacity with water. The model was checked daily for seven days for leakage.

Figure 3-9 (A-K) summarises the AutoCAD modelling and drafting of Saltus.

Figure 3-9. AutoCAD modelling and drafting. (A) Body: A reservoir that consisted of a 10° sloping surface, drainage outlet and tray-holding slots. (B) Tray: A base, onto which shelves sat, consisting of drainage holes, handles and shelf-holding slots. (C) Two shelves: Each shelf consisted of 4 cells. The shelf base is sloping towards the centre by 10°. (D) 8 Specimen disk holders: Each consisted of a handle, an anti-slope step and sample-holding slots. Each holder was designed to receive 1 specimen disk (E) of 3 mm in thickness and 30 mm in diameter. (F) Roof: This formed the tubing/model interface and consisted of tubing inlets, sample-set separator and adjustable height pedicles (G). (H) Top view. (I) Side view. (J) Mixer/specimen configuration (K) Source and clearance tubing.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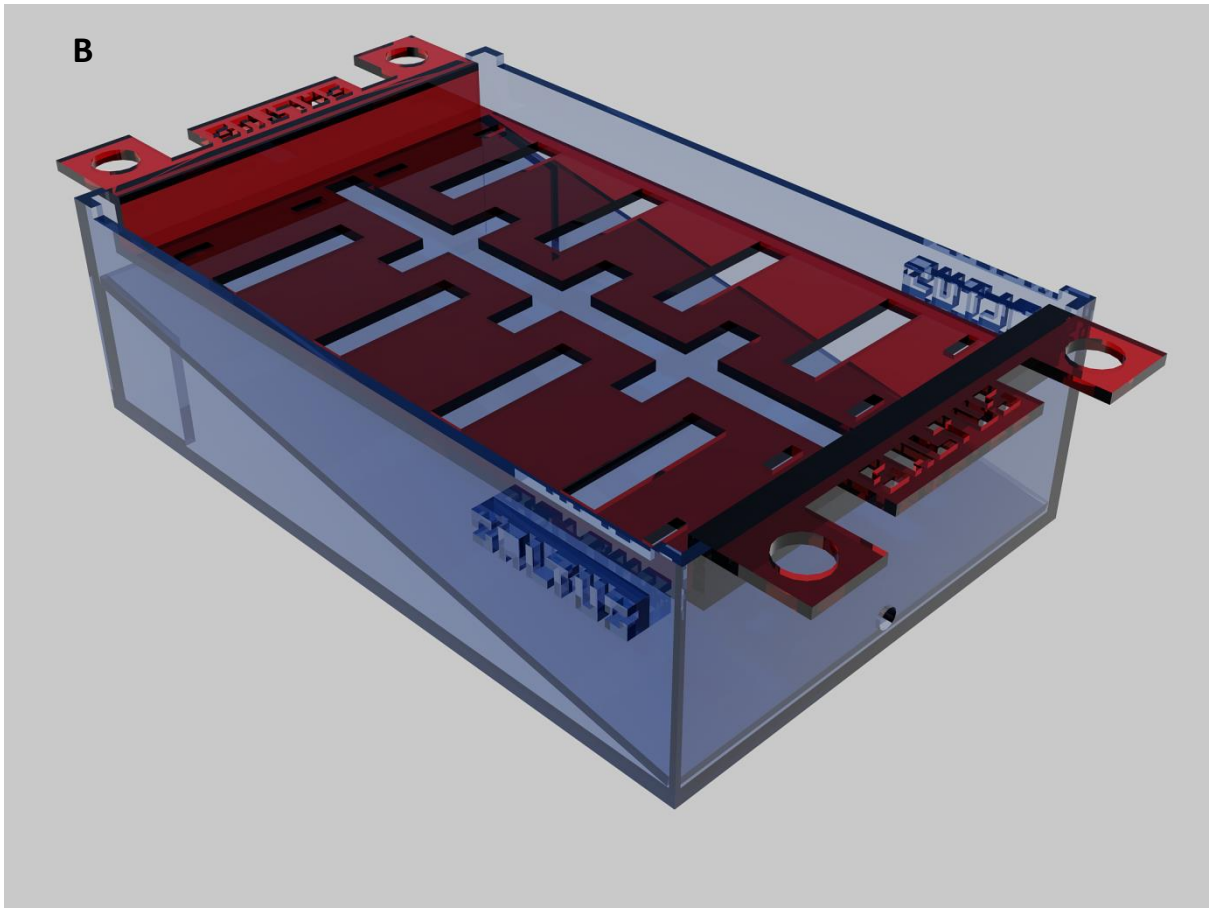
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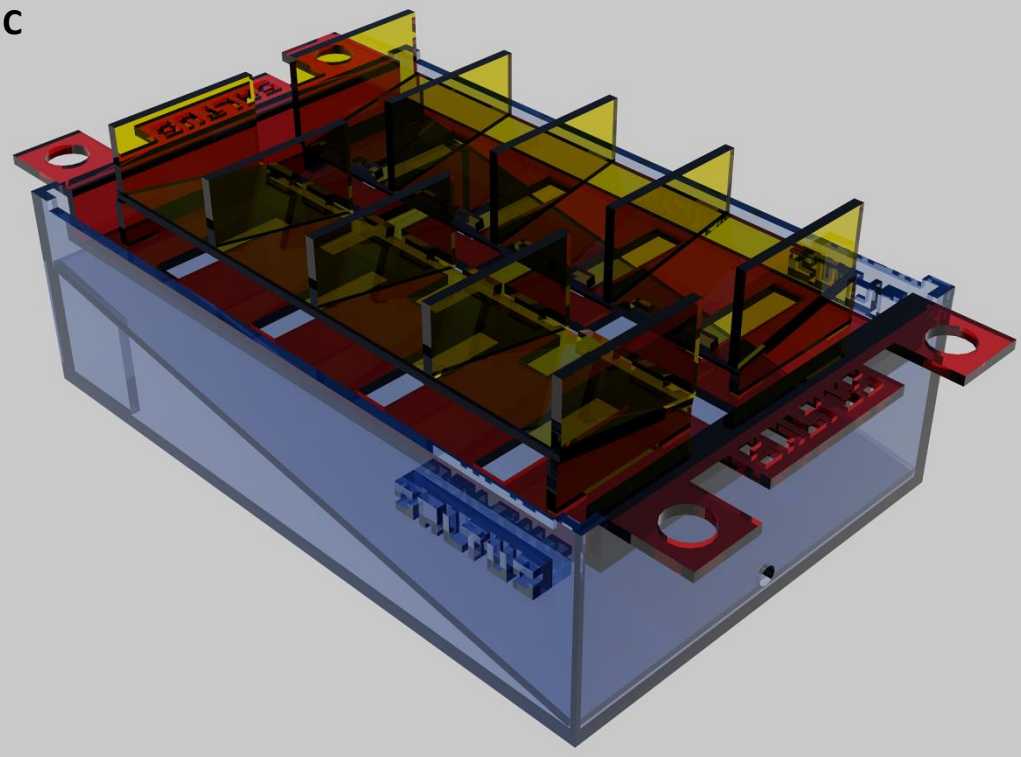
C



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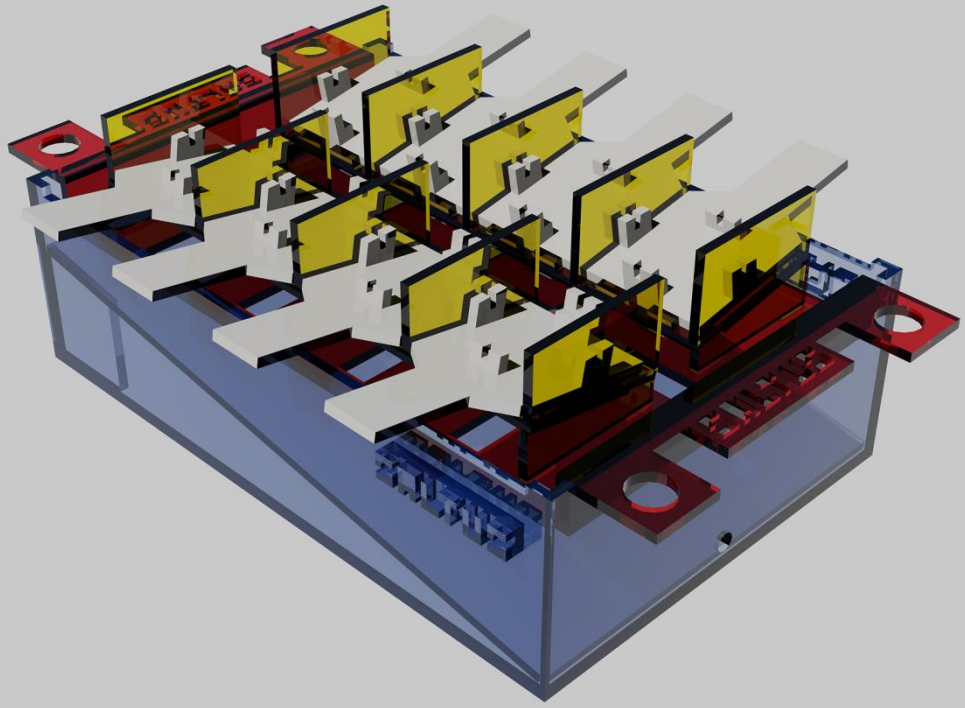

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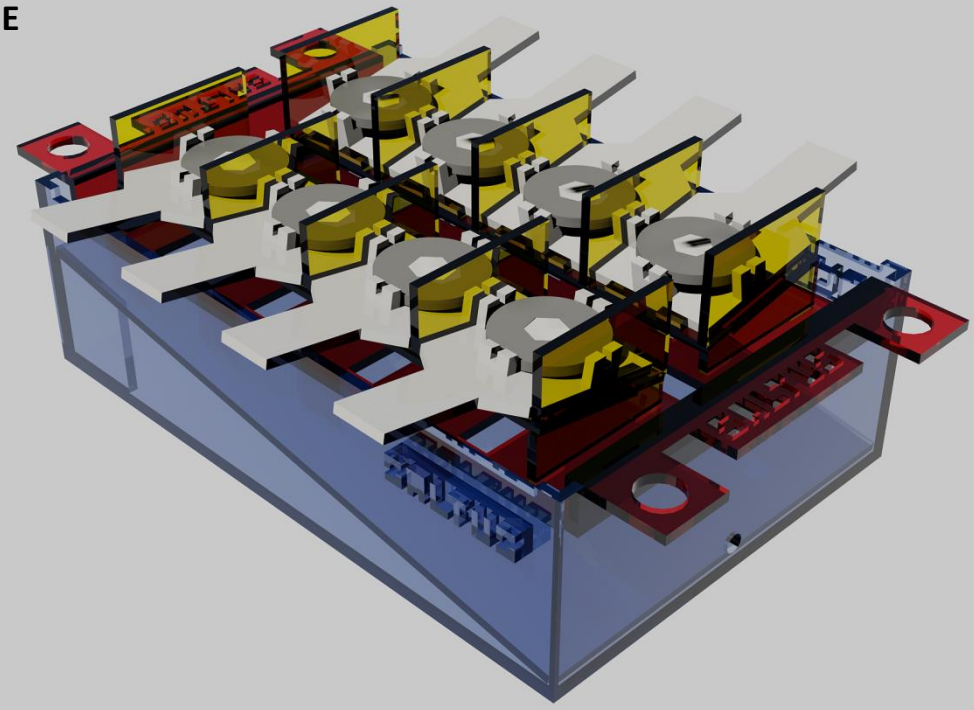

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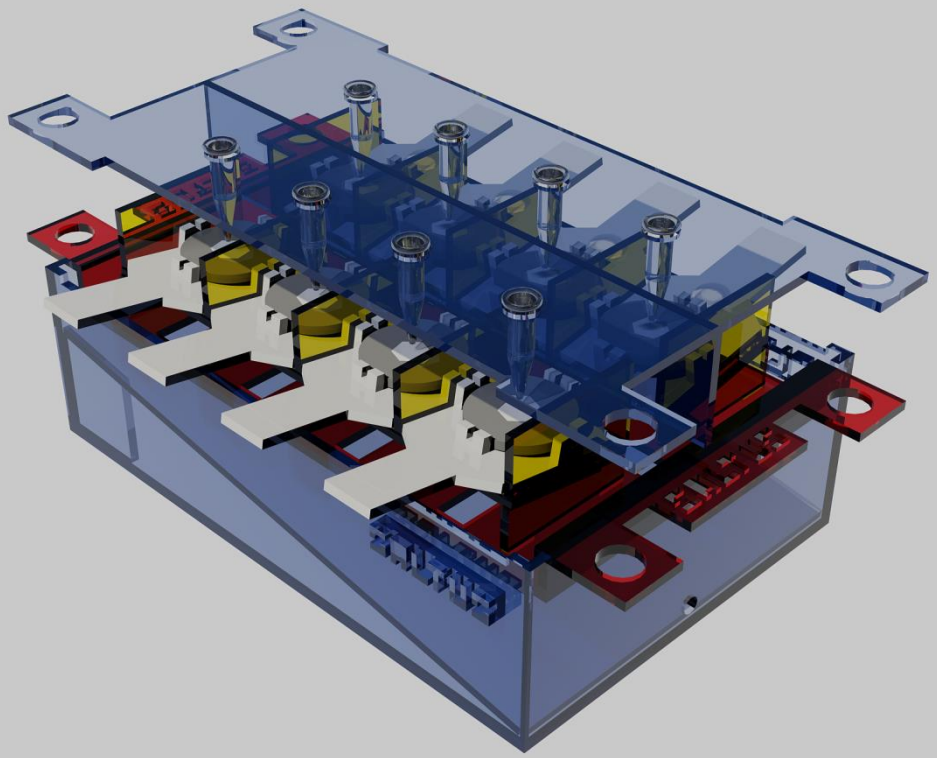

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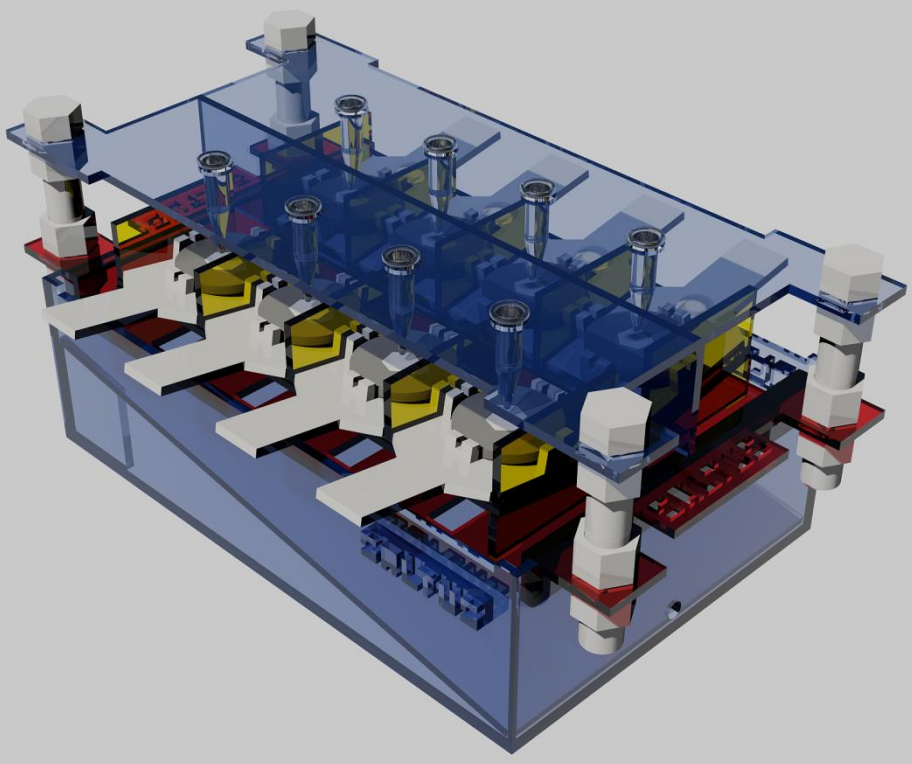
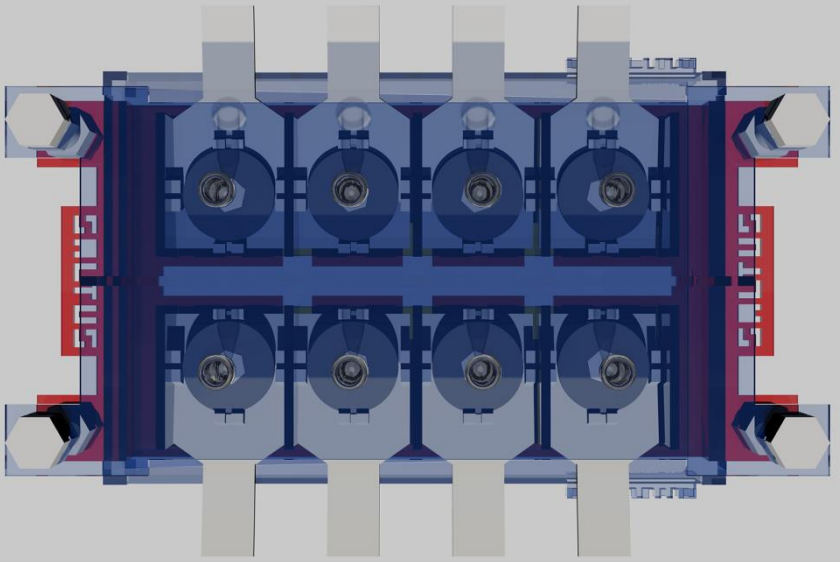


H



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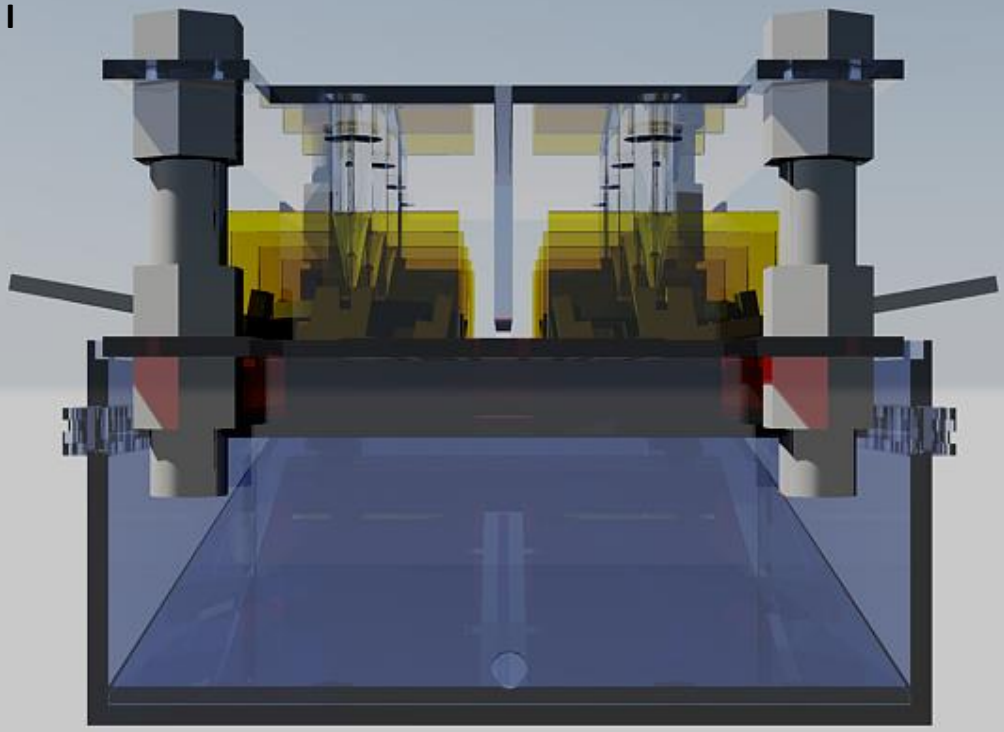

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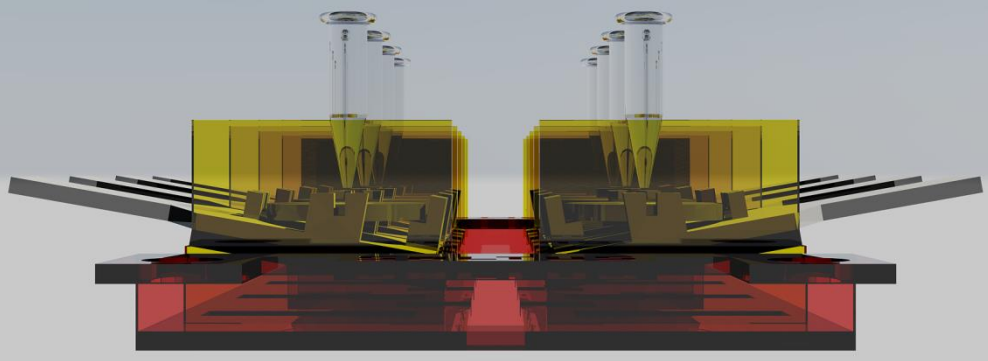
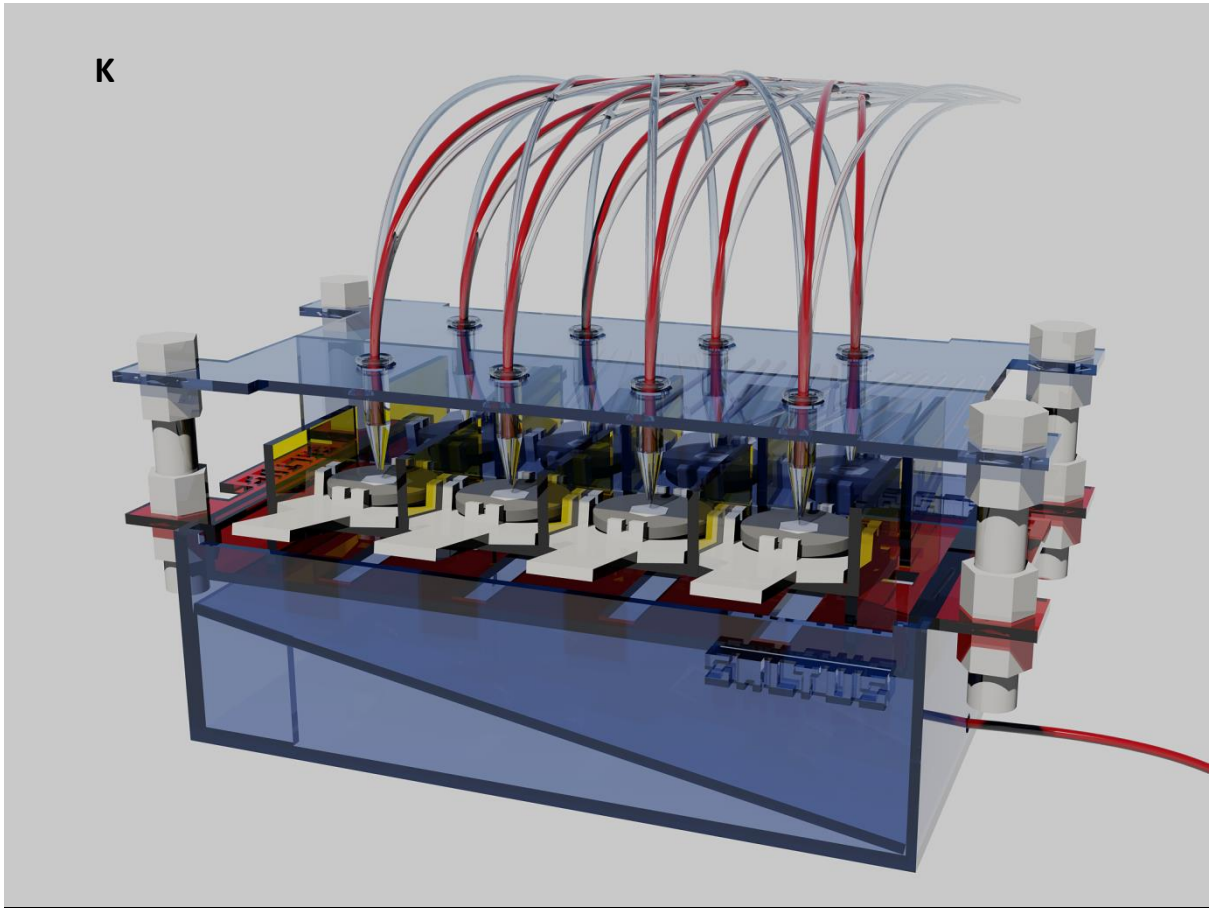



K

II. Pumps and Tubing

Following assembly of Saltus these were attached to transform the model into a fluid circulatory system capable of circulating stimulated and unstimulated saliva along with test beverages in a fully controllable manner. The principal pump was an Ismatec Peristaltic pump model, IPC 24, 24 channel drive (Michael Smith Engineers Ltd., Wetherby, West Yorkshire, UK).

Table 3-2 gives details of the tubing and its connectors.

Table 3-2. Tubing and connectors used in the set-up of pumps and tubing

Component	Manufacturer
Stainless Steel 316 Hypodermic tubing SMC-10T 10G/Thin wall 60" length	Shannon Coiled Springs Ltd., Limerick, Republic of Ireland
Stainless Steel 316 Hypodermic tubing SMC-14T 14G/Thin wall 60" length	Shannon Coiled Springs Ltd., Limerick, Republic of Ireland
Stainless Steel 316 Hypodermic tubing SMC-22R 22G/Regular wall 60" length	Shannon Coiled Springs Ltd., Limerick, Republic of Ireland
Pharmed® Ismaprene Ismatec peristaltic pump extension tubing, 0.38, 1.65 and 2.79 mm	Michael Smith Engineers Ltd., Wetherby, West Yorkshire, UK
Pharmed® Ismaprene Ismatec peristaltic pump 2 stop tubing, 0.38, 1.65 and 2.79 mm	Michael Smith Engineers Ltd., Wetherby, West Yorkshire, UK

The range of required functionality necessitated the utilisation of tubing lengths that were considerably longer than the ones provided by the pumping system manufacturer. To accommodate this stainless steel hypodermic tubing and ERGO adhesive (Primer and adhesive) (Michael Smith Engineers Ltd., Wetherby, West Yorkshire, UK) was used to link runs of tubing together to afford the desired lengths.

Connectors were selected to have no effects upon flow rate and fluid kinematics (inner diameter of connector) and for the tubing to fit the connector tightly (outer diameter of connector).

Table 3-3 gives the specifications of the tubing and connectors used.

Table 3-3. (A) Specifications of the SS 316 Hypodermic tubing. (B) Inner diameters of tubing and connectors. (C) Inner diameter of tubing versus outer diameter of connectors.

(A)

SS 316 Hypodermic tubing	Wall type	Outer diameter (inch)	Inner diameter (inch)
SMC-22R	Regular	0.028±0.000	0.016±0.000
SMC-14T	Thin	0.083±0.001	0.067±0.001
SMC-10T	Thin	0.134±0.001	0.114±0.002

Specifications of the SS 316 Hypodermic tubing

(B)

	Tubing inner diameter (mm)	Connector inner diameter (mm)	Ratio
Small	0.38	0.40	0.95
Medium	1.65	1.70	0.97
Large	2.79	2.89	0.96

Connectors' inner diameters were chosen so as to have a negligible effect on flow rate and fluid kinematics.

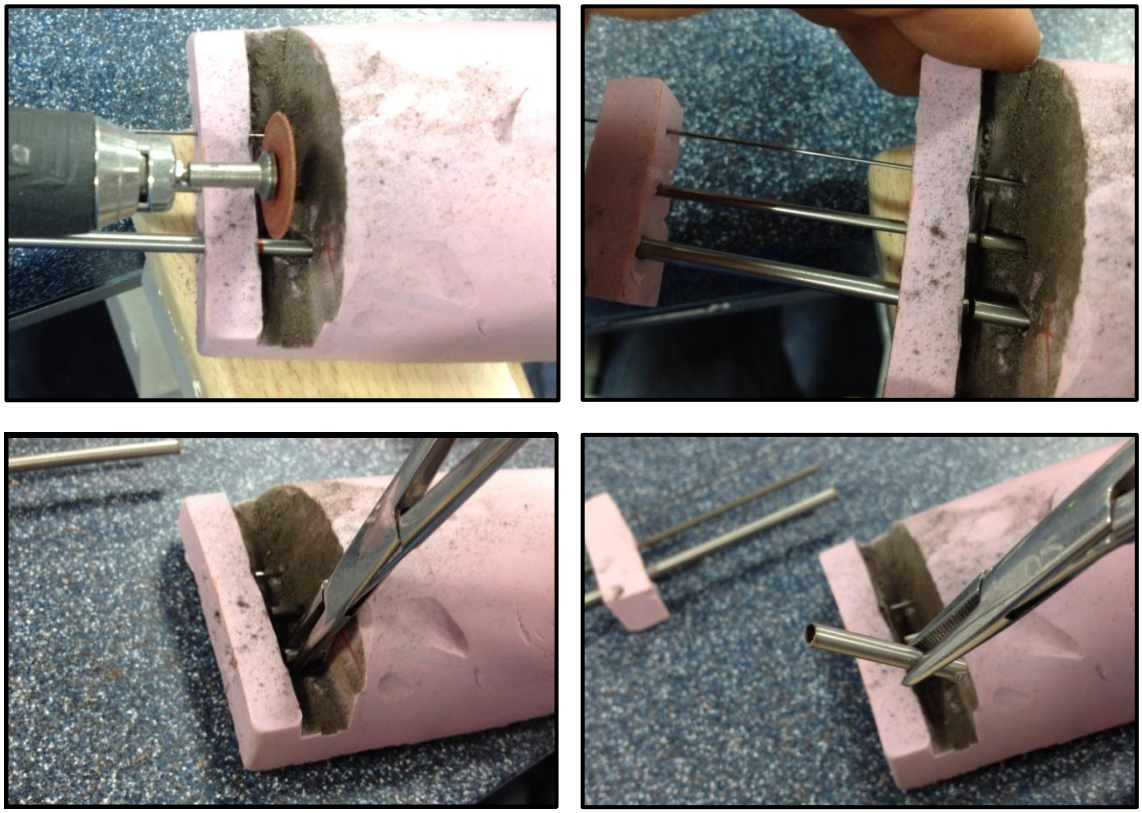
(C)

	Tubing inner diameter (mm)	Connector outer diameter (mm)
Small	0.38	0.71
Medium	1.65	2.10
Large	2.79	3.40

Connectors' outer diameters were chosen so as to ensure that the elastic tubing will fit the rigid connector tightly.

In order to fashion connectors from the 60" hypodermic tubing rods, these were cut to 1" lengths using steel cutting disks under a fume hood. A custom made rod holder was used to facilitate precise and safe cutting (figure 3-10). This was formed from condensation silicone impression putty moulded to the desired shape and configured to have special slots into which the rods of different diameters could snugly fit. Once cut, edges and lumens of the freshly cut lengths were inspected and polished using endodontic Hedstrom files.

Figure 3-10. Preparation of 1” lengths of SS hypodermic tubing.



Thereafter, Pharmed® Ismaprene 2-stop tubes were connected to the extension tubes using the aforementioned custom-made connectors. One half of the connector was inserted into the 2-stop tubing while the other half was inserted into the corresponding end of the extension tubing. Facilitation of insertion was assisted by warming the tubing ends in a hot water bath to make it easier to insert the connector. After inserting each connector into its corresponding tubing end, ERGO adhesive (Primer and adhesive) (Michael Smith Engineers Ltd., Wetherby, West Yorkshire, UK) was applied to the exposed surface of the connector and this was approximated to the length of tubing to be joined, until a convenient circumferential tube-to-tube contact was obtained.

Flow rate calculations

The Saltus model sought to investigate the effects of realistic human drinking behaviour (as informed by the pizza and soft drink observations of this work) upon the dentition and countered by saliva flow. To achieve this, the flow rates summarised in table 3-4 had to be calibrated for and achieved.

Table 3-4. Flow rate values that are to be adopted

Fluid variables	Justification	Flow rate
Carbonated beverage drinking rate	(Qutieshat et al. 2015)	13.3 ml/min
Stimulated Saliva flow rate	(Dawes, 1987)	5.0 ml/min
Unstimulated saliva flow rate (waking hours)	(Thomson et al., 2011; Dorion, 2011)	0.3 ml/min
Unstimulated saliva flow rate (sleeping hours)	(Dorion, 2011)	0.1 ml/min

The peristaltic Ismatec IPC 24 was selected for this because it can produce flow rates ranging from 0.002-44 ml/min per channel thus delivering the desired flow rates needed for both stimulated and unstimulated artificial saliva along with the desired drinking flow rate. Table 3-5, compiled from manufacturers data, summarises according to tubing type number the expected range of flow rates of fluid moving through the types of tubing propelled by the peristaltic pump operating at 45 revolutions per minute (RPM). It forms the basis upon which the following calculations were performed in order to select the appropriate tubing group to circulate the required fluids:

Table 3-5. Flow rate ranges (ml/min) for each tubing group (Pharmed® Ismaprene Ismatec peristaltic pump tubing). The values provided represent flow rates when the pump is running at its maximum drive speed of 45 rev/min.

Tubing type number	Min. flow rate (ml/min)	Max. flow rate (ml/min)	Internal diameter (mm)	Flow rate range (ml/min)
1	0.009	0.86	0.38	0.009 to 0.86
2	0.14	14	1.65	0.14 to 14.0
3	0.35	35	2.79	0.35 to 35.0

To obtain 13.3 ml/min to model beverage drinking rate:

This flow rate lies within group (3)'s range (0.35 < 13.3 < 35.0); therefore, tubing group number 3 was chosen to circulate the test beverage (table 3-5). The RPM required may be calculated as;

$$\text{RPM} = Expected\ flow\ rate \times \frac{Max\ pump\ drive\ speed}{Max\ tube\ flow\ rate}$$

$$= 13.3 \times \frac{45}{35}$$

= 17.1

∴ the pump will need to run at 17.1 RPM to achieve this during the day mode (waking hours).

To obtain 5 ml/min to model, the stimulated saliva rate, as the pump is committed to 17.1 RPM, substitution in the same equation yields a maximum tube flow rate of 13.158. This matches use of tube type number 2 as 13.158 is within the stated range of 0.14-14.

To obtain 0.3 ml/min to model, unstimulated saliva during waking hours, substitution in the same equation yields a maximum tube flow rate of 0.86. This matches use of tube type number 1.

∴ Tube number 1 was chosen because 0.86 is within the range of 0.009-0.86 ml/min and equal to the max flow rate value of 0.86

In the same way, to obtain a night sleeping unstimulated saliva flow rate of 0.1 ml/min if the same tubing (number 1) is used the RPM of the pump must fall to 5.23.

III. The mixer

Before the fluids contact the textured substrate the fluids must be mixed. To ensure this Saltus was equipped with mixers. Thus, at any point of time, the specimen surfaces should not be exposed to the beverage under investigation in the absence of artificial saliva.

To achieve this eight Eppendorf tubes (Cole-Parmer Instrument Co. Ltd., Hanwell, London, UK) were modified by removing their caps and trimming each tube's tip. This transformed them into tapered conical-shaped tubes. These had two openings; a large opening into which could be fitted three different sized Pharmed Ismaprene tubings and a small opening that was >2.79 mm in diameter for exit of the mixture. The modified tubes were bonded to the roof part of Saltus using Sheramega 2000 adhesive (Shera Werkstoff-Technologie GmbH & Co. KG., Lemforde, Germany).

- ***A pilot study of the mixer physics***

At any given point of time, no more than 2 solutions will be dispensed through Saltus' mixer unit. To test the efficiency of mixing, the following experiment was undertaken.

For this Bromophenol blue dye, that can be used as an acid-base indicator, was passed through the mixing tips of Saltus with a clear solution of citric acid. At a low pH, the dye absorbs both ultraviolet and blue light most strongly and appears yellow in

solution. The hypothesis tested was that mixing Bromphenol Blue (Blue) with Citric acid (Clear) using Saltus' mixer unit, would yield a yellow solution if mixing was efficient.

Table 3-6 details the chemicals and equipment used in this investigation.

Table 3-6. Chemicals and equipment used in the pilot study

100 ml of 1 M Citric Acid solution (Sigma-Aldrich Ltd., Gillingham, Dorset, UK).
0.005 g Bromphenol Blue (Sigma-Aldrich Ltd., Gillingham, Dorset, UK) in 500 ml H_2O solution (wavelength λ = 590 nm).
Spectrophotometer (WPA Lightwave S2000 UV/Vis, Biochrom Ltd., Cambridge, UK).

In preparation for the experiment a solution of Bromphenol Blue (0.005 g Bromphenol Blue in 500 ml H_2O solution) and Citric acid (100 ml of 1 M Citric Acid solution) was thoroughly mixed in the laboratory using a magnetic stirrer for 2 minutes (Stuart Scientific SM1, Keison Products, Chelmsford, Essex, UK). This was passed through the spectrophotometer and its absorption value (λ) was set to read 0.000. This therefore gave a reference value against which the passage of 500 µl of each solution dispensed simultaneously through Saltus' mixer unit into a cuvette also on the spectrophotometer device, was assessed on 4 occasions. The process was videotaped for quality control purposes.

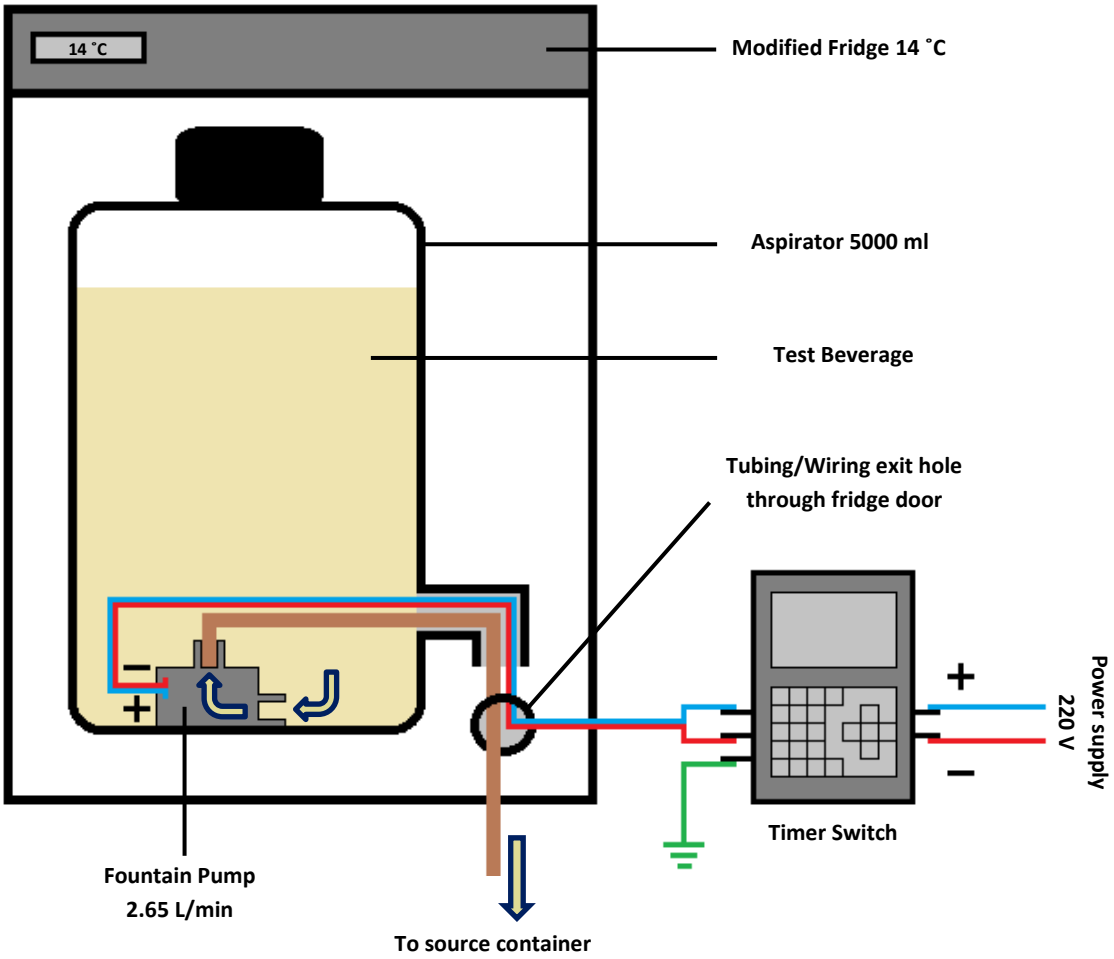
IV. Delivering the beverage to be tested with simulated saliva flow

The system was designed to circulate unstimulated and stimulated saliva together with a test beverage. These were stored separately and delivered to the erosion substrate through the mixing tips of the device in a controlled way. A variety of timing, mechanisms and pumps were used in this regard.

A. Beverage delivery

This consisted of a 5 litre capacity polyethylene aspirator with tap (Azlon, SciLabware Ltd., Stoke-on-Trent, Staffordshire, UK) housed in a mini-fridge (Thermoelectric cooler and warmer, Diplomat, Slemcka Ltd. Smethwick, Birmingham, UK) operating at 14 ˚C. A submersible low voltage direct current fountain pump (Vovyo Technology Co. Ltd., Shenzhen, Guangdong, China) with a flow rate of 2.65 litres per minute controlled movement of the beverage through Tygon pump tubing (Cole-Parmer Instrument Co. Ltd., Hanwell, London, UK) from this reservoir to a 5000 ml glass beaker (Fisher Scientific UK Ltd., Loughborough, UK) from which it was conveyed to the Saltus mixing tips, at the desired flow rate, by the action of a peristaltic pump (Michael Smith Engineers Ltd., Wetherby, West Yorkshire, UK) and its tubing (Pharmed® Ismaprene Ismatec peristaltic pump extension tubing group 2.79 mm, Michael Smith Engineers Ltd., Wetherby, West Yorkshire, UK). To prevent the source tubing from floating, due to the beverages fizzy nature, and also to prevent air entrapment the tubing was kept submerged by attachment to a 60 g fishing weight (WSB Tackle Ltd., Redruth, Cornwall, UK). A micrometer timer switch (ZYT16G Micrometer timer switch, Shanghai Zhuoyi Electronic Co. Ltd., Pudong, Shanghai, China) controlled the movement of the beverage to the beaker. For a “one can diet” (see section 3.3.3 [Validation of the artificial mouth]) this operated once for one minute but in the case of a “two can diet” operation was on two occasions. Its actions were monitored remotely by a webcam (Logitech webcam C200, Logitech, Newark, USA) by the researcher. All wiring and tubing openings/ interfaces were sealed with PTFE thread seal tape and silicone waterproof sealant (Unibond, Henkel Consumer Adhesives, Winsford, Cheshire, UK). Figure 3-11 illustrates diagrammatically the set-up.

Figure 3-11. An illustration showing the timer-controlled and temperature-regulated container system components and setting.



A.I Verifying the constancy of drink temperature in the time frame of an experimental run

The temperature that we wish the drink to be at when it contacts the substrate (based upon the observations from the pizza and soft drink parties) is of the order of 14.9 ˚C. This is supplied from the reservoir at around this temperature. This section seeks to investigate what happens to the temperature of the drink in the time frame of the experiment. A chilled can of coke (4 ˚C) was opened and its temperature monitored with time under room temperature (25 ˚C) using a digital thermometer (Basetech BT-

80, Conrad, Colchester, UK) and a digital timer (Debut Sport Timer, DebutSports, Bristol, UK). This procedure was repeated 6 times, all of which were video recorded.

B. Saliva delivery

The system, via the peristaltic pump (Michael Smith Engineers Ltd., Wetherby, West Yorkshire, UK) delivered at appropriate times and flow rates unstimulated and stimulated artificial saliva to the mixing tips of Saltus. These fluids were housed in separate 2000 ml laboratory glass bottles (Fisher Scientific UK Ltd., Loughborough, UK) modified to ensure stability of the saliva by preventing carbon dioxide from escaping from it, which, as well as undermining the stability of the solution, would also deplete its carbonate content. To achieve this, the caps of the laboratory glass bottles were modified by drilling 8 holes into each to permit 1" lengths of stainless steel hypodermic tubing (Shannon Coiled Springs Ltd., Limerick, Republic of Ireland) to snuggly fit. The unstimulated artificial saliva bottle cap was prepared to receive eight 22R-Gauged tubing lengths while the stimulated artificial saliva bottle cap was prepared to receive eight 14T-Gauged tubing lengths. The tubing/cap interfaces were sealed with PTFE Thread seal tape and silicone sealant. Stainless steel hypodermic tubing 1" lengths were inserted into the tubing so as to connect the main pump tubing with these reservoirs.

After inserting each connector into its corresponding tubing end, ERGO adhesive was applied to excess circumferentially around the 1" connector inserts allowing some excess adhesive to be applied on the exposed surface of connectors. This was to ensure an air tight seal so as to prevent CO_2 from escaping from the freshly prepared working solution. Figure 3-12 illustrates this set-up.

In operation the unstimulated artificial saliva was replenished on a daily basis while the stimulated saliva was prepared fresh just before the commencement of an experimental run.

To permit biochemical analysis of the post exposure fluid mix Saltus was equipped with an outlet connected to a pump (Watson Marlow 505U, Refer Scientific, Blackdog, Aberdeen, UK) supplying a 10 L aspirator (HDPE aspirator, Azlon, SciLabware Ltd., Stoke-on-Trent, Staffordshire, UK)(figure 3-13). A faucet in the aspirator allowed for the collection of 10 ml samples of the resultant saliva/beverage solution to be later tested for traces of calcium and phosphate ions by automated chemistry analysis (ADVIA® 2400 Clinical Chemistry System, Siemens Healthcare, Camberely, UK).

Figure 3-14 illustrates the role of the main pump in conveying the test beverage and artificial saliva from source reservoirs to Saltus.

Figure 3-12. An illustration showing the modified reservoir cap of the artificial saliva source container.

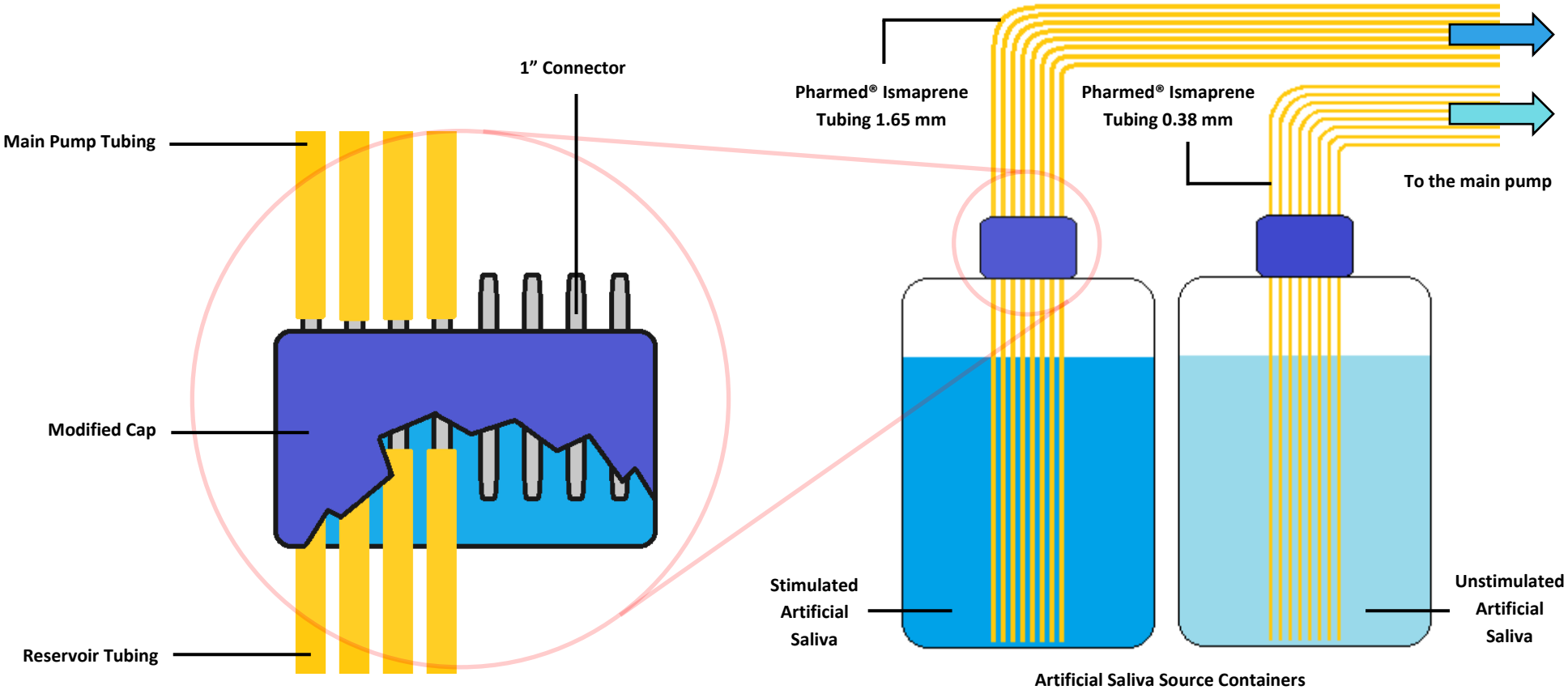


Figure 3-13. The output (clearance) pump (Watson Marlow U505) transports the saliva/beverage resultant solution (Green tubing) from the artificial mouth model (Saltus) to the collector reservoir.

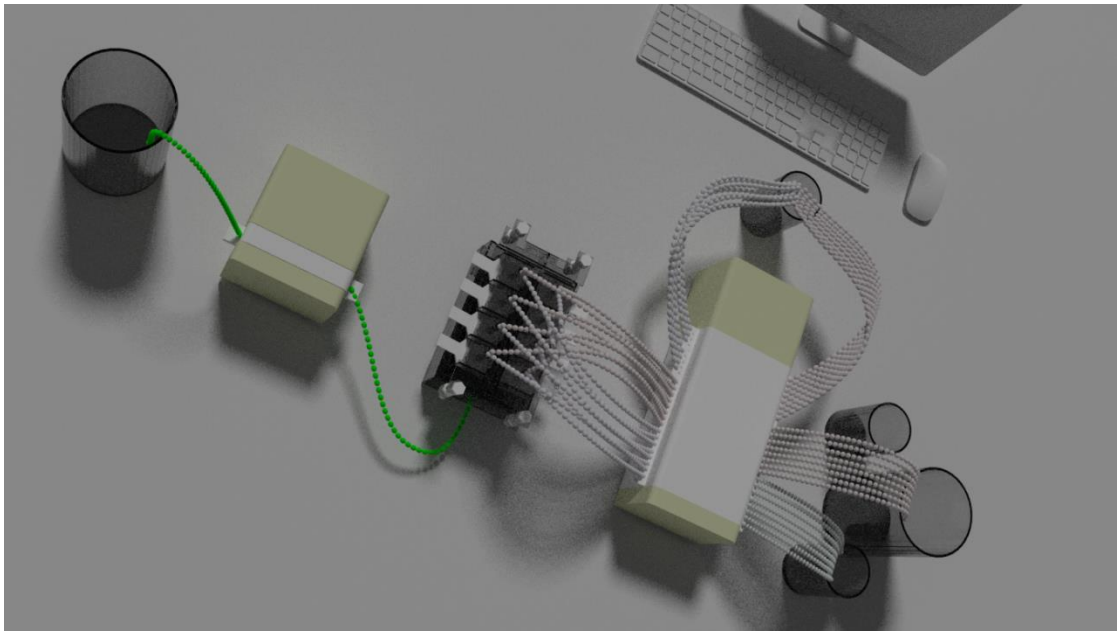
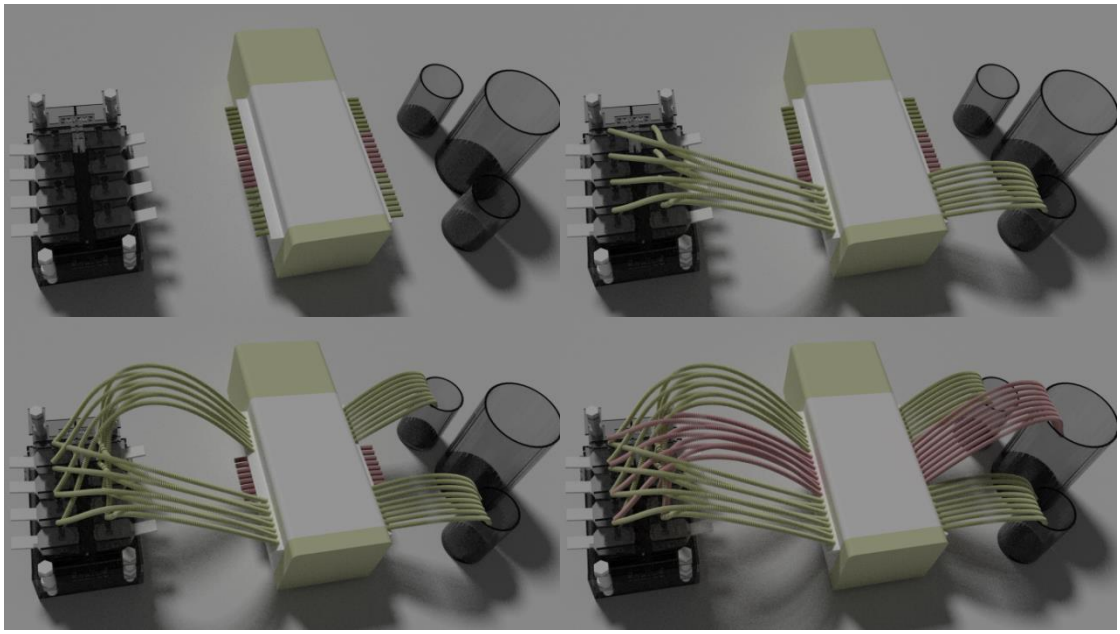


Figure 3-14. The main pump (Ismatec IPC-24) transports the test beverage (red tubing) and artificial saliva (Beige tubing) from source reservoirs to the artificial mouth model (Saltus).



V. Formulation of Artificial Saliva

The work relied originally upon the formation described by Leung and Darvel (1991)(Leung and Darvell, 1991) (table 3-7). Due to its lack of calcium however, this section describes the investigation of the addition of this element in various forms to promote remineralisation of the erosion substrate. To achieve this, three stock solutions were prepared from which a final working solution was prepared by dilution and mixing. These stock solutions were used to provide bicarbonate stability and indefinite longevity of solutions when stored. This would not be the case if mixed in advance to prepare the working solution.

Table 3-7. Darvel's artificial saliva original recipe.

Darvel's Original Recipe	Concentration	
	g/L	Mol/L
Stock solution A		
NaH_2PO_4	28.0	0.233
KCl	86.8	1.164
NaCl	7.21	0.123
NH_4Cl	11.0	0.205
Trisodium citrate di-hydrate	1.1	3.74×10^{-3}
Lactic acid	3.5	0.039
Stock solution B		
Urea	10.0	0.167
Uric acid	0.75	4.46×10^{-3}
NaOH	0.2	5.00×10^{-3}
Stock solution C		
KSCN	12.0	0.123

All stock solutions were made up at 50 times the working concentration; the working solution prepared from these was therefore prepared by dilution; a volume of 500 ml was used for titration. All preparations were held under room temperature conditions. Their pH was adjusted by mixing additions of 5 M KOH (Sigma-Aldrich Ltd., Gillingham, Dorset, UK) whilst stirring thoroughly.

On a daily basis stock solutions were pipetted into well-stirred distilled water at 4 ˚C to prepare the working solution. At 90 % final volume the pH was adjusted to the required level. The final working solution was then made up to the desired volume. In order to minimize the premature loss of CO_2 the stock solutions were added in the order A, B and C. As the artificial saliva when in contact with air loses some of its CO_2 content; special precautions were adopted to limit this effect namely:

1. Custom-made container caps were used to seal the artificial saliva containers.
2. Artificial saliva was prepared on a daily basis.
3. Stimulated saliva was prepared just ahead of its use.
4. The distilled water used was kept at 4 ˚C.

In this investigation four different recipes were piloted (table 3-8). The selection criteria for the formulation used in the main body of this work was dependent upon the ability of the formulation to enhance the process of remineralisation of the erosion substrates. This was assessed by measuring the hardness percentage gain of already eroded tooth and eggshell specimens.

The chemical constituents and recipes are shown in table 3-8. All shared the same constituents except the compound considered as the “Calcium-ion vehicle”. Several potential “vehicles” were tested namely: $CaCO_3$, CaO, and $CaCl_2.2H_2O$.

One recipe was intentionally deprived of Calcium so as to permit tracing of the Calcium ions released from the demineralisation process of different substrates (recipe 1).

Table 3-8. Artificial saliva recipes. (1) Calcium deprived. (2) Stock solutions A and B are the same. $CaCO_3$ was added to stock solution C. (3) Stock solutions A and B are the same. CaO was added to stock solution C. (4) Stock solutions A and B are the same as above. $CaCl_2.2H_2O$ was added to stock solution C. (5) Stock solutions A, B and C are the same $CaCl_2.2H_2O$ was added to the working solution.

	Concentration	
	g/L	Mol/L
Saliva Recipe 1		
Stock solution A		
NaH_2PO_4 (Sigma-Aldrich Ltd., Gillingham, Dorset, UK)	32.13	0.233
KCl (Sigma-Aldrich Ltd., Gillingham, Dorset, UK)	86.0	1.164
NaCl (Sigma-Aldrich Ltd., Gillingham, Dorset, UK)	7.21	0.123
NH_4Cl (Fisher Scientific UK Ltd., Loughborough, UK)	11.0	0.205
Trisodium citrate di-hydrate (Sigma-Aldrich Ltd., Gillingham, Dorset, UK)	1.1	3.74×10^{-3}
Lactic acid (Acros organics, Fisher Scientific UK Ltd., Loughborough, UK)	2.9 ml	0.039
Stock solution B		
Urea (Sigma-Aldrich Ltd., Gillingham, Dorset, UK)	5.0	0.167
Uric acid (Acros organics, Fisher Scientific UK Ltd., Loughborough, UK)	0.375	4.46×10^{-3}
NaOH (Sigma-Aldrich Ltd., Gillingham, Dorset, UK)	0.1	5.00×10^{-3}
Stock solution C		
KSCN (Fisher Scientific UK Ltd., Loughborough, UK)	12.0	0.123
Saliva Recipe 2		
Stock solution A		
Stock solution B		
Stock solution C		
KSCN (Fisher Scientific UK Ltd., Loughborough, UK)	12.0	0.123
$CaCO_3$ (Fisher Scientific UK Ltd., Loughborough, UK)	35.75	0.714
Saliva Recipe 3		
Stock solution A		
Stock solution B		
Stock solution C		
KSCN (Fisher Scientific UK Ltd., Loughborough, UK)	12.0	0.123
CaO (Fisher Scientific UK Ltd., Loughborough, UK)	20.025	0.4
Saliva Recipe 4		
Stock solution A		
Stock solution B		
Stock solution C		
KSCN (Fisher Scientific UK Ltd., Loughborough, UK)	12.0	0.123
$CaCl_2.2H_2O$ (Sigma-Aldrich Ltd., Gillingham, Dorset, UK)	2.4 g/L for stimulated 2.2 g/L for unstimulated	
Saliva Recipe 5		
Stock solution A		
Stock solution B		
Stock solution C		
KSCN (Fisher Scientific UK Ltd., Loughborough, UK)	12.0	0.123
Working Solution		
$CaCl_2.2H_2O$ (Sigma-Aldrich Ltd., Gillingham, Dorset, UK)	2.4 g/L for stimulated* 2.2 g/L for unstimulated*	

*Final pH 6.85 ± 0.05 for unstimulated artificial saliva and 7.15 ± 0.05 for stimulated artificial saliva.

VI. Preparation of Erosion Substrates

This *in vitro* work sought to assess the erosive effects of consumption of beverages upon erosion substrates of:

1. Human enamel: obtained from caries-free extracted human teeth
2. Ostrich eggshell (Amazon.com, Inc., UK)

A. Human enamel specimen preparation

Caries-free extracted molars were selected from the anonymous Dundee Dental School tooth collection of teeth collected for dental research prior to September 2006 (The Conservation Collection registered with Tayside Tissue Bank), and housed in Dundee Dental School. The roots of each tooth were removed just above the cemento-enamel junction and the remaining tooth portion was embedded in acrylic resin aligning it vertically in 3 cm diameter cylindrical moulds. 1 mm thick sagittal slices were cut in a mesial-distal direction using a slow speed diamond saw running at 450 rpm (Isomet Buehler Ltd, USA) until the first signs of enamel was observed. Then 3 mm thick slices were produced. For experimental diets involving ion loss tracing, enamel-only samples were chosen that were dentin-free. The prepared specimens were kept in tap water until use. Once used the teeth were disposed of anonymously according to the protocols of NHS Tayside.

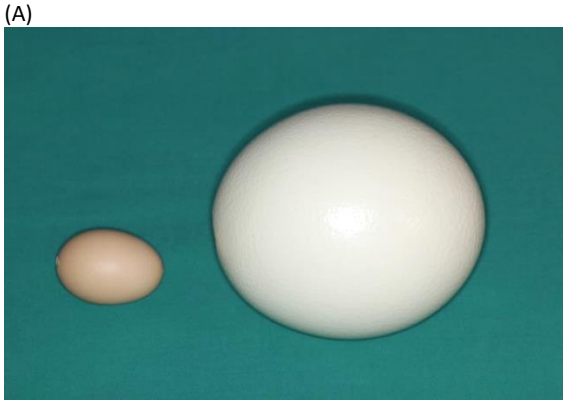
B. Ostrich eggshell specimen preparation

Ostrich eggshell specimens were prepared by cutting a blown Ostrich eggshell into 1 cm^2 sized squares using a dental high-speed handpiece and a diamond bur with continuous water coolant spray. Specimens (2 mm thick) were then bonded to 1 mm thick cylindrical acrylic bases to obtain an overall thickness of 3 mm (figure 3-15).

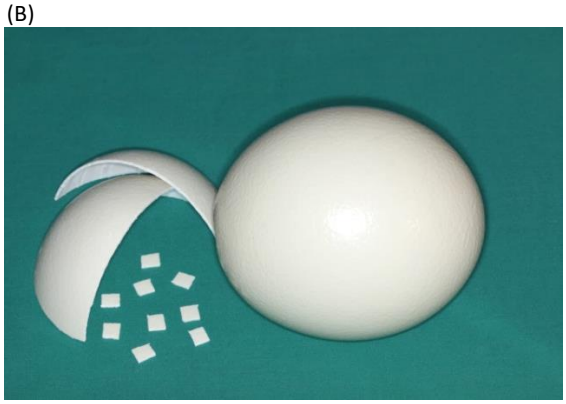
Prior to commencing any testing the surface microhardness and specimen profile were determined.

Figure 3-15. (A) Ostrich egg versus chicken egg for scale appreciation (B) The preparation of Ostrich eggshell specimens (C) The bonding of eggshell specimens to acrylic bases.

(A)



(B)



(C)

VII. Erosion testing regime

Based on previous work where natural human drinking behaviour was observed (Qutieshat et al., 2015); several drinking behaviour values were adopted for the purposes of this experiment namely: test beverage flow rate and quantity; sip volume and temperature; and consumption time period. Moreover, normal physiological stimulated and unstimulated artificial saliva flow rates were adopted (Table 3-9).

Table 3-9. (A) Test beverage kinematic behaviour values, consumption quantity, time period and temperature based upon observing human drinking behaviour in a social environment (Qutieshat et al., 2015). (B) Artificial saliva flow rate values used in this work.

(A)

Flow rate	13.3 ml/min
Sip volume	16.8 ml
Total daily volume	660 ml (full dose) or 330 ml (half dose)
Temperature	14.9°C
Offensive time period	44 minutes (full dose) or 22 min (half dose)

(B)

Fluid variables	Justification	Flow rate
Stimulated Saliva flow rate	(Dawes, 1987)	5.0 ml/min
Unstimulated saliva flow rate (waking hours)	(Thomson et al., 2011; Dorion, 2011)	0.3 ml/min
Unstimulated saliva flow rate (sleeping hours)	(Dorion, 2011)	0.1 ml/min

The enamel or Ostrich eggshell specimens were loaded into Saltus. Based upon the findings of the pizza and soft drink parties of this work, this was programmed to deliver the beverage to the substrate according to the values summarised in table 3-9(b).

Operation of Saltus was continuous and 24/7 consisting of 3 daily periods namely:

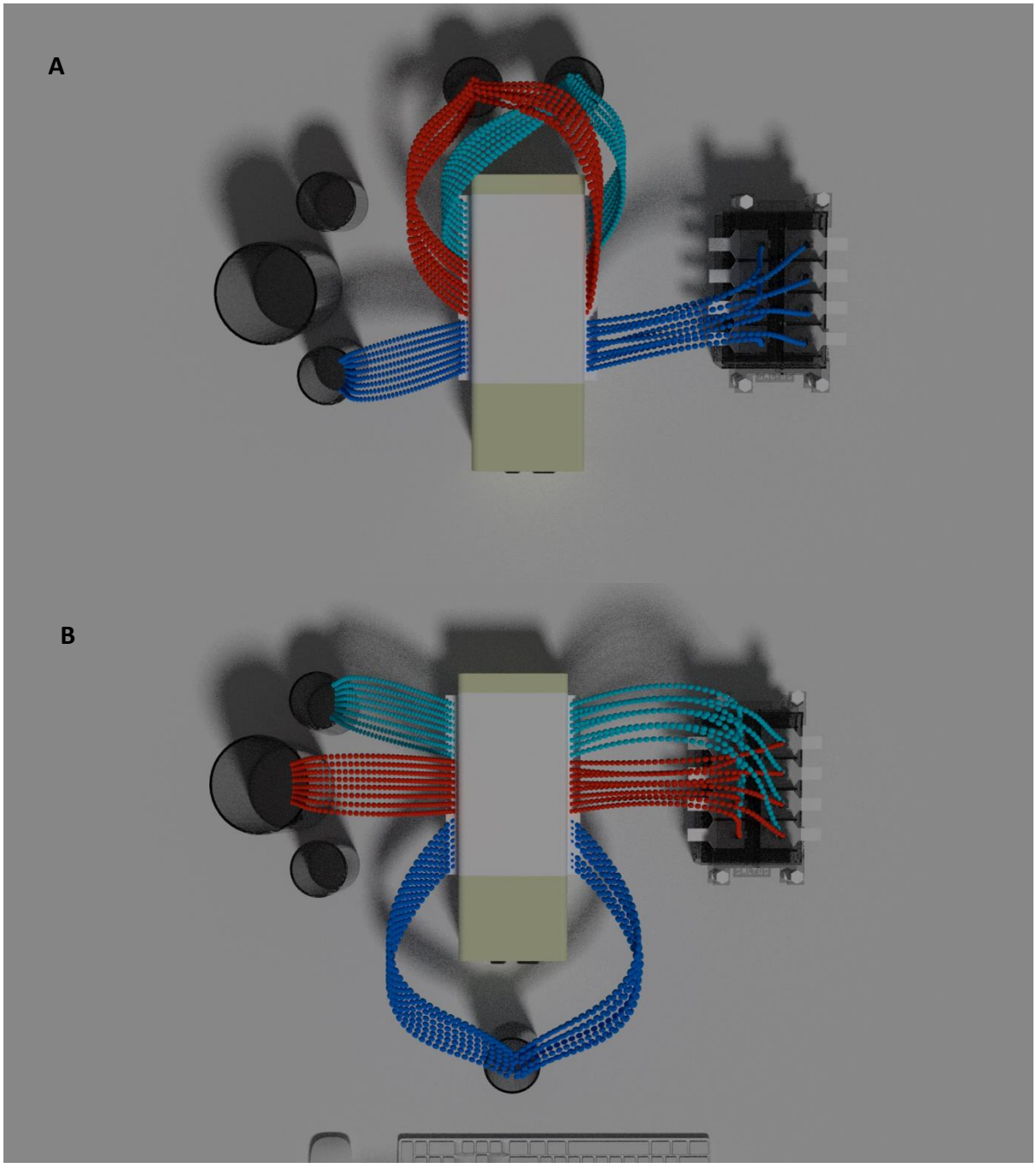
1. Day “waking hours” period.
2. Night “sleeping hours” period.
3. Stimulated period.

During the day and night periods artificial unstimulated saliva circulated through the system at physiological flow rates, while during the stimulated period artificial stimulated saliva flowed at higher rates simultaneously with a test beverage (figure 3-16). In this work this was Coca-Cola regular (pH 2.47 ± 0.02).

Before commencing any testing cycles, a series of mock cycles were performed to fine-tune the parameters that govern the kinematic behaviour of the artificial saliva and the test beverage.

An operation manual that describes the step-by-step instructions for operation is appended (Appendix 5).

Figure 3-16. (A) Tubing setting during the day and night periods. RPM is set to 17.1 for the former and 5.23 for the latter. Unstimulated saliva tubing (Dark Blue) is active while stimulated (Light blue) and test beverage (Red) tubing are set to loop (B) Tubing setting for the stimulated period. RPM is set to 17.1. Stimulated saliva (Light blue) and test beverage (red) tubing are active while unstimulated saliva (Dark blue) tubing is set to loop.



3.3.3 Validation of the Artificial mouth (Saltus)

To validate the model, experimental diets were performed repeatedly using specimens prepared from ostrich eggshells, and the results compared to those obtained from extracted human teeth specimens in the same experimental setup. Each run used 8 samples of erosion substrate.

For these runs the pump drive speed values were calculated to approximate values from their corresponding RPM values according to the formula 2.1 shown above. Thereafter, the exact value was determined by calibration due to the fact that the flow rate and pump speed values provided by the manufacturers are theoretical. Validations of the outcome drive speed values were based upon a pilot study that followed a standardised algorithmic procedure as detailed in Appendix 6.

A Description of the diets that Saltus was programmed to deliver

Based on the observations from the pizza and soft drink parties, a range of exposure conditions and measurement opportunities were developed. These are summarized in the Gantt charts depicted in figure 3-17. These were subdivided into two types; one which looked at the initial surface characteristics of the specimen and following the diet, termed "the immediate effect method" and the other approach where at various stages throughout the diet, substrate surface characteristics were assessed "the accumulative effect method".

I. Definitions

Diet: A program delivered by Saltus that is comprised of a series of cycles through which erosion substrates were exposed to artificial saliva and a test beverage. Diets were of the duration of 5, 7 or 9 days.

Rest Cycle: a single day (24 hrs) of a diet where a test beverage was not introduced. This cycle consists of day 'waking hours' and night 'sleeping hours' periods only (i.e. no stimulated period).

Test Cycle: a single day (24 hrs) of a diet where a test beverage was introduced for either 22 min (1 can) or 44 min (2 cans). This cycle consists of day 'waking hours', night 'sleeping hours', and stimulated periods.

Figures 3-18 and 3-19 illustrate the Rest Cycle and the Test Cycle respectively.

Figure 3-17. In order to replicate the behaviour observed in the pizza and soft drink parties, Saltus was programmed to deliver a variety of diets. These are represented in the following Gantt

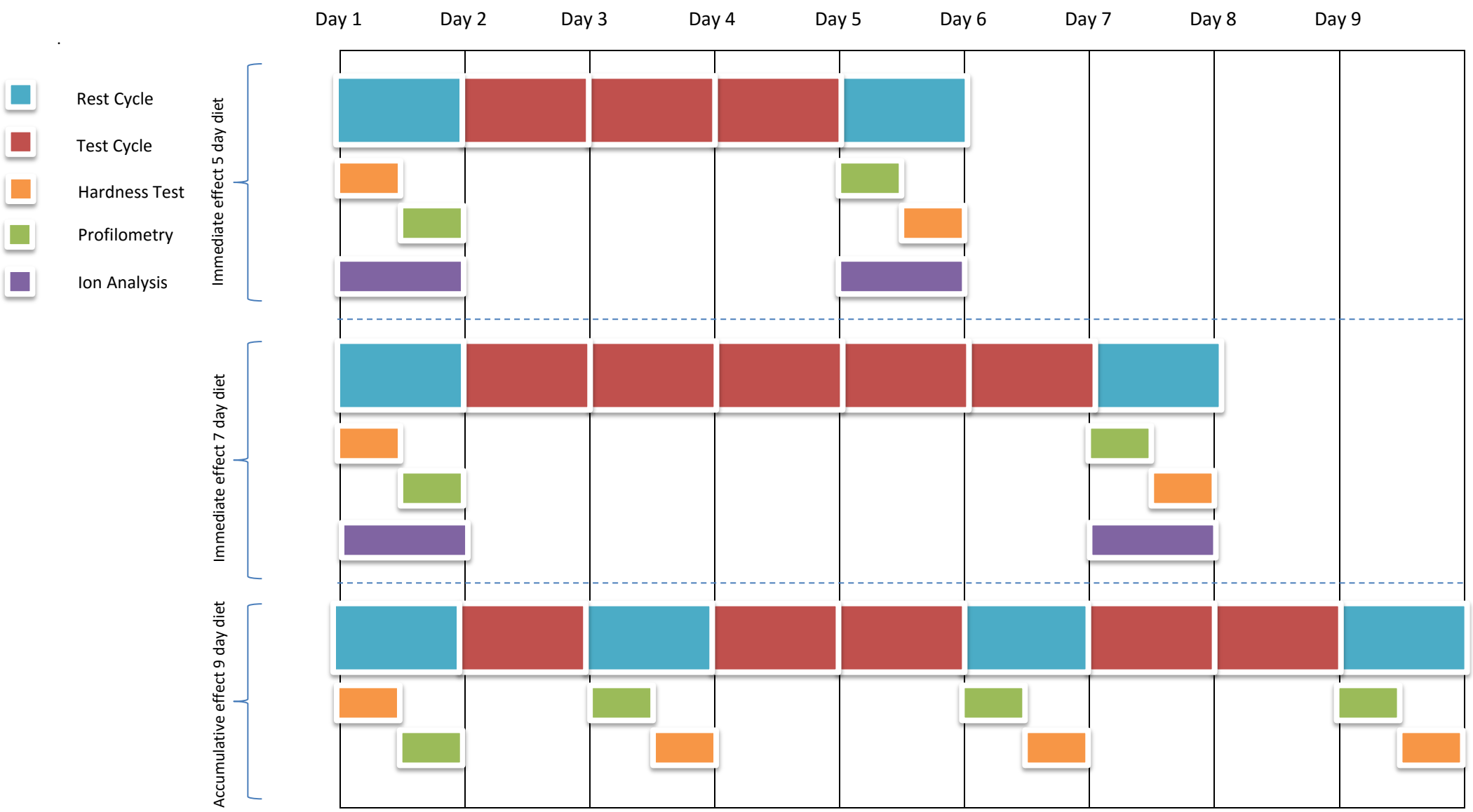


Figure 3-18. A Gantt chart representing the fluid control during a **Rest Cycle** over a time frame of 24 hours.

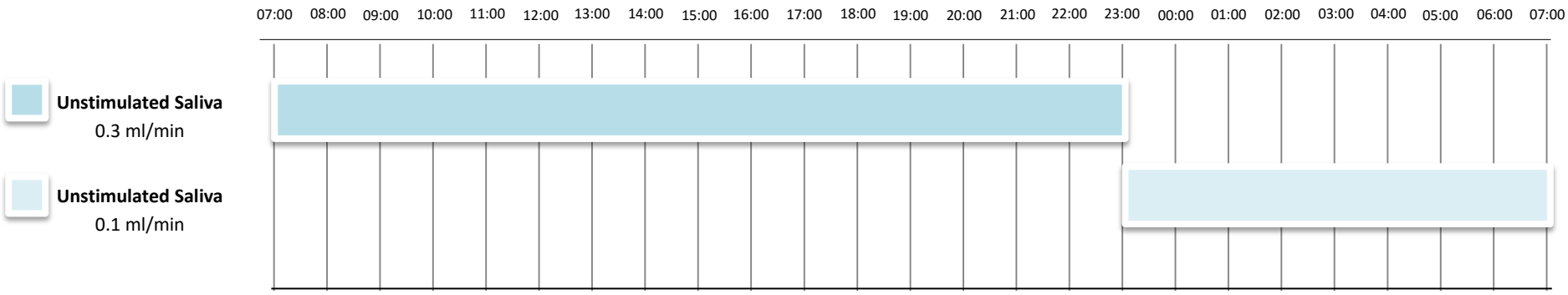
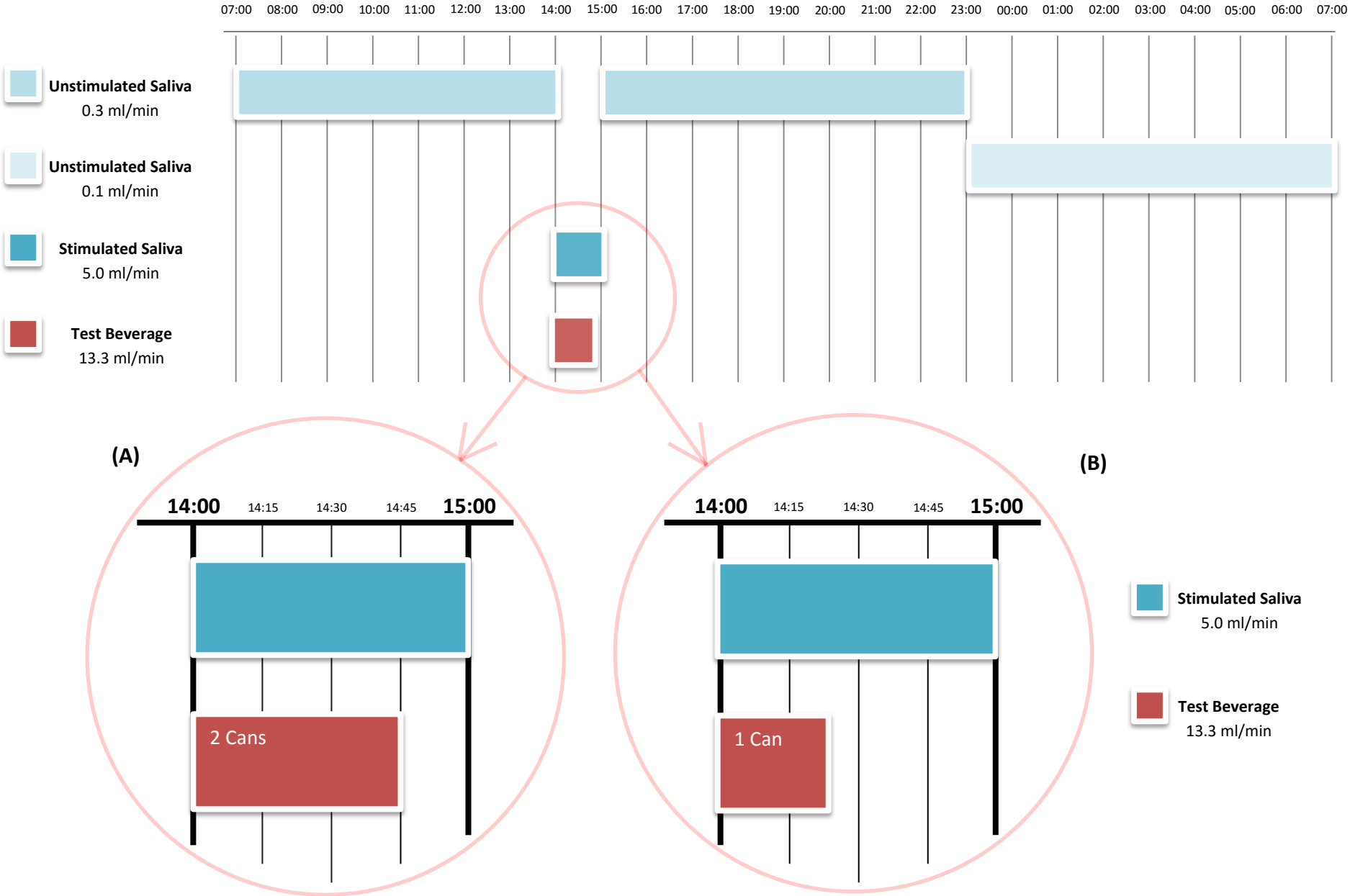


Figure 3-19. A Gantt chart representing the fluid control during a **Test Cycle** over a time frame of 24 hours. (a) The 2 can diet [double dose]. (b) The 1 can diet [single dose].



II. Dietary effects

The effects of various diets were investigated using Saltus. These were used to validate the procedures. Compare and contrast the behaviour of the erosion substrates and to explore the potential of using Saltus to evaluate erosion preventive regimes. Where, for assessment of reproducibility, an experiment is repeated. The repetition of a diet is denoted by placing the suffix R after the diet code. E.g. Diet 1R is a repeat run of diet 1.

The effects of the diets described below were observed upon both human enamel and Ostrich eggshell (8 samples of human enamel and 8 samples of Ostrich eggshell). These comprised:

- Immediate effect method
- Accumulative effect method

For this work the default test beverage was Coca-Cola (Coca-Cola Great Britain, London, UK) unless otherwise stated.

A. Immediate effect method

For this method, Saltus was capable of delivering 4 diets. The default test beverage was Coca-cola (Coca-Cola Great Britain, London, UK) unless otherwise stated.

Scenario 1:

Short-Single dose diet: this diet was executed over a period of 5 days, the 1st and the last days were rest cycles while the 2nd, 3rd and 4th days were test cycles. Test cycles consisted of 1 can (330 ml) of the test beverage administered over the duration of 22 min. Thus in all, 3 cans were consumed per sample in this diet. Specimens were analysed for surface hardness and loss twice; prior to commencement of the diet and after the diet was terminated. This diet was coded 'Diet 1'

Scenario 2:

Long-Single dose diet: this diet was executed over a period of 7 days, the 1st and the last days were rest cycles while the 2nd, 3rd, 4th, 5th and 6th days were test cycles. Test cycles consisted of either 1 can (330 ml) of test beverage administered over the duration of 22 min a day. Thus in all, 5 cans were consumed per sample in this diet. Specimens were analysed for surface hardness and loss twice; prior to commencement of the diet and after the diet was terminated. This diet was coded 'Diet 2'.

Scenario 3:

Short-Double dose diet: this diet was executed over a period of 5 days, the 1st and the last days were rest cycles while the 2nd, 3rd and 4th days were test cycles. Test cycles consisted of 2 cans (660 ml) of the test beverage administered over the duration of 44 min. Thus in all, 6 cans were consumed per sample in this diet. Specimens were analysed for surface hardness and loss twice; prior to commencement of the diet and after the diet was terminated. This diet was coded 'Diet 3'.

Scenario 4:

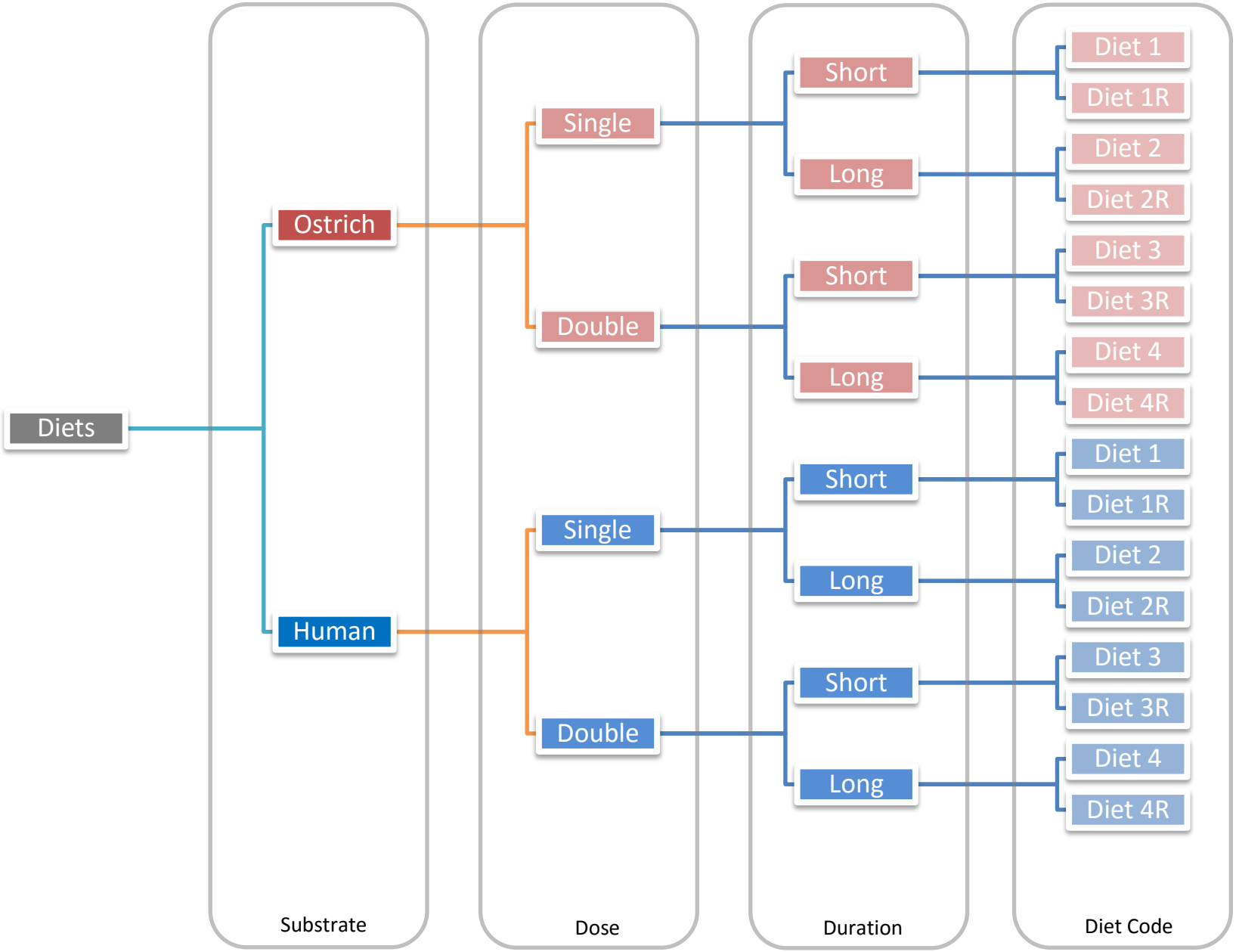
Long-Double dose diet: this diet was executed over a period of 7 days, the 1st and the last days were rest cycles while the 2nd, 3rd, 4th, 5th and 6th days were test cycles. Test cycles consisted of 2 cans (660 ml) of test beverage administered over the duration of 44 min a day. Thus in all, 10 cans were consumed per sample in this diet. Specimens were analysed for surface hardness and loss twice; prior to commencement of the diet and after the diet was terminated. This diet was coded 'Diet 4'.

For these diets, representing the immediate effect method, specimen surface characteristics were also analysed by means of ion loss (see section 3.4 [Methodology: Assessment of dental erosion]).

Table 3-10 summarises all the diets delivered by Saltus.

In addition, these diets were repeated once so that consistency could be checked. The repetitive iterations were coded ‘R’. Figure 3-20 demonstrates this.

Figure 3-20. A flow chart illustrating the experimental diets delivered by Saltus for the immediate effect method



*Where R is a repeat experimental run

Table 3-10. Saltus Diets: (a) Immediate effect method diets. (b) Accumulative effect method diet. (c) Preventive measures diets. (d) Testing a different beverage diet.

(a)

Diet	Beverage	Duration	Rest cycles	Test cycles	Test cycle dose	Total number of cans per specimen	Preventive measure	Code
Short-Single dose	*Coca-Cola*	5 days	2	3	1 can	3 cans	None	**Diet 1**
Long-Single dose	*Coca-Cola*	7 days	2	5	1 can	5 cans	None	**Diet 2**
Short-Double dose	*Coca-Cola*	5 days	2	3	2 can	6 cans	None	**Diet 3**
Long-Double dose	*Coca-Cola*	7 days	2	5	2 can	10 cans	None	**Diet 4**

(b)

Diet	Beverage	Duration	Rest cycles	Test cycles	Test cycle dose	Total number of cans per specimen	Preventive measure	Code
Extended-Single dose	*Coca-Cola*	9 days	4	5	1 can	5 cans	None	**Diet 5**

(c)

Diet	Beverage	Duration	Rest cycles	Test cycles	Test cycle dose	Total number of cans per specimen	Preventive measure	Code
Short-Single dose	*Coca-Cola*	5 days	2	3	1 can	3 cans	Regenerate™	**Diet 6**
Short-Single dose	*Coca-Cola*	5 days	2	3	1 can	3 cans	Fluor Protector™ S	**Diet 7**

(d)

Diet	Beverage	Duration	Rest cycles	Test cycles	Test cycle dose	Total number of cans per specimen	Preventive measure	Code
Short-Single dose	*Sprite*	5 days	2	3	1 can	3 cans	None	**Diet 8**

B. Accumulative effect method

For this method Saltus was set to deliver the following scenario:

Extended-Single dose diet: this diet was executed over a period of 9 days, the 1st, 3rd, 6th and last days were rest cycles while the 2nd, 4th, 5th, 7th and 8th days were test cycles (figure 3-17). Test cycles consisted of 1 can (330) of test beverage administered over the period of 22 min a day. Thus in all, 5 cans were consumed per sample in this diet. This program allowed the specimens to be analysed for surface characteristics (i.e. surface hardness and profilometry) 4 times; prior to commencement of the diet, two times throughout the diet (day 3 and 6) and after the diet was terminated. This method was coded 'Diet 5'.

For this method, specimen surface characteristics were analysed by means of surface hardness and surface profilometry only (see section 3.4 [Methodology: Assessment of dental erosion]).

In addition Saltus was also used to:

(A) Assess the effectiveness of two erosive preventive measures [application of Regenerate™ NR-5 Boosting Serum (Unilever UK Limited, Leatherhead, Surrey, UK) and application of Fluor Protector™ S (Ivoclar Vivadent Limited, Enderby, Leicester, UK)] upon 8 samples of human enamel and 8 samples of Ostrich eggshell.

(B) Evaluate the erosiveness of a different carbonated beverage [Sprite (Coca-Cola Great Britain, London, UK) (pH 2.73 ± 0.02) upon 8 samples of human enamel and 8 samples of Ostrich eggshell.

The specific methodology for these aspects of the work is described in the following subsections:

I. Preventive measure 1: Regenerate™ NR-5 Boosting Serum

For this evaluation, a diet consisting of 3 test cycles was adopted (short-single dose diet). Apart from introducing a preventive measure, the diet was that of 'Diet 1' (see section 4.6.1 [Immediate effect method: Scenario 1]). This diet was coded 'Diet 6'.

Regenerate™ Boosting Serum (Unilever UK Limited, Leatherhead, Surrey, UK) was applied according to the manufacturer's instructions to each of the 8 human enamel and Ostrich eggshell samples used in this work: A thin layer of the NR-5 Serum was applied in a custom-made specimen tray to the specimen surface. A layer of Activator Gel was then added in to the tray on top of the NR-5 Serum. The two ingredients were accurately mixed using the mixing stick provided with the kit before the trays were placed on top of each specimen for 3 minutes. Thereafter, the specimens were rinsed with distilled water using a syringe to remove product residues. This application was repeated daily for 3 days as recommended by the manufacturer. As a result, serum applications preceded test days and were introduced just before the 'sleeping hours' period of that day.

Over the experimental period of 5 days, the test cycles started on day 2 and were terminated on day 4 thus allowing 1 challenge-free day at the start and at the end of each diet. Test cycles consisted of the application of 1 can (330 ml) of the test beverage consumed over 22 min. In all 3 cans were consumed per specimen in this work. Specimens were analysed for surface hardness and loss twice; prior to commencement of the diet and after the diet was terminated (see section 3.4

[Methodology: Assessment of dental erosion]). The results from Diet 1 and Diet 1R served as controls.

II. Preventive measure 2: Fluor Protector™ S

A Diet consisting of 3 test cycles was adopted (short duration diet and single dose beverage). Apart from introducing a preventive measure, the diet was that of 'Diet 1'. (see section 4.6.1 [Immediate effect method: Scenario 1]). This diet was coded 'Diet 7'.

Fluor Protector™ S (Ivoclar Vivadent Limited, Enderby, Leicester, UK) was applied according to the manufacturer's instructions to each of the 8 human enamel and Ostrich eggshell samples used in this work. The varnish was directly applied in thin layers onto the surfaces of specimens using the Vivabrush G (Ivoclar Vivadent Limited, Enderby, Leicester, UK) provided with the kit. The varnish was allowed to dry for 1 minute before placing the specimens in a 37 ˚C incubator for 1 hour.

Over the period of 5 days, the test cycles started on day 2 and were terminated on day 4 thus allowing 1 challenge-free day at the start and at the end of each diet. Test cycles consisted of the application of 1 can (330 ml) of test beverage consumed over 22 min. In all 3 cans were consumed per specimen in this work. Specimens were analysed for surface hardness and loss twice; prior to commencement of the diet and after the diet was terminated (see section 3.4 [Methodology: Assessment of dental erosion]). The results from Diet 1 and Diet 1R served as controls.

III. Testing a different beverage

A Diet consisting of 3 test cycles was adopted (short duration diet and single dose beverage). For this diet, Sprite (Coca-Cola Great Britain, London, UK) was used as the test beverage. All other variables were those of 'Diet 1'. (see section 4.6.1 [Immediate effect method: Scenario 1]). This diet was coded 'Diet 8'.

Over the period of 5 days, the test cycles started on day 2 and were terminated on day 4 allowing 1 challenge-free day at the start and at the end of each diet. Test cycles consisted of the application of 1 can (330 ml) of test beverage consumed over 22 min. In all 3 cans were consumed per specimen in this work. Specimens were analysed for surface hardness and loss twice; prior to commencement of the diet and after the diet was terminated (see section 3.4 [Methodology: Assessment of dental erosion]). Diet 1 and Diet 1R served as the control.

The codes (Diet #) will be used in the remainder of this work to conveniently illustrate the experimental diet used.

3.4 Assessment of dental erosion

The design and build of the model allowed the samples preloaded on the system to be tested for surface characteristics (i.e. surface hardness and surface profilometry) before and after exposure to determine the extent of erosion if any. The model also allowed the resultant solution to be analysed for traces of calcium and phosphate ions to give an assessment of relative mineral loss.

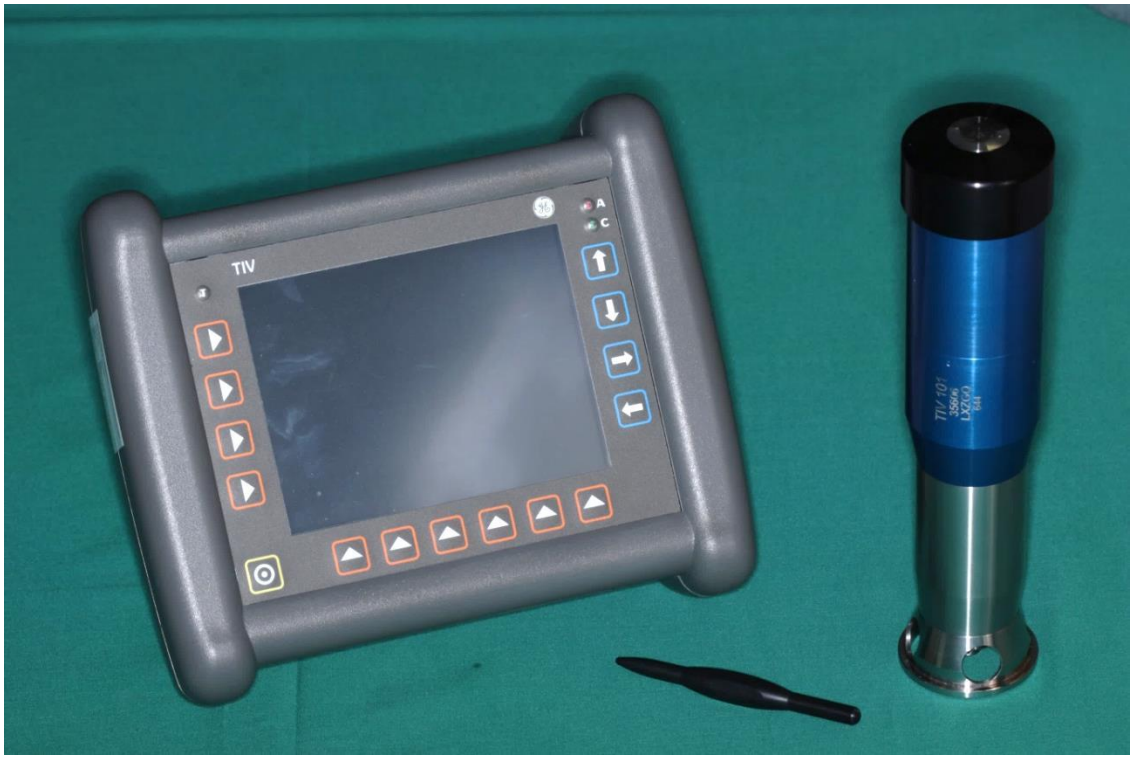
3.4.1 Surface Hardness

Specimen surface hardness was measured quantitatively and qualitatively using the TIV (Through Indenter Viewing) hardness tester (GE Measurement & Control, Groby, UK)(figure 3-21). With the TIV method, the test load is applied manually via a spring. A Vickers-diamond is used as an indenter, and the hardness is measured under load. The CCD camera integrated into the probe uses special optics to generate high quality images of the Vickers diamond penetrating into the surface. The camera can be also used to view through the diamond during the indentation process as it happens (Through-Indenter Viewing). These qualities can be used as an adjunctive qualitative tool in the evaluation of specimen surfaces.

For each test the following parameters were used:

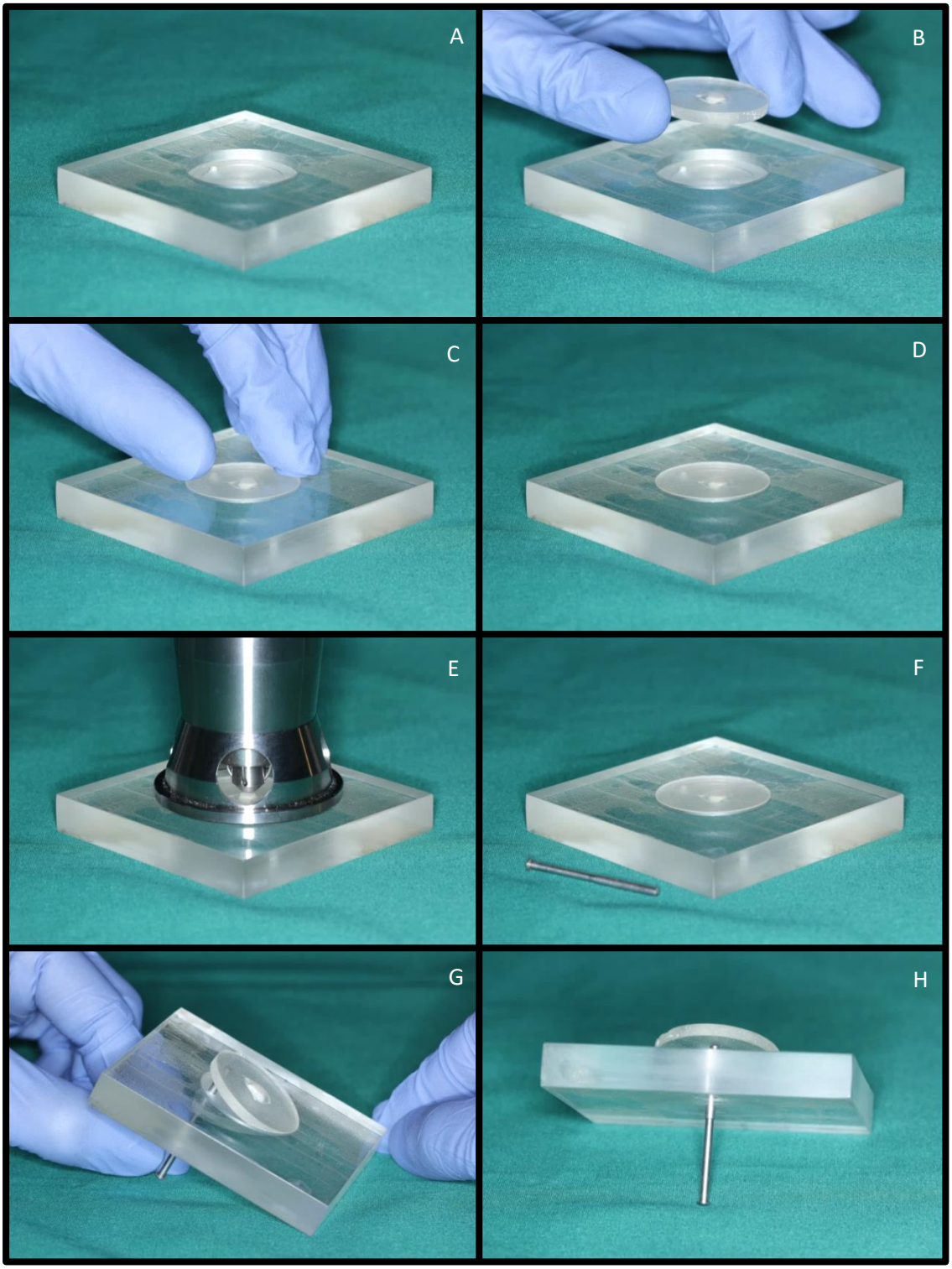
- Conversion: DIN 50150
- Dwell time: 0
- Diagonal ratio 5%
- Probe type: 101
- Load: 9.8 N

Figure 3-21. The TIV hardness tester.



Reproducible orientation of specimens was achieved by fabricating a custom made jig into which the specimen disk could be firmly placed. This was designed using a computer-aided design and drafting software, AutoCAD 2012 (Autodesk Inc., San Rafael, California, US) and prepared via the ILS-III NM Laser cutting system (Laser Tools and Technics Corp, Hsin Chu City, Taiwan). This jig base was 5 mm thick and it had a 3 mm deep circular slot where the specimen disk could be easily and snugly placed. Once tested, specimen disks where dislodged using a metallic rod that could be inserted through a hole from underneath the base pushing the disk out of its slot. Thereafter, specimen disks were kept for further analysis. Figure 3-22 summarises the operation of this jig.

Figure 3-22. A summary of how to operate the custom-made jig used in hardness testing. (a-d) placement of the specimen. (e) Hardness testing. (f-h) removal of the specimen.



Prior to each diet, 10 baseline surface hardness values along with their corresponding images were obtained from all specimens to serve as reference points against which loss of surface hardness percentage was calculated.

The relative hardness percentage was calculated for each specimen using the formula:

$$Percentage\ of\ relative\ hardness = \frac{\text{Hardness value}}{\text{Mean initial hardness}}\ \text{X}\ 100$$

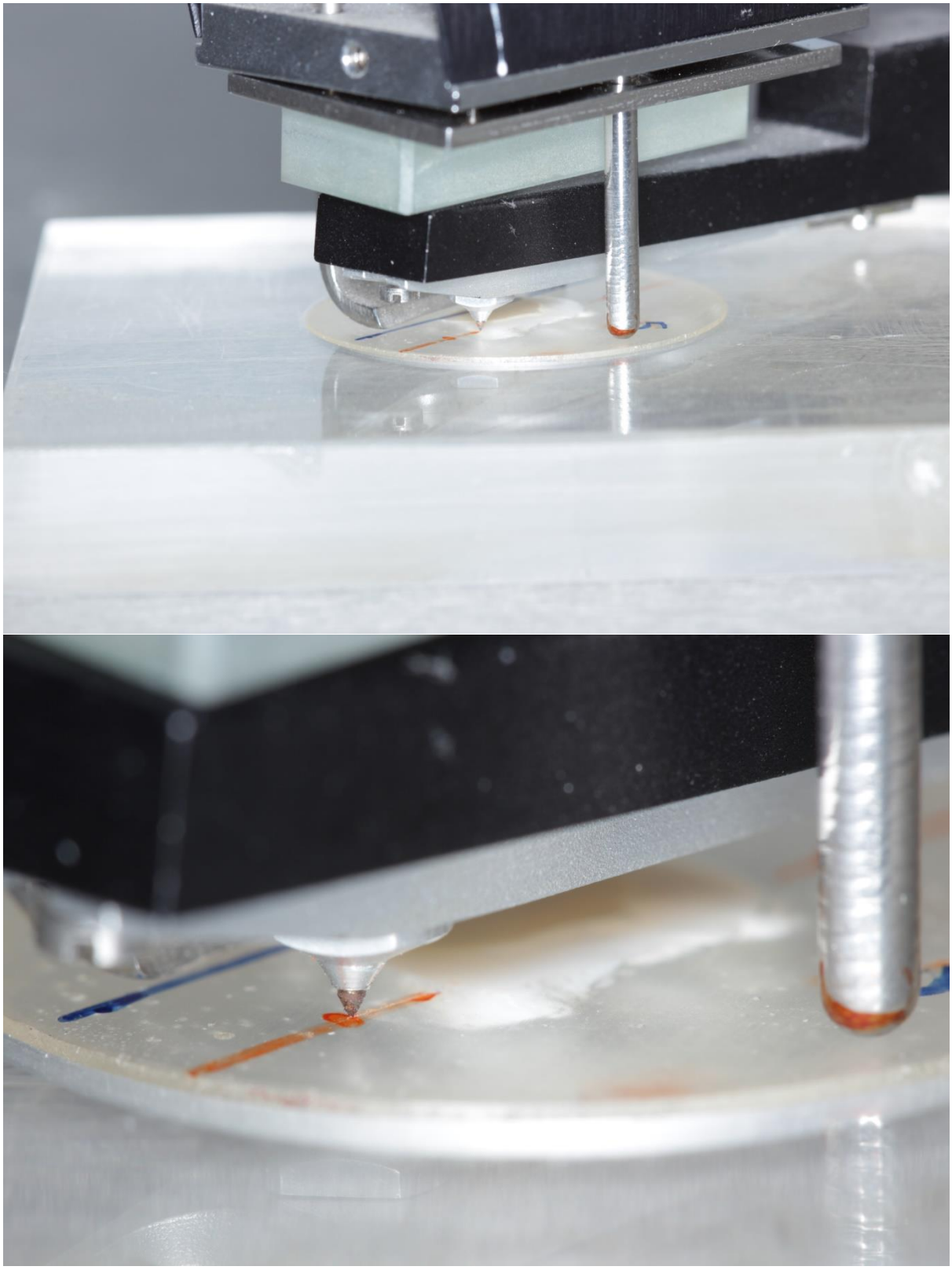
3.4.2 Profilometry

Specimen surface loss was quantitatively measured using a profilometer (Planer SF220 Surface Profiler, Planer Products Ltd., Sunbury-on-Thames, UK)(figure 3-23) with a diamond stylus moving in a straight line across the specimen surface along with the reference and guidance lines that are marked on the acrylic resin surface.

An acid resistant permanent marker pen (Staedtler UK Ltd., Bridgend, UK) was used to draw guidelines and reference points on specimen surfaces using a template to facilitate their accurate placement when performing profilometry measurements.

Prior to the commencement of the experimental diet, all specimens were subjected to 3 baseline surface profiles to serve as reference points against which any surface loss was calculated. After the experimental diet, another 3 surface profiles were performed, and the mean value of surface loss depth relative to the baseline readings was calculated.

Figure 3-23. Planer SF220 diamond stylus moving in a straight line across the specimen surface along with the reference guidance lines.



3.4.3 Calcium and phosphate Ion loss

The saliva/beverage mixture solution was analysed for traces of calcium and phosphate ions using an automated chemistry analyser, the ADVIA® 2400 Clinical Chemistry System (Siemens Healthcare, Camberely, UK)

For calcium determination

- Method principle: CPC (o-cresolphthalein complexone)
- Specimen type: Human serum, plasma and urine
- Reaction type: Endpoint (EPA)
- Measurement wavelength: 545/658 nm
- Standardisation: NIST atomic absorption reference method
- Analytical range: 0.25-3.75 mmol/L
- Reagent code: 74712
- Calibrator: Siemens Chemistry Calibrator REF 09784036

The Calcium Concentrated Reagent (CA_c) method is based on the work of Gitelman (o-cresolphthalein complexone without deproteinization) (Gitelman, 1967). Calcium ions form a violet complex with o-cresolphthalein complexone in an alkaline medium. The reaction is measured at a wavelength of 545/658 nm.

The equation of the reaction is

$$CPC + 2Ca^{2+} \rightarrow CPC(Ca^{2+})_2 Complex$$

For inorganic phosphate (Pi) determination

- Method principle: Phosphomolybdate/UV
- Specimen type: Human serum, plasma and urine
- Reaction type: Endpoint (EPA)
- Measurement wavelength: 340/658 nm
- Standardisation: Ammonium molybdate reference method
- Analytical range: 0-6.46 mmol/L
- Reagent code: 74060
- Calibrator: Siemens Chemistry Calibrator REF 09784096

The ADVIA Chemistry Inorganic Phosphorus (IP) method is based on the Daly and Ertinghausen procedure (Daly and Ertingshausen, 1972), which relies on the formation of a UV absorbing complex between phosphorus and molybdate. Inorganic phosphorus reacts with ammonium molybdate in the presence of sulfuric acid to form an unreduced phosphomolybdate complex, which is measured as an endpoint reaction at λ of 340/658 nm.

The equation of the reaction is

$$Phosphate + Molybdate \xrightarrow{H^+} Phosphomolybdate\ complex$$

Prior to testing experimental diets, three erosion substrate-free diets were circulated through Saltus and the resultant saliva/beverage mixture solution was collected and analysed to serve as a baseline upon which ion concentrations was calculated when performing full diets.

To carry out daily resultant saliva/beverage mixture solution analyses, 10 ml of this solution was collected in three 10 ml universal vials each and stored at 4°C until the complete experimental diet was finished.

After the experimental diet, 3 vials were chemically analysed, and the mean value of ion loss was calculated. Ion concentrations already present in the artificial saliva/test beverage solution were subtracted.

CHAPTER 4

4. Results

These are presented in the same order as the methods that were used to acquire them. Throughout conventional statistical significance is used $P < 0.05$. The following statistical packages were used: GraphPad Prism (Version 6, GraphPad Software Inc., San Diego, USA), IBM SPSS Statistics (Version 22, International Business Machines Corp, Armonk, NY, USA), and MedCalc (Version 13, MedCalc Software, Mariakerke, Belgium). Parametric methods were used after carrying out D'Agostino and Pearson omnibus normality test demonstrating that the data was distributed normally. Where this is not the case, all the data was categorical then a different method was used as stated in the text.

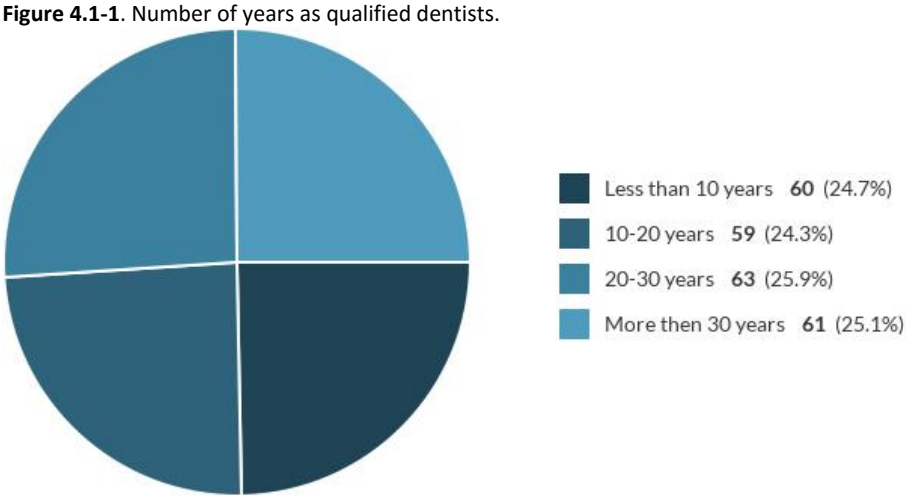
4.1 The Human Tissue Act Questionnaire

The questionnaire results are presented in figures 4.1-1 – 4.1-13. Their order matches approximately the corresponding questions in the questionnaire. For clarity however, the results of some questions are grouped and interpreted together where their theme is common.

In response to the invitation to participate in the questionnaire a total of 254 responses (225 mail and 29 online) were received. This figure represents an overall percentage return rate of 50.8%. It is clear that mail (88.5 %) was the preferred method of response.

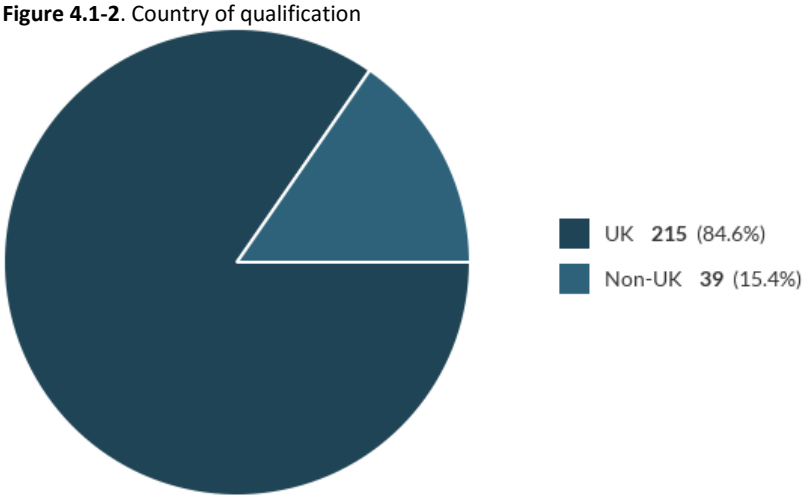
The mean length of time respondents had been practicing dentistry was 21.2 years. Figure 4.1-1 illustrates the number of years the respondents had been practicing dentistry as qualified dentists.

Figure 4.1-1. Number of years as qualified dentists.



Most of the respondents (84.6%) had qualified as dentists in the UK while 15.4% qualified in other countries (figure 4.1-2).

Figure 4.1-2. Country of qualification



39.4% of respondents indicated that they collected extracted teeth in their practices (figure 4.1-3). 33.2% of respondents also indicated that they are currently storing extracted teeth in their practice (figure 4.1-4a). Among the dentists that stored the extracted teeth in their practice, 57.1% indicated that they have in storage less than 20 teeth while 42.9% have in excess of 20 teeth (figure 4.1-4b).

Figure 4.1-3. Collection of extracted teeth

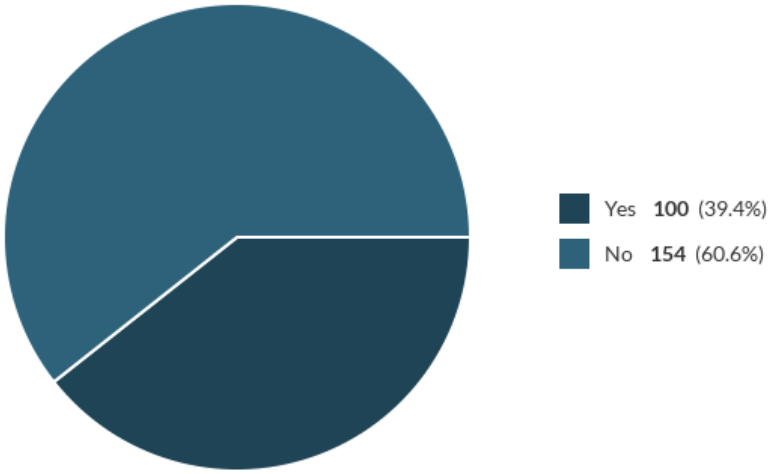
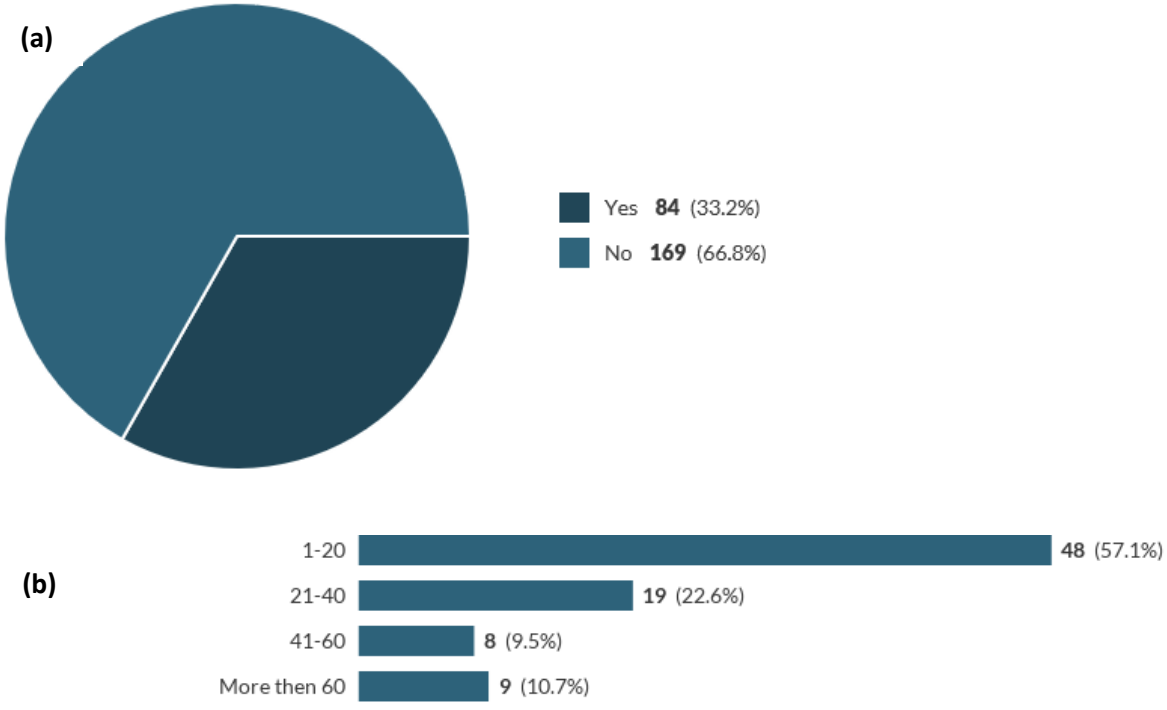
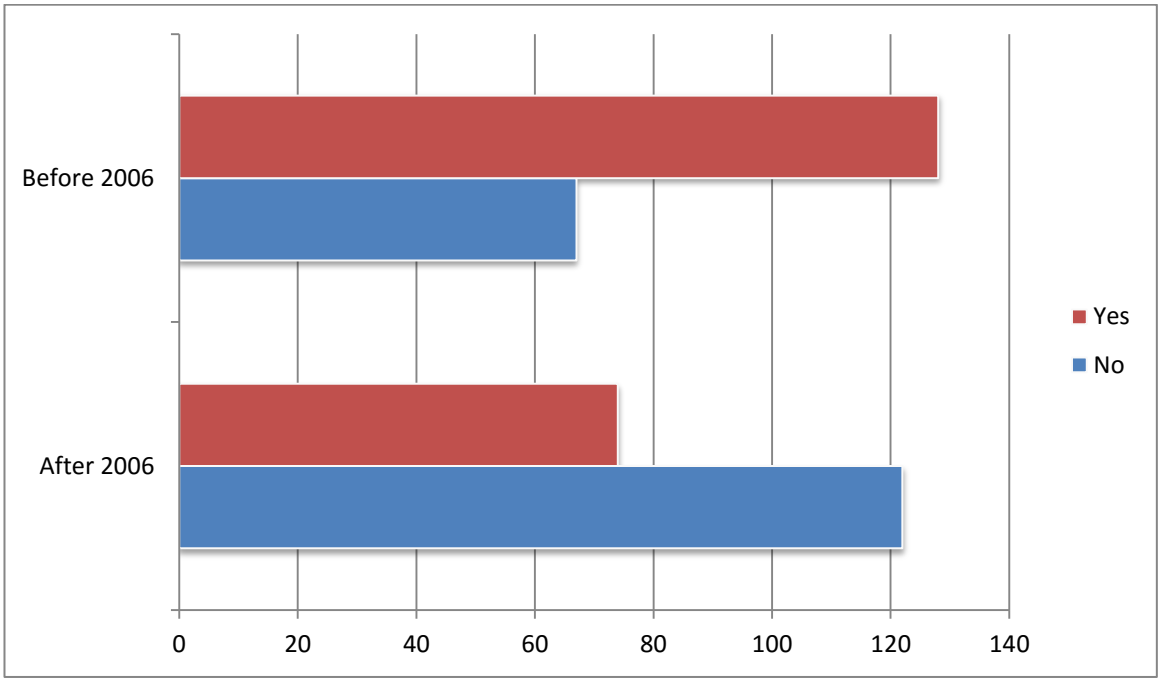


Figure 4.1-4. (a) Current storage of extracted teeth. (b) Number of extracted teeth stored.



Prior to 2006, about two thirds of dentists had been collecting extracted teeth in their practice. However, this figure had considerably reduced to about one third after 2006 (figure 4.1-5). Statistical analysis (Fisher's exact test) showed extremely significant differences between the two groups ($p<0.0001$) (Table 4.1-1a) and this was also borne out by a Chi-square test as well ($P<0.0001$) (Table 4.1-1b).

Figure 4.1-5. Collection of extracted teeth before and after the year 2006 (by number of respondents).



*Dentists who got qualified after 2006 were filtered.

Table 4.1-1 (A). a Fisher's exact test of tooth collection before and after 2006

	Yes	No	Total
Before 2006	128 (65.6%)	67 (34.4%)	195
After 2006	74 (37.8%)	122 (62.2%)	196
Total	202	189	391
The two-tailed P value equals 0.0001 The association between time period and tooth collection is considered to be extremely statistically significant.			

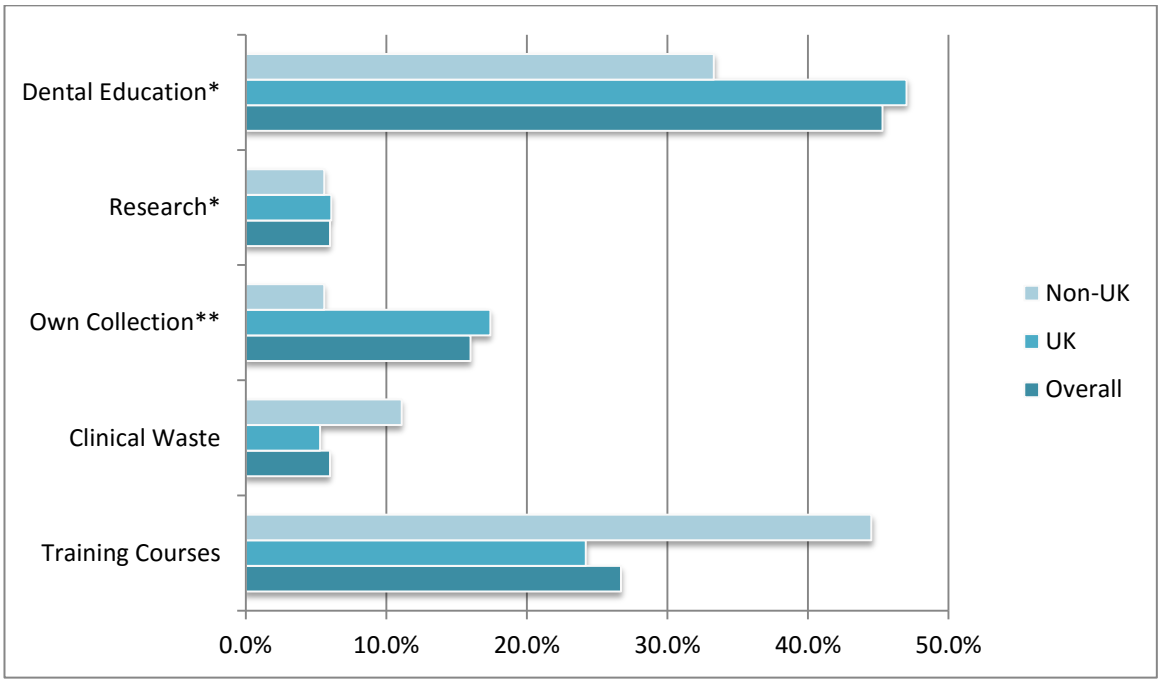
Table 4.1-1 (B). Chi-square with Yates correction

	Yes	No	Total
Before 2006	128 (65.6%)	67 (34.4%)	195
After 2006	74 (37.8%)	122 (62.2%)	196
Total	202	189	391
Chi squared equals 29.332 with 1 degrees of freedom The two-tailed P value equals 0.0001 The association between time period and tooth collection is considered to be extremely statistically significant.			

When the respondents were asked to state the reasons for collecting extracted teeth in their practice, collecting teeth for dental education on behalf of students or dental institutions was foremost, followed by collecting teeth for self-training and dental courses (figure 4.1-6). However, dentists who did not qualify in the UK were keener on

collecting teeth for training purposes compared to their peers who qualified in the UK. Collecting teeth for research on behalf of dental institutions on the other hand came at the bottom with only 6% of respondents claiming to do so (figure 4.1-6).

Figure 4.1-6. Reasons for collecting extracted teeth

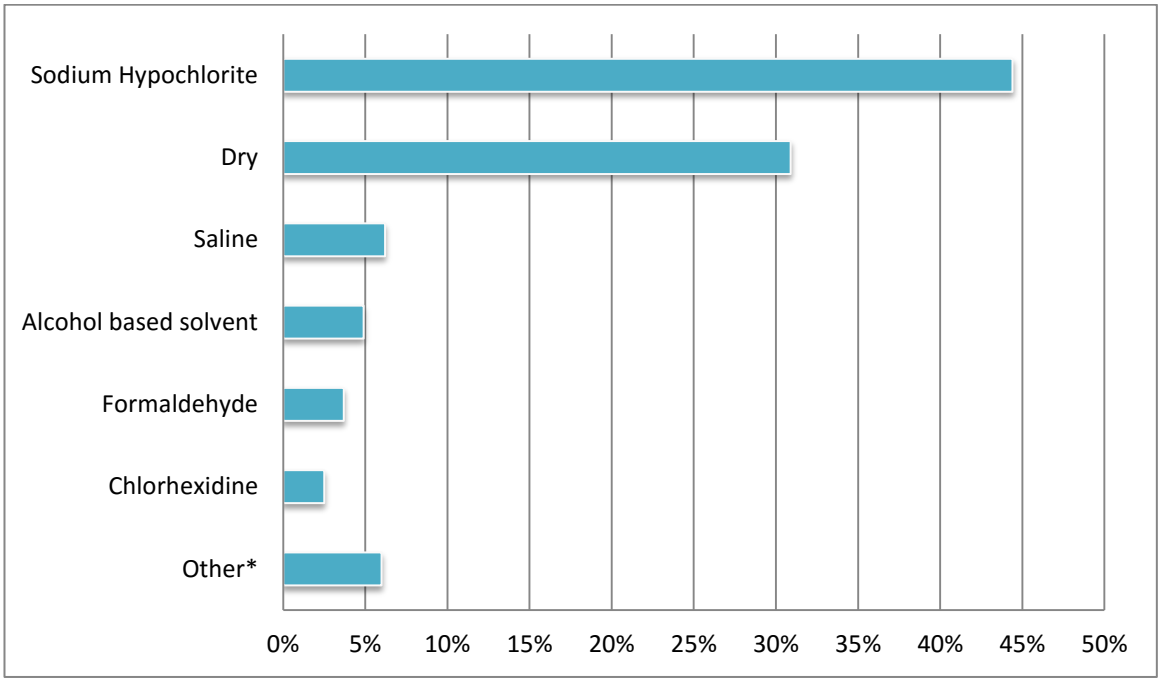


*On behalf of students and/or dental institutions.
**Difficult extractions, rare cases, anomalies etc...

However, the differences seen between UK versus non-UK qualified dentists were found to be of no statistical significance (P>0.05).

Sodium hypochlorite was the most popular storage medium of extracted teeth among dentists who collect extracted teeth in their practice, followed by dry storage (30.9%) (Figure 4.1-7).

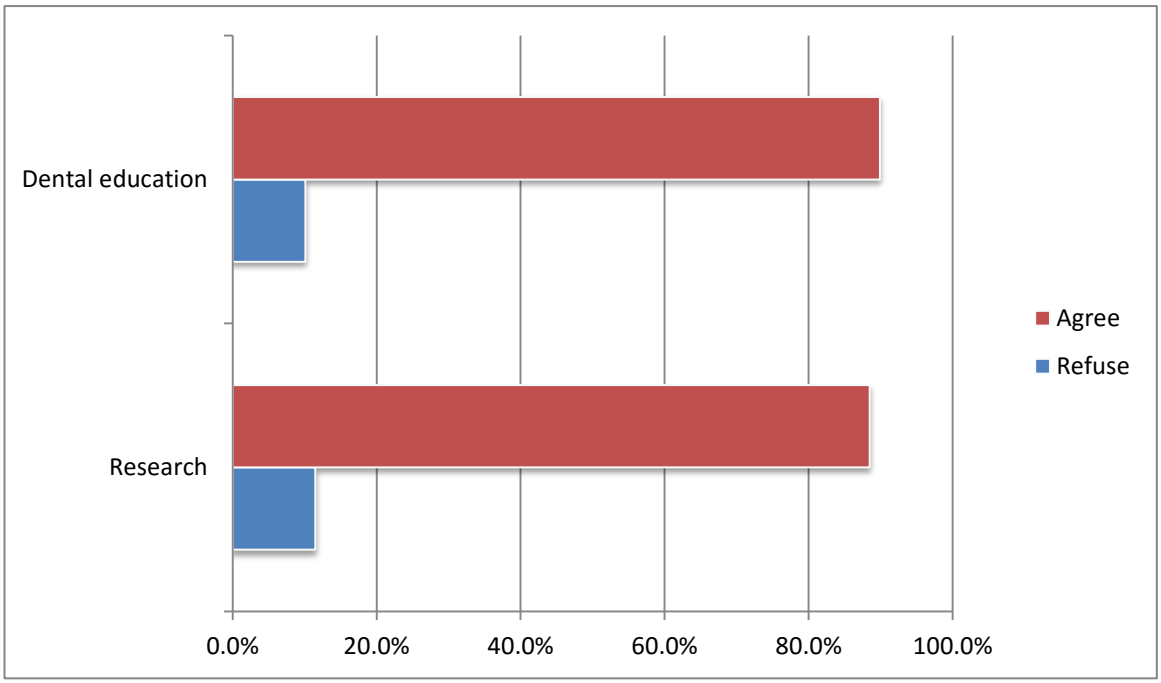
Figure 4.1-7. Extracted teeth storage medium



*Other media included hydrogen peroxide, thymol, ozonated water, tap water, sulphur granules and mercury.

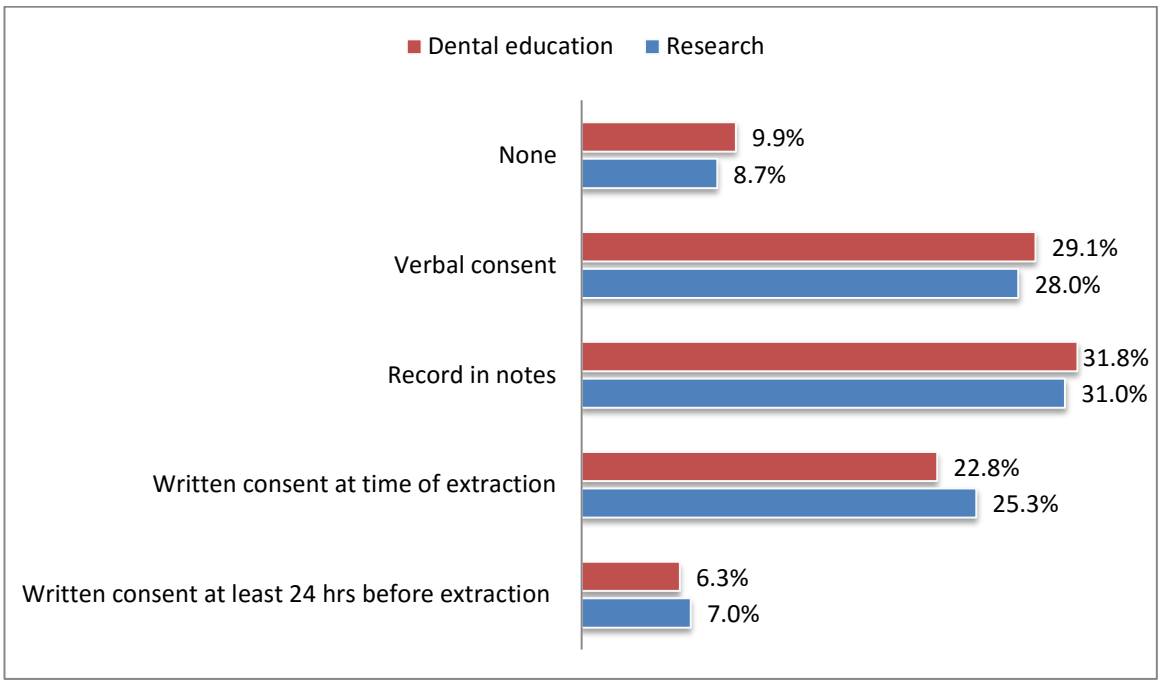
The majority of respondents would agree to collect teeth for dental education and research if approached by an institution (figure 4.1-8). However, only the dentists qualified before the year 2006 would refuse to do this (12.1 % for dental education and 13.4 % for research purposes).

Figure 4.1-8. The attitude towards collecting teeth on behalf of institutions for the purposes of dental education and research



The type of consent dentists believed was required for tooth collection for the purposes of dental education and research was most commonly “record in notes” followed by verbal and written consents (figure 4.1-9). However, among the respondents who believed that the dentist owned the freshly extracted teeth (13.4 %) immediately after their extraction (figure 4.1-12), “None” was the most chosen answer as the type of consent required for tooth collection.

Figure 4.1-9. The type of consent required for tooth collection for the purposes of dental education and research.



The majority of respondents (79.1%) had found practicing upon extracted human teeth very helpful during their undergraduate training (figure 4.1-10). More than half of the respondents had attempted to collect teeth during their dental undergraduate study (figure 4.1-11a). Among those, 59.6% found the people they approached on seeking to collect teeth helpful (figure 4.1-11b).

Figure 4.1-10. Attitude towards practicing clinical skills upon extracted human teeth

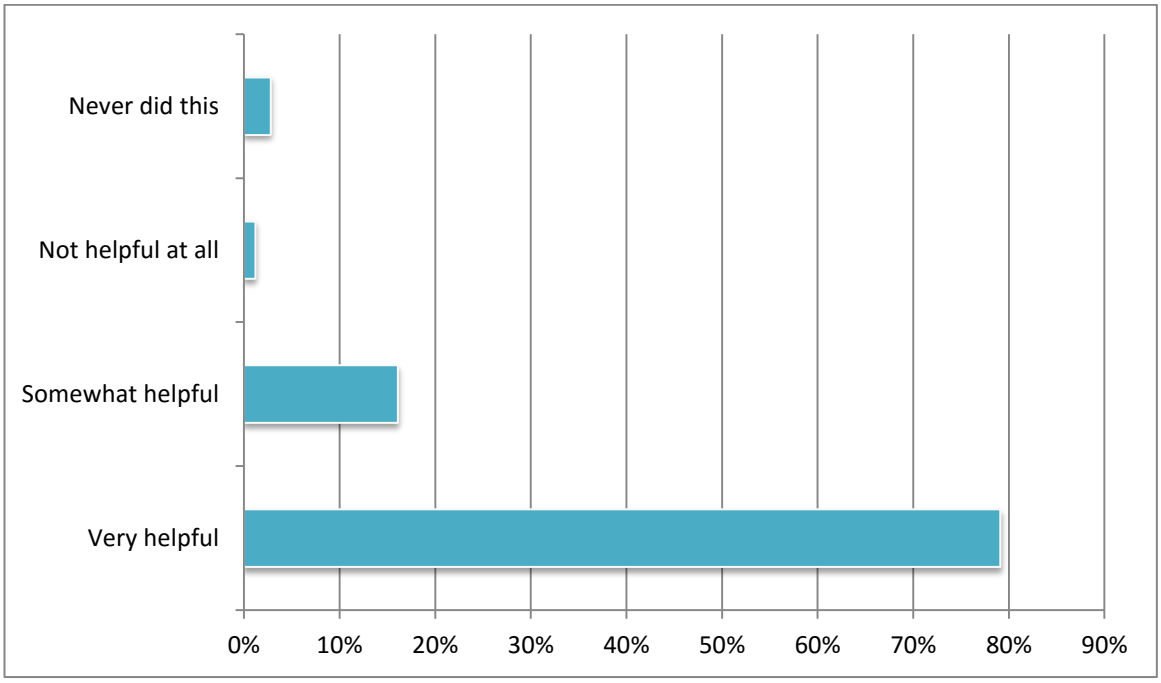
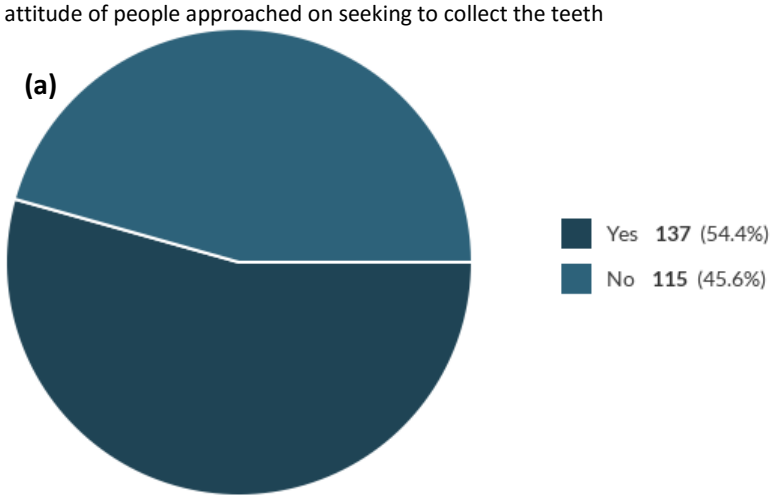
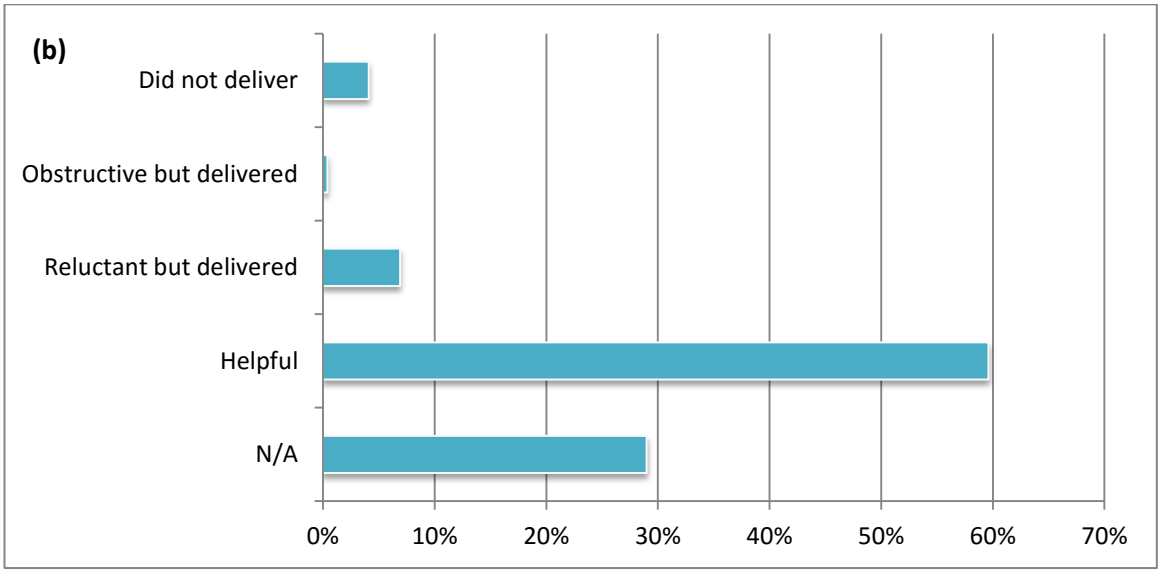


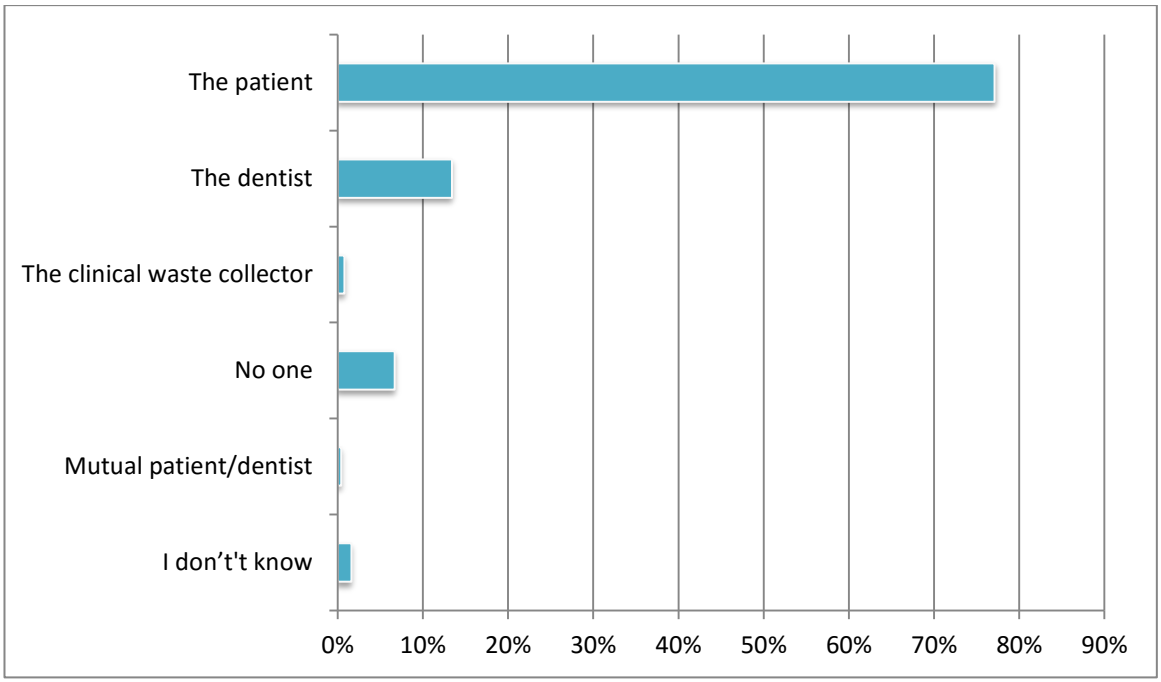
Figure 4.1-11. (a) Collection attempts of extracted teeth during dental undergraduate study. (b) The reported attitude of people approached on seeking to collect the teeth





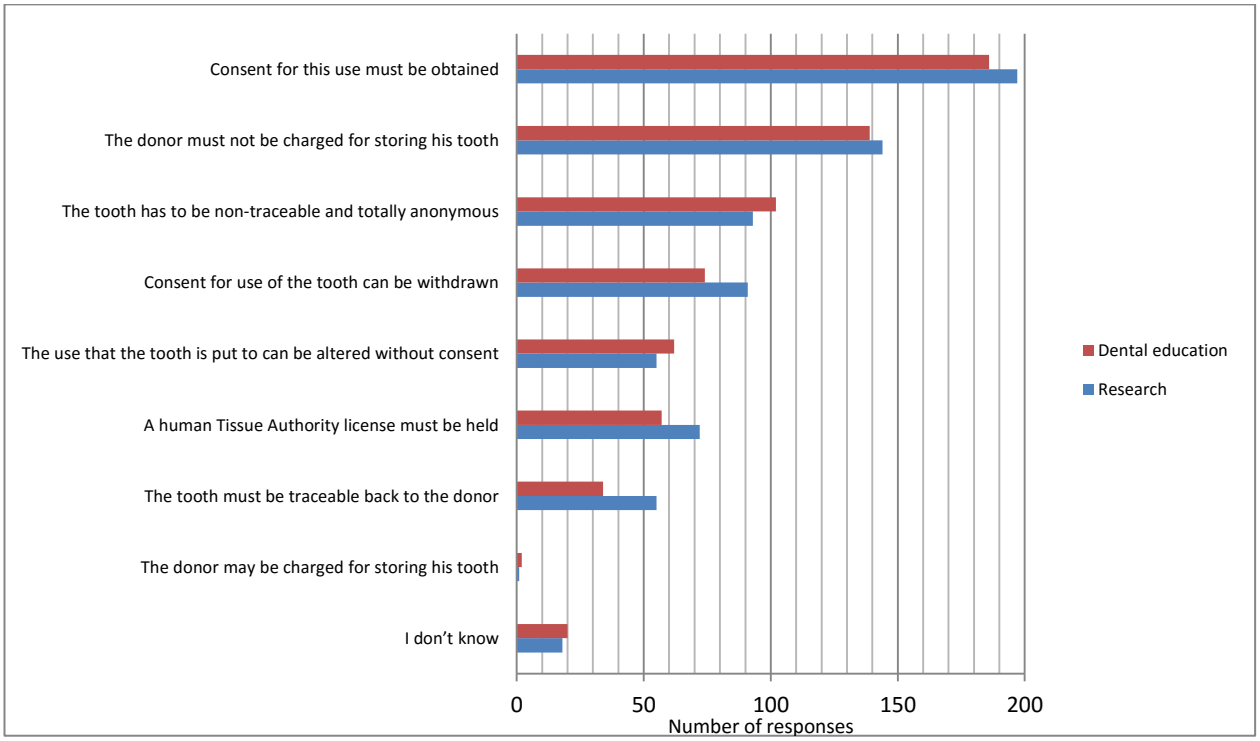
Most of the respondents (77.1%) believed that the patient owned the freshly extracted teeth immediately after extraction, while only 13.4% believe the ownership rested with the dentist and/or the dental clinic or institution (figure 4.1-12). The correct position on this is the freshly extracted tooth is the property of the patient unless consent is given for retention by the dentist.

Figure 4.1-12. Freshly extracted teeth ownership



Respondents were provided with a list of statements relating to possible conditions for the collection of teeth for the purposes of dental education and dental research and were asked to tick all sentences they agreed with. The top three agreed statements were: "consent must be obtained", "the donor must not be charged for storing his tooth" and "the tooth has to be non-traceable and totally anonymous (figure 4.1-13).

Figure 4.1-13. Statements that dentists believe are true regarding the collection of teeth for the purposes of dental education and research (by number of respondents).



In some cases these were at odds with legal requirements. For example a Human Tissue Authority license is not required where teeth are collected for the purposes of dental education.

From the foregoing results it is clear that;

- There is a misunderstanding about the Human Tissue Act among dentists.
- A considerable number of dentists have ceased to collect extracted teeth after the year 2006.
- Dentists in the UK nowadays tend to be too cautious with regard to the collection of teeth regardless of the reason.

4.2 Pizza and soft drink party

4.2.1 Pre-experimental Questionnaire

Prior to the pizza and soft drink party a survey of the participants was conducted to ascertain their favourite drinks and if they had any allergies. It also built up a picture of the drinking habits of the participants.

For intra-respondent reliability a convenience sample (i.e. University of Dundee Dental postgraduate students) (n=6) was given the questionnaire again within 2 weeks. The reliability of these responses was assessed by calculating the Kappa statistic.

Statistical analysis (weighted Kappa) showed almost perfect agreement (K =0.961) for the participants who filled out the questionnaire on two occasions. Table (4.2-1) illustrates this.

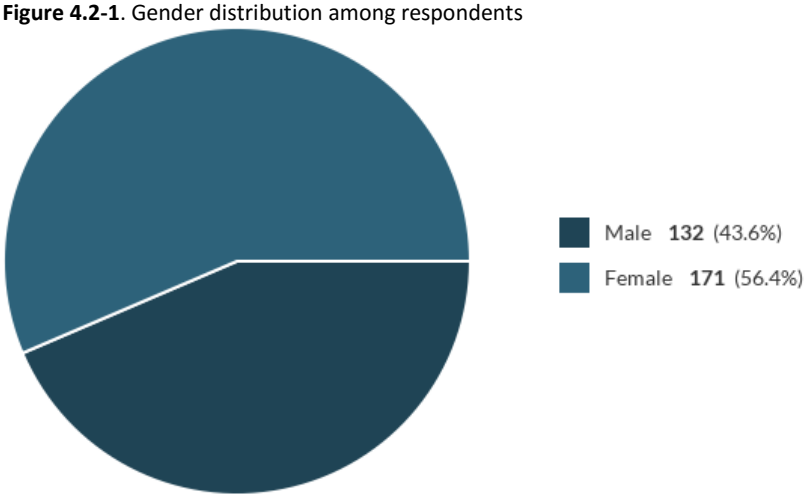
Table 4.2-1. Weighted Kappa for participants of the pizza and soft drink party questionnaire

Weighted Kappa (Linear weights)	0.961
Standard error	0.014
95% CI	0.933 to 0.989

The questionnaire findings are presented in figures (4.2-1 - 4.2-9). The order of result presentation matches approximately the order of corresponding questions in the questionnaire. For clarity however, some questions are grouped and interpreted together.

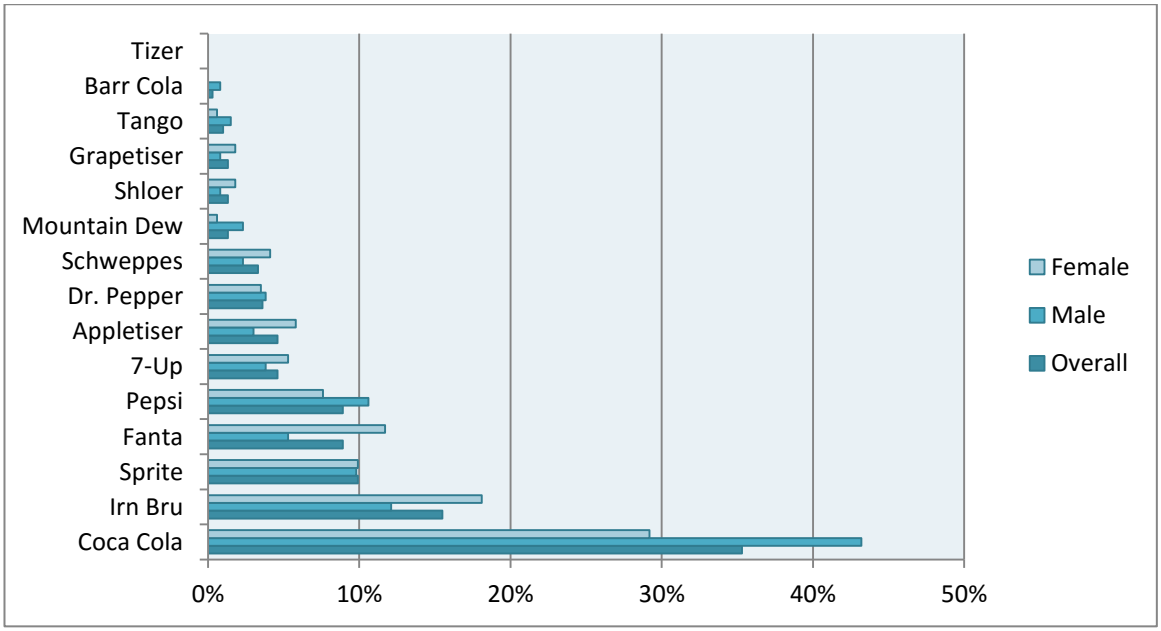
In response to the invitation to complete the online questionnaire a total of 303 responses were received from University of Dundee students (132 males and 171 females) (figure 4.2-1). The mean age of respondents was 22.8 ± 4.7 (min 17 and max 43).

Figure 4.2-1. Gender distribution among respondents



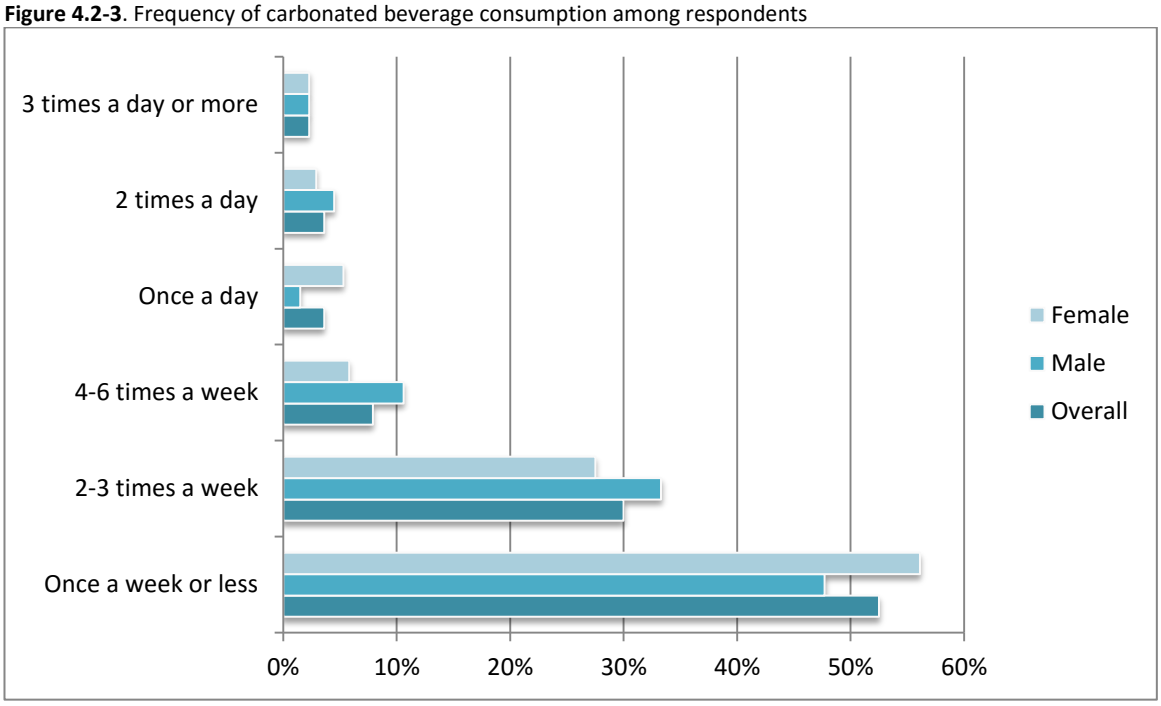
Coca-Cola was the most preferred beverage among respondents (35.3%) followed by Irn Bru (15.5%) and Sprite (9.9%) (figure 4.2-2). Only the difference between males versus females in terms of Coca-Cola preference was found to be statistically significant ($P<0.05$) with significantly more males preferring this drink than females.

Figure 4.2-2. Carbonated beverage preference among respondents



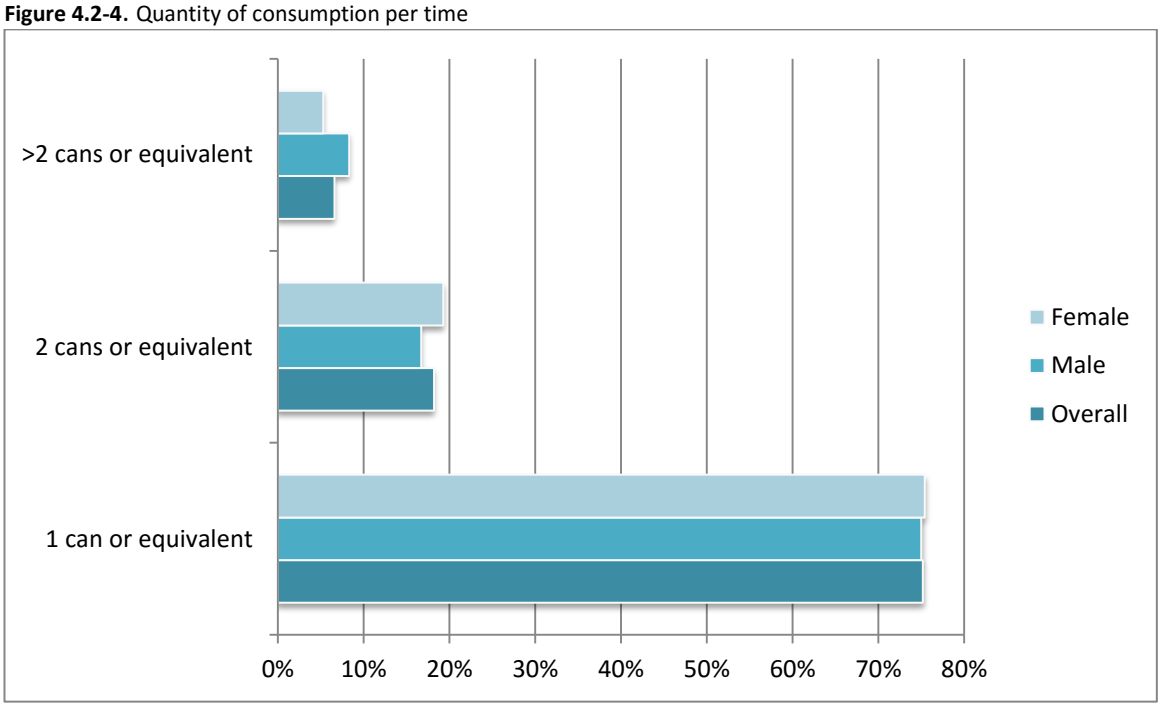
Overall, about one tenth of the respondents consumed a carbonated beverage at least once a day while the majority (90.4%) drank one 6 times a week or less (figure 4.2-3).

Figure 4.2-3. Frequency of carbonated beverage consumption among respondents



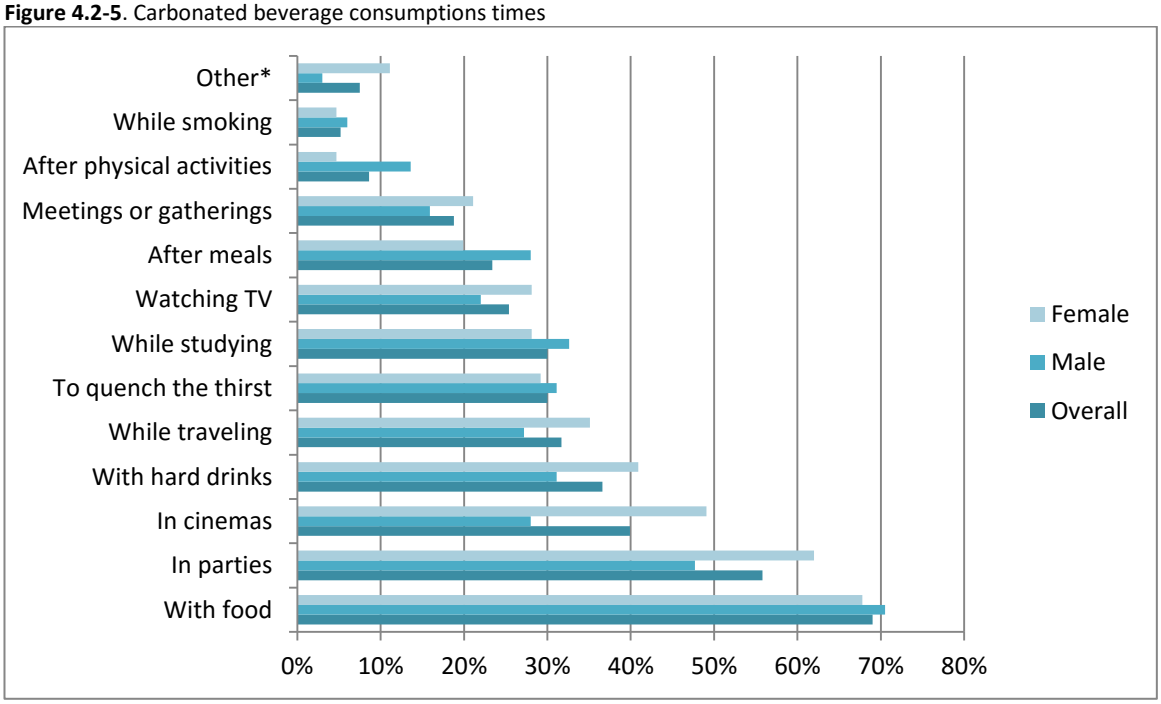
About three quarters of respondents consumed 1 can or equivalent per drinking session and one fifth of them consumed 2 cans or equivalent (figure 4.2-4). 6.6% of respondents consumed more than 2 cans per drinking episode.

Figure 4.2-4. Quantity of consumption per time



Most respondents consumed their beverages with food, at parties and in cinemas (69%, 56% and 40% respectively) but some other occasions came to light for this activity as shown in figure 4.2-5.

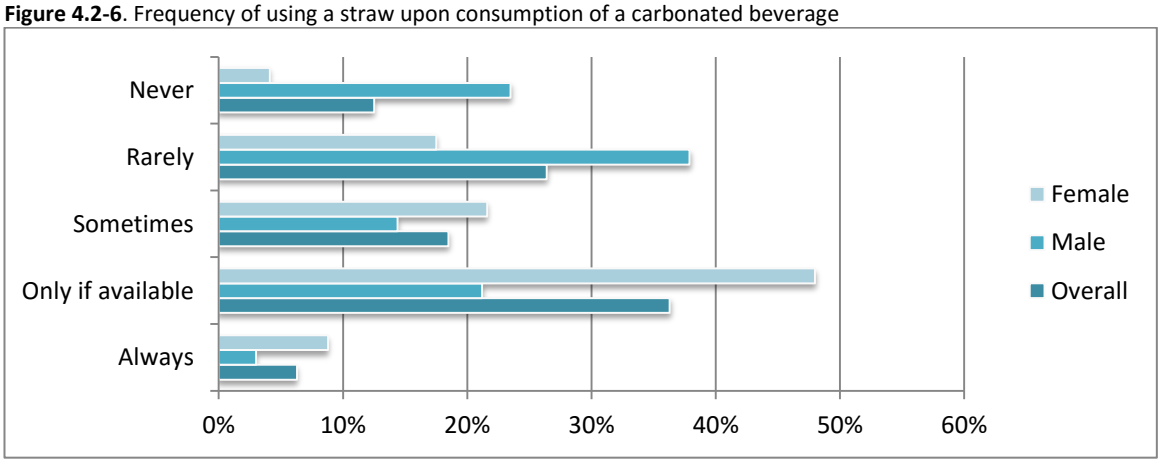
Figure 4.2-5. Carbonated beverage consumptions times



*other included (overall percentage): when hungover (1.3%), at work (1.3%), during picnics (1%), first thing in the morning (1%) and as a treat (1%).

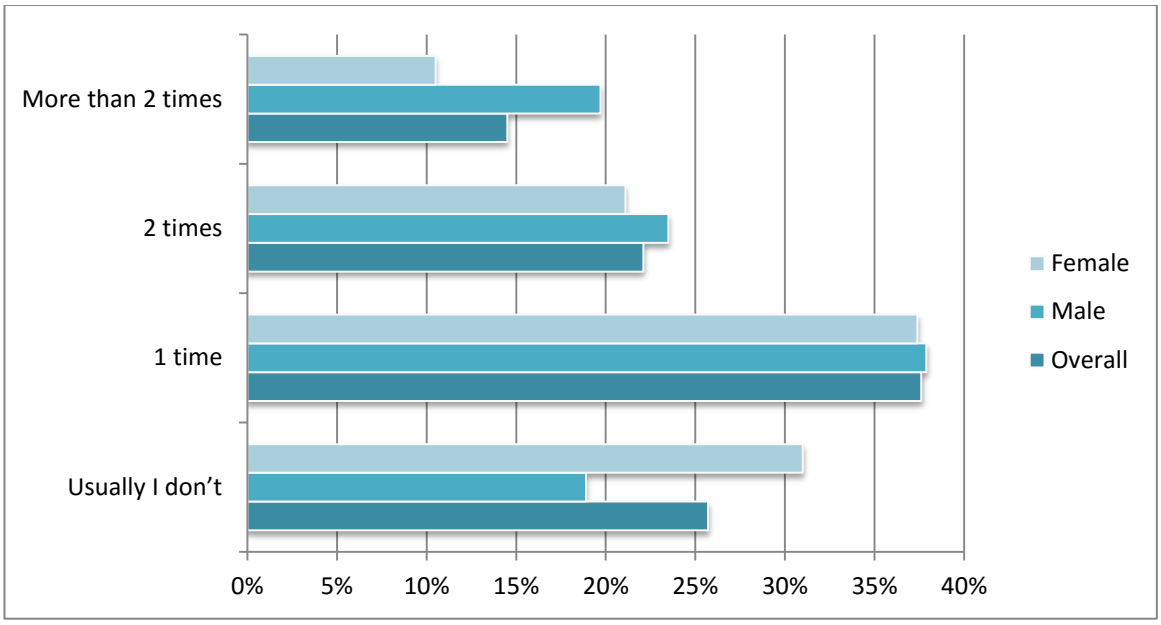
The responses to a variety of questions (figures 4.2-6) demonstrated a higher tendency for females to use a straw to consume a carbonated beverage in comparison to males. Chi-square statistical analysis showed that this difference was statistically significant ($P<0.05$).

Figure 4.2-6. Frequency of using a straw upon consumption of a carbonated beverage



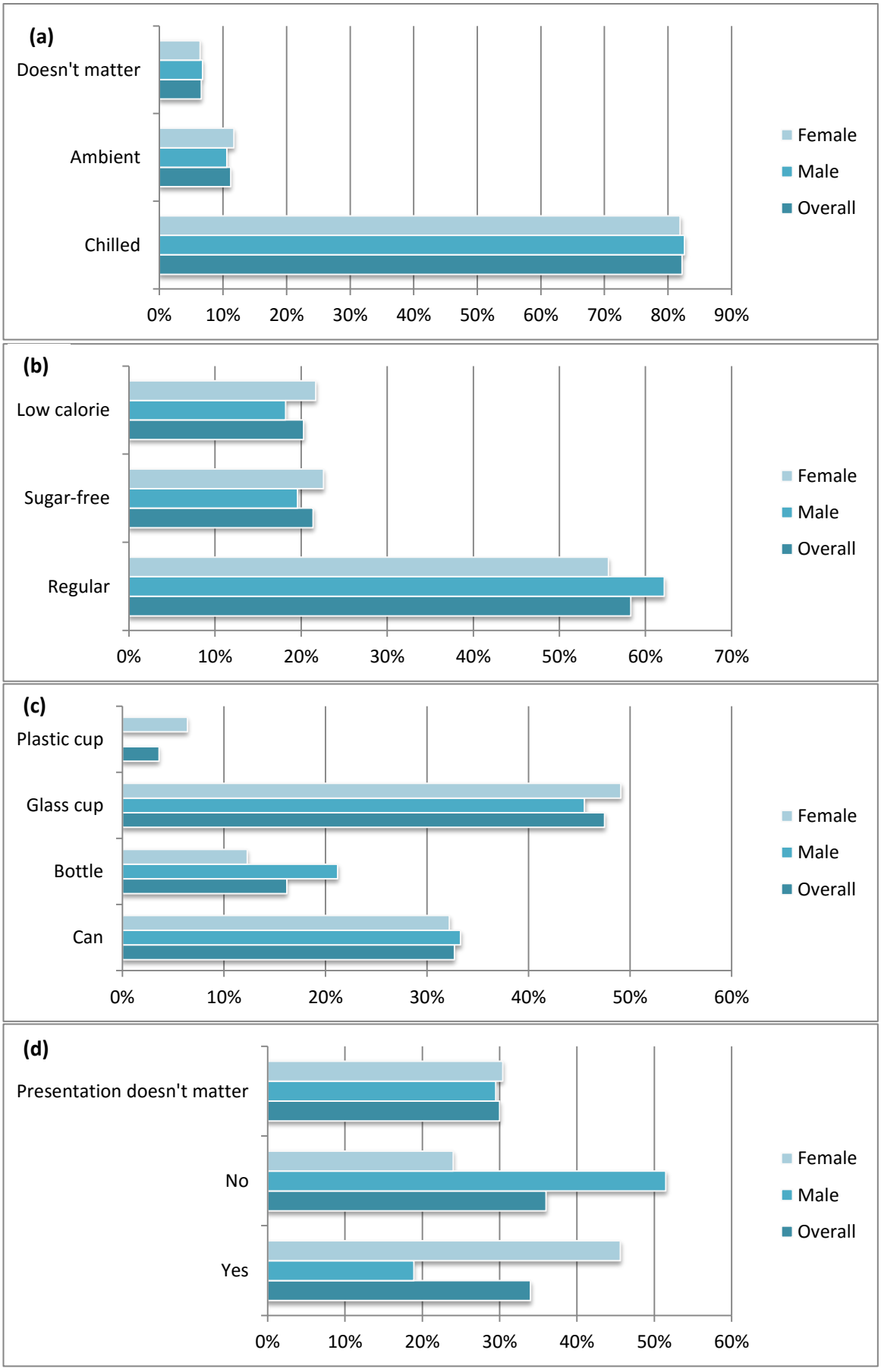
When having a meal in a restaurant, about three quarters of the respondents indicated that they would refill their drinks at least once (figure 4.2-7). Females were more inclined not to refill their drink compared to males. Chi-square statistical analysis found this difference to be statistically significant (P<0.05).

Figure 4.2-7. Frequency of refilling a carbonated beverage while having a meal in a restaurant



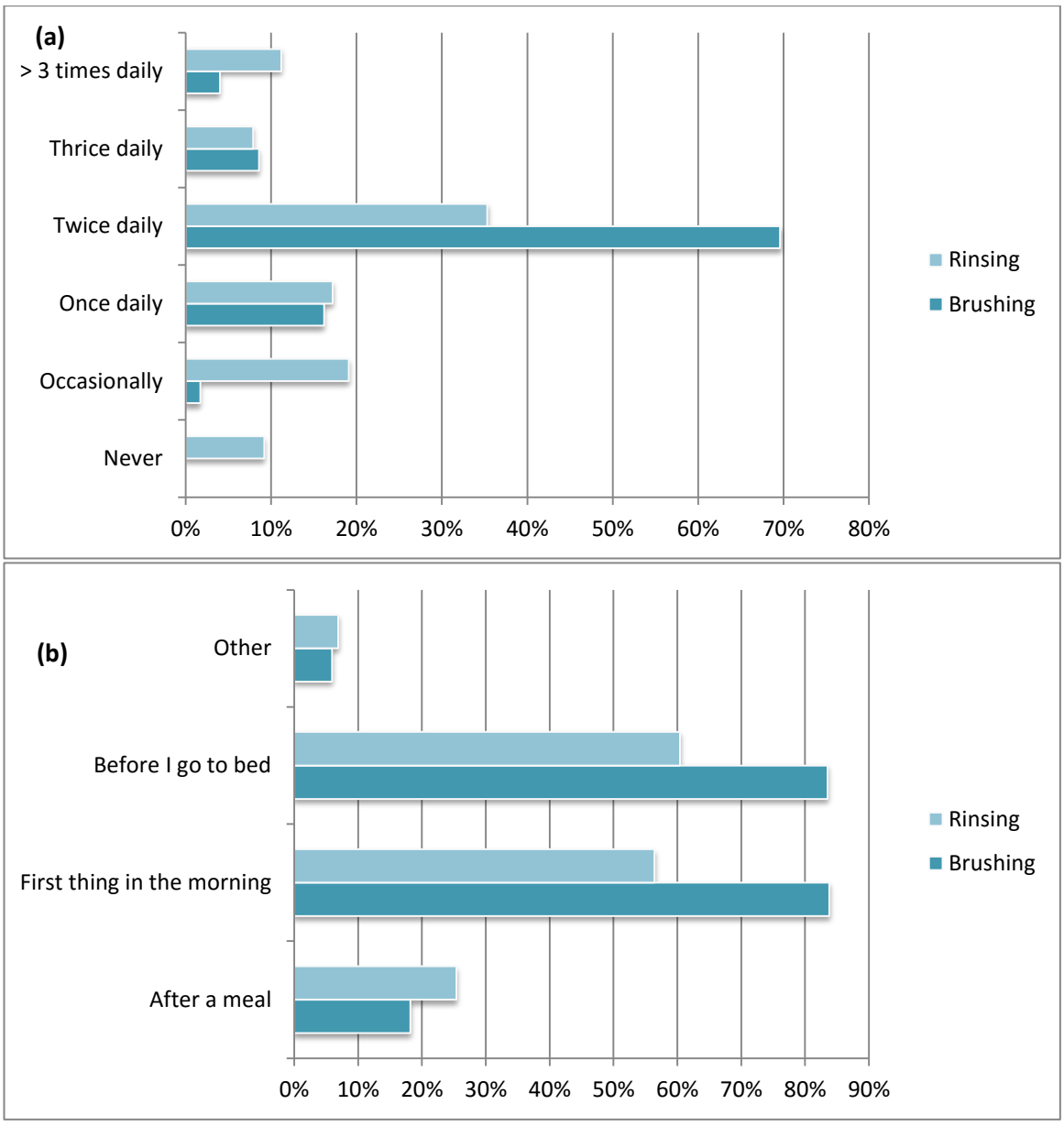
The questionnaire offered the respondents several serving options for their drink, under different categories namely: temperature, dietary requirements, presentation and the use of straw, and were asked to choose the option/s they most preferred (figures 4.2-8a, 4.2-8b, 4.2-8c and 4.2-8d)). The majority of respondents chose a chilled, regular drink poured in a glass cup. Among those who chose 'bottle'; a higher percentage of male respondents was found. On the contrary, among those who chose 'plastic cups' none were males. These differences were found by Chi-square to be statistically significant $P < 0.05$ and $P < 0.01$ respectively. Moreover, females were found to be pickier in terms of how their drinks were presented with 46 % of females stating that presentation does matter while only 19 % of males stated so. This difference was found to be very highly statistically significant ($P < 0.0001$)[Chi-square].

Figure 4.2-8. Ideal serving options for a carbonated drink (a) Temperature (b) dietary requirements (c and d) presentation



The majority of respondents brushed and rinsed their teeth twice daily, mainly two times, first thing in the morning and before going to bed (figures 4.2-9a and 4.2-9b).

Figure 4.2-9. Brushing and rinsing teeth (a) frequency and (b) timing



From the foregoing results it is clear within the limitations of the sample that;

- The Coca-Cola company is currently the leading soft drink manufacturer.
- One tenth of the respondents are consuming soft drinks at least once a day, among which one third consume at least two cans per drinking episode.
- The way drinks are presented seems to be of great significance to females.

4.2.2 Observations

A total of 303 students responded to the recruitment advert to attend a pizza and soft drink party. Of these 132 (43.6 %) were male and 171 (56.4%) female. On receipt of the timetable of experimental sessions eighty one potential participants, 48 (59.3%) males and 33 (40.7%) females aged 17–31 were able to attend the experiment and did so. The four individual sessions were attended by 20, 21, 19 and 21 participants respectively. The ratio of the invited to participating volunteers was thus 0.27.

I. Directly measured values

Table 4.2-2 gives the mean expectorated volumes for each of the two expectorated sips for the participants collectively and according to sex. The standard deviations of these observations are also given. Although in all cases the first expectorated sip volume was lower than the second expectorated one this difference was of no statistical significance ($P > 0.05$)[t-test]. The expectorated sip volume mean value for females (14.8 ± 6.9 ml) was considerably less than that for males (19.1 ± 8.2 ml) and this was statistically significantly ($P < 0.05$)[t-test] different.

Table 4.2-2 also gives for each expectorated sip the beverage temperatures for the participants collectively and according to sex. There was no difference between the overall expectorated beverage temperature mean values for the first two sips ($P > 0.05$)[t-test]. According to gender however, the expectorated temperatures were higher for the sips of females compared to those of the males (15.3 ± 1.9 °C and 15.0 ± 1.9 °C versus 14.8 ± 2.1 °C and 14.6 ± 2.3 °C respectively) though this was of no statistical significance ($P > 0.05$).

Table 4.2-2. Summary of the first, second and overall expectorated beverage volumes and temperatures for the participants collectively and according to gender (direct measurement).

		Male (*n* = 48)			**Female** (*n* = 33)			**Overall** (*n* = 81)		
		1st sip	2nd sip	Mean	1st sip	2nd sip	Mean	1st sip	2nd sip	Mean
A. Sip vol. (ml)		18.3	19.9	19.1	14.0	15.6	14.8*	16.4	18.0	**17.2**
	SD	8.6	8.6	8.2	6.2	8.6	6.9	7.9	8.8	7.9
B. Sip temp. (˚C)		14.8	14.6	14.7	15.3	15.0	15.1	15.0	14.8	**14.9**
	SD	2.1	2.3	2.1	1.9	1.9	1.8	2.0	2.1	2.0

*Significant difference among gender, $p < 0.05$.

II. Calculated values

Table 4.2-3 contains the standard deviations of all observations within it. It gives the mean volume of beverage consumption for the participants collectively and according to sex. The maximum and minimum values are also given. The mean consumption per person was 654.9 ± 348.8 ml. No statistically significant (P > 0.05)[t-test] gender differences were found despite the lower consumption mean value for females in respect of this quantity. This table also contains the mean period of time over which the beverages were consumed for all participants collectively and according to sex. The maximum and minimum values of this quantity are also given. The subjects consumed their beverages over considerably different time periods ranging from 10.6 to 95.4 min with a mean of 44.2 ± 17.4 min. Comparable time period mean values were noticed for both sexes with no statistical difference (P > 0.05)[t-test] between them. Observation of the video footage showed sip (single intake) duration to range from a fraction of a second to a maximum of 6.5 s.

Table 4.2-3 also gives the mean time and mean sip count for the participants to consume a 330 ml can collectively and according to sex. Although it is clear that females spent more time drinking a can than males this is of no statistical significance (P > 0.05)[t-test]. Females however took more sips per can compared to males (23.3 ± 12.1 sips and 18.4 ± 8.0 sips respectively); and this difference was statistically significant (P < 0.05)[t-test]. The mean sip volume and mean consumption rates as

calculated using the parameters observed (sip count (*S*) and the time period from first to last sip (*t*)) in the video footage are also in Table 4.2-3. Both calculated sip volumes and consumption rates were lower for females compared to the values for males. Statistical analysis however showed that only sip volume was significantly different among the sexes ($P < 0.05$)[t-test].

The measured overall expectorated volume (17.2 ± 7.9 ml) did not differ significantly ($P > 0.05$)[t-test] from the calculated sip volume value (16.8 ± 5.9 ml). This too was the case when comparing the measured expectorated volume and calculated sip volume values for males (19.1 ± 8.2 ml versus 18.0 ± 5.9 ml) and females (14.8 ± 6.9 ml versus 15.1 ± 5.5 ml). This gives validity to the method of video observation used in this study.

Table 4.2-3. Summary of human drinking behaviour mean values for the participants collectively and according to gender

		Male (*n* = 48)	**Female** (*n* = 33)	**Overall** (*n* = 81)
A. Consumed volume per subject (ml)		719.9	562.3	**654.9**
	SD	393.8	249.9	348.8
	Max	1625	1200	1625
	Min	162	181	162
B. Time period of consumption (min)		43.1	45.8	**44.2**
	SD	14.7	17.7	17.4
	Max	95.4	85.7	95.4
	Min	13.5	10.6	10.6
C. Time period per can (min)		21.4	24.4	**22.6**
	SD	11.4	10.9	11.2
D. Sip count per can		18.4	23.3*****	**20.4**
	SD	8.0	12.1	10.1
E. Sip volume "*calculated*" (ml)		18.0	15.1*****	**16.8**
	SD	5.9	5.5	5.9
	Max	30.0	28.5	30.0
	Min	7.4	7.2	7.2
F. Consumption rate (ml/min)		14.4	11.8	**13.3**
	SD	6.4	5.0	6.0
	Max	30.2	31.2	31.2
	Min	4.4	4.9	4.4

*Significant difference among gender, $p < 0.05$.

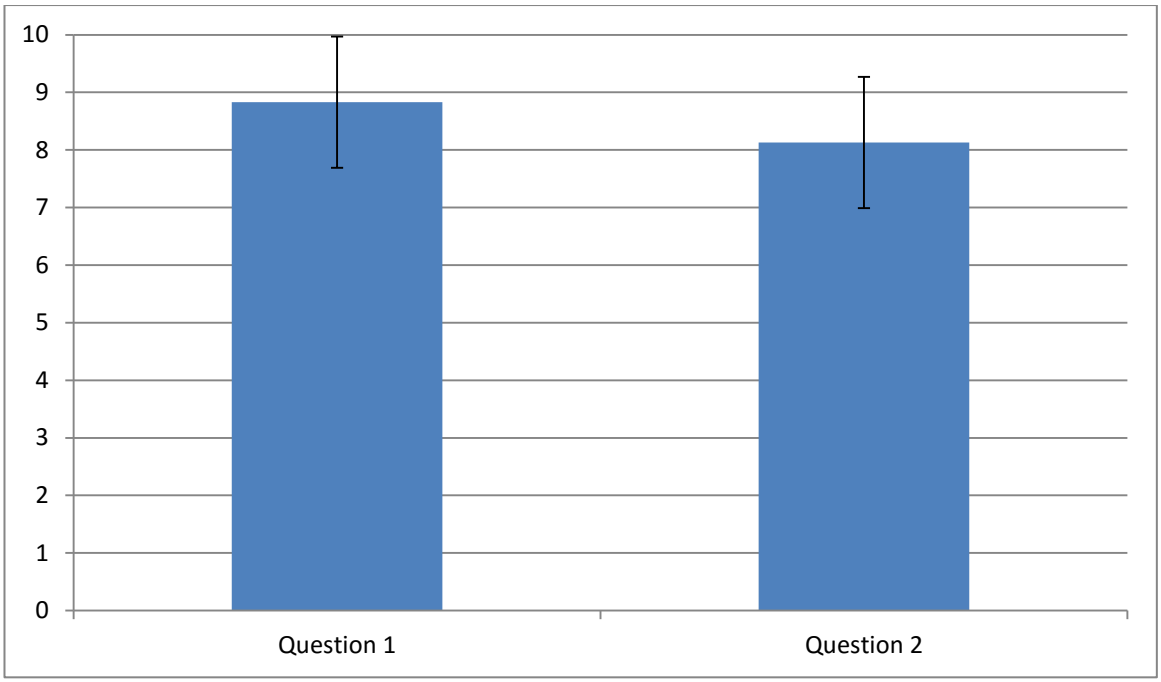
From the foregoing results it is clear that;

- Several human drinking behaviour values were reported such as sip volume and consumption rate.
- There are differences in the drinking behaviour of males and females with respect to sip volume and count.
- The values derived from video observation agree with those measured directly validating this technique for use in further studies.
- Sipped beverages attain a temperature of only 14.9 °C.

4.2.3 Post-experimental Questionnaire

A post-experimental questionnaire was given to the pizza and soft drink party participants to ascertain their perceptions of the environment in which they were conducted. Party participants gave a rank of 8.83 to 'Question 1' and 8.13 to 'Question 2'. Figure 4.2-10 summarises these findings and their standard deviation..

Figure 4.2-10. Post-experimental Questionnaire rank scores.



Question 1:

On a scale of 1 to 10, how did you feel during the experiment?

"10 = Relaxed and acting normal"

Question 2:

On a scale of 1 to 10, how would you describe your performance during the experiment?

"10 = Consuming exactly the same amount of drink you would usually consume in a similar real-life scenario"

It is thus clear that the environment in which the experiment was held was perceived as more normal than artificial.

4.3 A pilot study of Saltus mixer physics

This section reports upon the study undertaken to determine the efficacy of Saltus mixers used in later work by the erosion substrates with beverage and saliva types. Efficient mixing was signified by both a yellow appearance of the mixed Bromphenol blue and citric acid solutions leaving the mixers and a reading of 0.000 % absorption in the spectrophotometry analysis.

Table (4.3-1) gives the observed spectrophotometer readings for the mixture and Bromphenol Blue alone and when mixed with citric acid. It can be seen that:

The spectrophotometer's reading for Bromphenol Blue alone (0.005 g Bromphenol Blue in 500 ml H_2O solution) was 0.987 ± 0.000 %. The mean reading value following the mixing of citric acid and Bromphenol blue was 0.003 ± 0.003 %. This is close to the reference value of 0.000 demonstrating efficient mixing.

Table 4.3-1. Spectrophotometry readings for the mixture and Bromphenol Blue alone. Bromphenol blue % absorption value was set to read 0.000 (reference value).

Occasion	Reading		Reference
	Bromphenol blue	Mixed Solution	
1	0.987	0.002	
2	0.987	0.004	
3	0.987	0.007	
4	0.987	-0.001	
Mean	**0.987**	**0.003**	**0.000**
SD	0.000	0.003	

Furthermore, slow motion analysis of the video footage of the mixing process shows that the solution exiting the mixers orifice is yellow in colour at all times (CD-ROM disk). It was thus concluded that the Saltus' mixer unit achieved the desired efficient mixing of citric acid and Bromphenol blue.

4.4 Formulation of artificial saliva

This section describes the investigation, in terms of pH, of the final artificial saliva recipe that was able to promote remineralisation of the erosion substrates ‘recipe 5’.

Whilst preparing the artificial saliva it was noted that the initial pH of the mixed solution was about 3.5. It was essential for the pH to stabilize before proceeding with the addition of KOH to adjust the pH and subsequently the addition of $CaCl_2.2H_2O$. KOH was added to raise the pH to 7.2 for stimulated and 6.9 for unstimulated saliva. The addition of $CaCl_2.2H_2O$ resulted in a drop in pH level of about 0.05 resulting in a pH of 7.15 for stimulated and pH 6.85 for unstimulated saliva.

4.5 Verifying the constancy of drink temperature in the time frame of an experimental run

In order to determine the potential temperature rise of a beverage during an experimental run in Saltus the temperature of a beverage was tracked with time, 6 times, under the laboratory conditions that Saltus was to be operated in (i.e. Saltus will run the beverage at 14.0 ˚C rather than 4.0 ˚C). Figure 4.5-1 shows a temperature versus time plot where a can of coke at 4 ˚C was opened and its temperature monitored from a period of 0 to 22 minutes. After 22 minutes, the temperature rose from 4.0 ± 0.5 ˚C to 7.1 ± 0.6 ˚C (Δ 3.1 ˚C) (figure 4.5-1).

At a later time when the temperature has reached 14.0 ˚C, the monitoring continued for a further period of 22 minutes as shown in the figure 4.5-2. The same period of time (22 min) was only able to raise the temperature from 14.0 ± 0.0 to 15.7 ± 0.3 (Δ 1.7 ˚C) (figure 4.5-2). In summary, after 22 minutes the temperature of the beverage rose from 4.0 to 7.1 ˚C and from 14.0 to 15.7 ˚C under room temperature conditions (25 ˚C).

Figure 4.5-1. Temperature versus time plot where a can of coke at 4 °C was opened and its temperature monitored for 22 minutes.

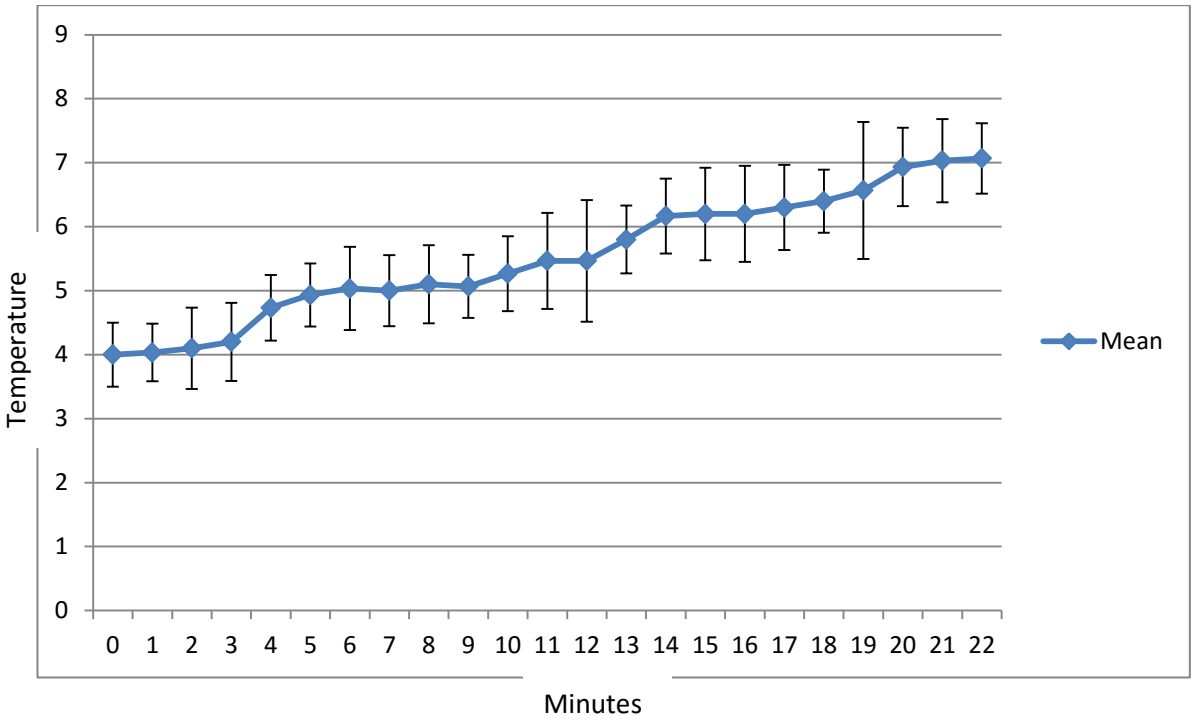
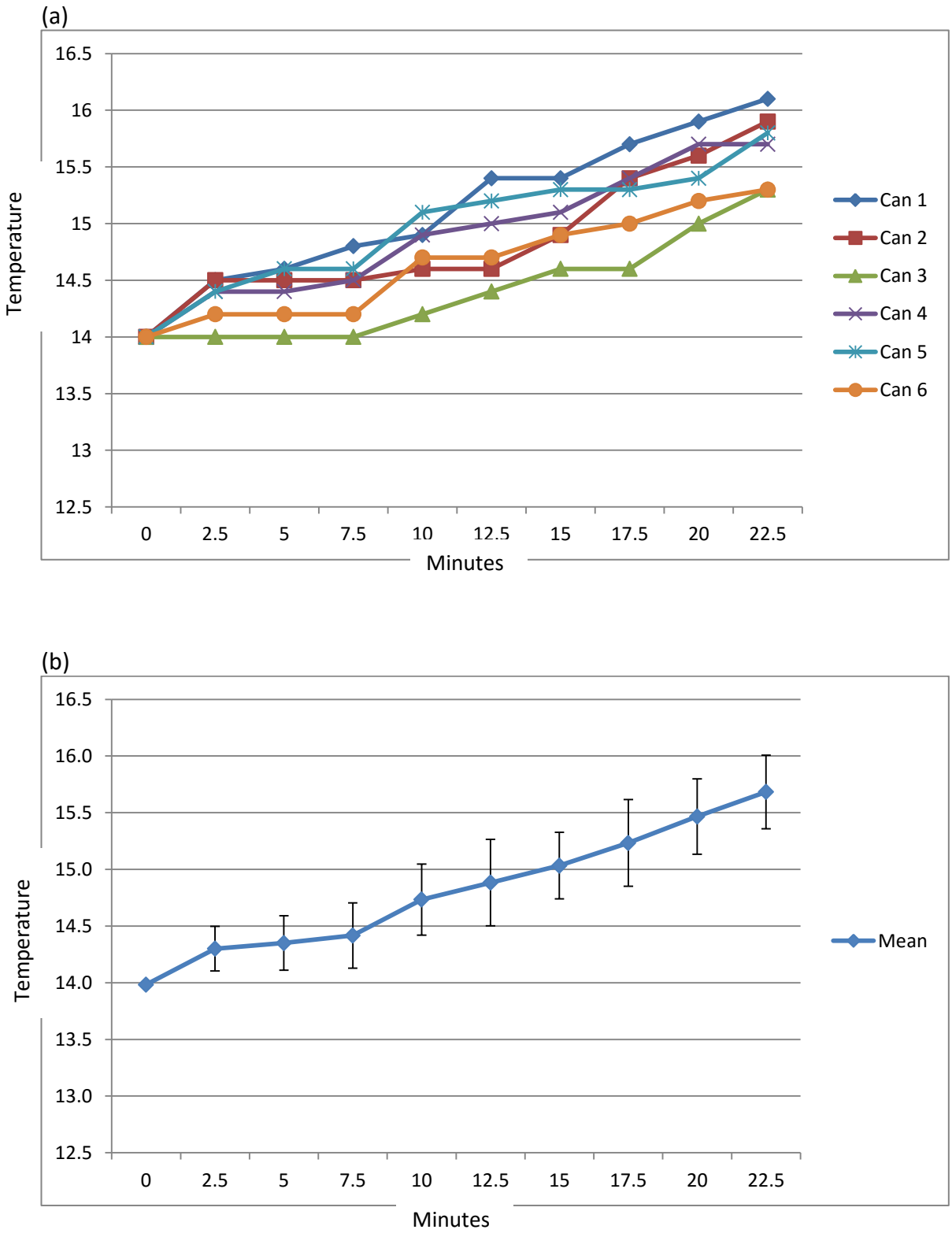


Figure 4.5-2. Temperature versus time plot where a can of coke's temperature was monitored for 22 minutes starting from an initial temperature of 14 °C. (a) The plots over 6 iterations. (b) The mean temperature versus time plot.



It is thus clear that the temperature will only raise about 1.7 ˚C during the time frame of an experimental run.

4.6 Saltus Diets I

In order to ascertain there was no alteration in artificial saliva composition and to ensure the constancy of calcium and phosphate ions when the prepared saliva was conveyed through Saltus; a substrate- and beverage-free single day diet was run. The effect of this diet on the calcium and phosphate ion concentrations is summarised in table 4.6-I. No effect of diet was found on the ionic composition of saliva ($P>0.05$)[t-test].

Table 4.6-I. A comparison between artificial saliva's calcium and phosphate ion content before and after a substrate- and beverage-free single day diet. (a) Calcium content. (b) Phosphate content.

(a)

Artificial Saliva Samples	Original Readings Ca^{2+} mmol/l	Substrate- and Beverage-free Diet Ca^{2+} mmol/l
1	1.10	1.10
2	1.12	1.08
3	1.10	1.09
4	1.12	1.11
5	1.11	1.10
Mean	**1.11**	**1.10**
SD	0.01	0.01

(b)

Artificial Saliva Samples	Original Readings Pi mmol/l	Substrate- and Beverage-free Diet Pi mmol/l
1	4.73	4.73
2	4.74	4.77
3	4.76	4.77
4	4.78	4.75
5	4.80	4.75
Mean	**4.76**	**4.75**
SD	0.03	0.02

Table 4.6-II summarises the outcome of the different recipes piloted in terms of remineralisation. Recipe 5 was stable and had the ability to remineralise both human enamel and Ostrich eggshell and therefore, recipe 5 was used for all experimental runs.

Table 4.6-II. Calcium ion vehicles in different recipes and their role in the remineralisation of Human enamel and Ostrich eggshells.

Recipe #	Calcium Vehicle	Human Enamel	Ostrich Eggshell
1	None	No	No
2	$CaCO_3$	Yes	No
3	CaO	No	No
4	$CaCl_2$ added to stock solution C	Unstable	Unstable
5	$CaCl_2$ added to working solution	Yes	Yes

4.6.1 Immediate effect method

In considering the results of the immediate effect method it is helpful to summarise the testing conditions of this aspect of work that was conducted upon both samples of human enamel and Ostrich eggshell.

Table 4.6-1 summarises Diet 1, Diet 2, Diet 3 and Diet 4

Table 4.6-1. Diet 1, Diet 2, Diet 3 and Diet 4 summary.

Diet	Beverage	Duration	Rest cycles	Test cycles	Test cycle dose	Total number of cans per specimen	Preventive measure	Code
Short-Single dose	*Coca-Cola*	5 days	2	3	1 can	3 cans	None	**Diet 1**
Long-Single dose	*Coca-Cola*	7 days	2	5	1 can	5 cans	None	**Diet 2**
Short-Double dose	*Coca-Cola*	5 days	2	3	2 can	6 cans	None	**Diet 3**
Long-Double dose	*Coca-Cola*	7 days	2	5	2 can	10 cans	None	**Diet 4**

Where a diet is repeated in a second experimental run this is signified by the suffix R. Thus a repeat run of Diet 1 is reported as Diet 1R.

I. Human enamel

A. Surface hardness

Tables (A7-1 to A7-4)(appendix 7) give the raw pre- and post-diet surface hardness values (Vickers Hardness) for all human enamel specimens tested using the immediate effect method.

The effect of each diet on the surface hardness of enamel (i.e. diets 1-4) over 2 experimental runs is summarised in tables 4.6-2 – 4.6-5, and is illustrated graphically in figure 4.6-1. In these tables the raw hardness values have been converted to % change in hardness relative to the pre-diet hardness value (100 %).

Table 4.6-2. Percentages of Surface hardness of human enamel relative to the pre-diet readings (100% Hardness) after a 5 day, single dose diet.

Readings Diet 1	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	93.20	102.81	114.28	99.85	94.30	112.06	106.51	120.93	105.03	114.64	106.36	8.67
Sample 2	103.54	107.60	96.51	96.51	92.44	90.21	96.86	98.73	95.01	97.60	97.50	4.77
Sample 3	116.46	116.09	94.42	79.72	101.03	106.17	106.17	113.89	106.17	116.83	105.69	11.13
Sample 4	110.64	107.46	109.93	95.09	85.54	116.30	93.32	95.09	111.70	104.98	103.00	9.52
Sample 5	115.73	92.88	98.50	88.39	109.48	111.61	92.51	108.35	92.88	90.26	100.06	9.65
Sample 6	93.16	102.18	108.94	110.44	84.52	91.28	93.16	114.95	106.69	95.04	100.04	9.46
Sample 7	91.36	90.65	96.70	95.36	102.29	101.94	99.47	89.59	93.47	105.01	96.58	5.13
Sample 8	95.08	106.57	115.54	96.88	83.24	89.70	95.08	93.65	101.18	104.77	98.17	8.72
Mean											**100.93**	
SD											3.71	

Readings Diet 1R	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	105.38	115.34	98.61	118.55	98.97	97.19	121.04	108.22	107.51	94.70	106.55	8.86
Sample 2	77.53	90.84	76.74	109.24	104.54	112.76	119.81	127.34	108.46	110.81	103.81	16.07
Sample 3	99.09	89.56	91.08	113.19	108.61	118.90	107.85	114.33	123.00	108.99	107.46	10.56
Sample 4	84.32	90.31	85.08	85.97	88.89	103.10	107.83	92.17	104.92	102.00	94.46	8.58
Sample 5	87.22	90.12	113.64	100.25	97.72	101.34	101.34	102.06	87.59	102.42	98.37	7.70
Sample 6	106.43	109.49	112.92	102.62	98.80	107.20	90.03	112.16	96.13	102.24	103.80	6.94
Sample 7	106.01	94.23	107.58	105.61	88.34	92.27	113.47	95.41	111.90	118.18	103.30	9.59
Sample 8	106.18	79.64	113.82	101.82	108.00	92.01	91.27	88.00	82.91	81.82	94.55	11.49
Mean											**101.54**	
SD											5.11	

Table 4.6-3. Percentages of Surface hardness of human enamel relative to the pre-diet readings (100% Hardness) after a 7 day, single dose diet.

Readings Diet 2	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	86.87	99.82	97.56	87.63	89.51	96.28	95.52	88.38	95.52	86.87	92.40	4.74
Sample 2	93.27	103.72	108.32	96.19	115.01	115.01	115.85	96.61	100.38	102.89	104.73	8.01
Sample 3	95.97	84.31	87.64	95.97	84.97	82.97	88.30	81.64	86.97	94.30	88.30	5.06
Sample 4	105.74	106.58	108.89	102.37	101.09	90.17	96.90	107.95	100.69	104.90	102.53	5.40
Sample 5	96.03	99.96	90.98	107.15	93.93	87.39	99.30	107.95	102.72	88.84	97.42	6.88
Sample 6	92.88	99.29	110.68	96.80	93.95	87.90	101.42	101.07	86.12	113.88	98.40	8.49
Sample 7	102.27	103.96	114.06	112.37	103.96	103.96	92.17	106.48	122.47	107.32	106.90	7.67
Sample 8	96.28	98.91	109.44	93.64	87.25	93.49	100.04	110.57	105.08	101.17	99.59	6.98
Mean											**98.78**	
SD											6.19	

Readings Diet 2R	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	108.31	94.77	103.42	81.99	84.62	95.52	91.39	77.10	91.76	100.04	92.89	9.22
Sample 2	90.76	97.45	106.99	97.45	111.67	111.25	104.98	107.94	115.85	100.38	104.47	7.41
Sample 3	80.97	86.30	94.64	81.97	94.30	99.97	97.30	96.63	78.31	84.31	89.47	7.50
Sample 4	118.27	118.27	115.42	115.42	101.43	117.42	113.52	106.48	115.00	107.74	112.90	5.43
Sample 5	90.61	97.81	105.00	99.12	90.28	105.33	73.60	87.99	87.34	90.61	92.77	8.99
Sample 6	93.24	106.76	105.69	102.85	93.63	91.14	103.20	91.89	100.00	118.15	100.66	8.08
Sample 7	118.69	113.64	95.96	109.85	118.69	103.11	93.43	116.58	106.48	109.85	108.63	8.49
Sample 8	74.84	74.09	91.01	88.76	94.77	86.87	87.63	85.75	100.41	95.90	88.00	8.03
Mean											**98.72**	
SD											9.30	

Table 4.6-4. Percentages of Surface hardness of human enamel relative to the pre-diet readings (100% Hardness) after a 5 day, double dose diet.

Readings Diet 3	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	78.55	90.77	75.18	71.52	103.91	80.38	75.79	87.10	78.15	92.60	83.40	9.49
Sample 2	85.29	83.21	91.23	78.95	82.62	79.05	75.08	83.21	97.77	66.57	82.30	8.07
Sample 3	86.48	104.74	91.61	102.82	97.69	92.89	93.21	96.09	93.53	87.12	94.62	5.65
Sample 4	96.97	99.04	109.35	93.19	100.07	98.35	106.26	106.26	92.50	80.81	98.28	7.87
Sample 5	115.23	101.89	110.35	101.56	92.12	104.82	96.35	101.56	103.84	105.47	103.32	6.17
Sample 6	89.07	98.10	90.05	104.66	105.97	106.30	96.13	100.39	98.10	96.13	98.49	5.75
Sample 7	89.79	90.76	90.50	92.53	98.28	95.10	93.33	96.86	96.07	92.17	93.54	2.76
Sample 8	88.77	100.00	94.04	96.14	93.33	98.25	91.58	93.33	103.86	97.19	95.65	4.16
Mean											**93.70**	
SD											7.34	

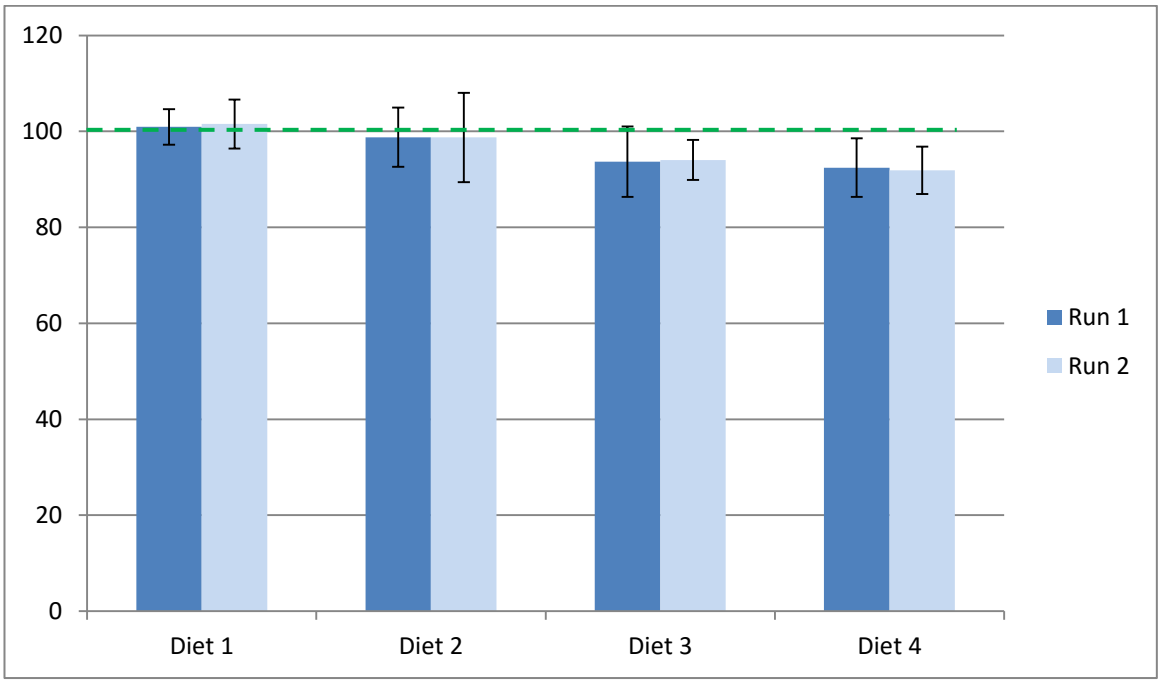
Readings Diet 3R	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	105.02	91.85	90.07	95.41	92.56	92.92	79.39	82.24	97.54	103.95	93.09	7.76
Sample 2	80.66	74.78	92.01	100.63	113.55	103.76	87.31	104.93	104.93	80.66	94.32	12.38
Sample 3	102.13	85.75	84.60	68.98	77.36	120.05	92.61	105.95	69.74	111.28	91.84	16.70
Sample 4	90.71	80.87	89.62	97.27	93.99	74.68	72.86	72.86	99.45	84.52	85.68	9.53
Sample 5	119.75	94.05	107.08	108.17	116.49	112.51	82.83	83.19	96.22	82.11	100.24	13.72
Sample 6	85.08	105.68	89.66	99.58	107.59	92.33	105.68	103.01	89.28	79.36	95.73	9.37
Sample 7	78.92	110.72	96.98	80.49	109.54	74.20	107.18	108.76	98.55	92.27	95.76	13.10
Sample 8	80.00	99.64	92.01	110.91	92.00	107.64	80.00	98.91	100.73	94.18	95.60	9.73
Mean											**94.03**	
SD											4.18	

Table 4.6-5. Percentages of Surface hardness of human enamel relative to the pre-diet readings (100% Hardness) after a 7 day, double dose diet.

Readings Diet 4	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	97.16	86.11	96.49	87.78	108.56	104.20	104.87	88.12	101.52	102.86	97.77	7.61
Sample 2	108.12	98.59	75.14	111.79	104.09	119.85	100.43	68.54	105.93	96.76	98.92	15.03
Sample 3	95.74	83.93	92.46	98.03	100.98	103.93	96.72	85.25	101.97	77.38	93.64	8.32
Sample 4	83.40	75.06	89.89	71.36	83.09	96.07	86.80	65.18	88.65	87.42	82.69	8.94
Sample 5	88.68	85.44	108.83	99.08	84.46	70.82	110.12	94.53	65.94	105.58	91.35	14.45
Sample 6	76.26	75.17	91.08	118.18	91.44	72.28	91.44	88.91	86.38	112.04	90.32	14.22
Sample 7	87.77	96.44	74.93	97.48	101.65	106.16	96.10	106.16	115.87	107.55	99.01	10.91
Sample 8	79.93	62.93	112.24	94.22	71.09	88.78	83.67	96.94	92.52	75.85	85.82	13.51
Mean											**92.44**	
SD											6.09	

Readings Diet 4R	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	95.11	90.46	100.43	80.81	87.80	82.47	90.12	89.79	94.11	95.44	90.66	5.69
Sample 2	100.37	87.18	97.44	87.18	97.07	87.18	84.25	81.32	94.87	95.60	91.25	6.20
Sample 3	100.43	100.10	101.76	96.77	98.10	95.11	91.12	89.13	86.80	87.13	94.65	5.40
Sample 4	94.70	92.92	97.90	85.80	97.54	89.36	86.51	96.12	74.76	101.46	91.71	7.43
Sample 5	109.08	98.77	96.44	104.09	100.10	108.75	102.76	95.44	93.45	102.76	101.16	5.05
Sample 6	88.28	88.28	100.00	95.60	105.86	87.91	75.82	98.17	95.97	100.00	93.59	8.17
Sample 7	82.59	91.14	92.92	75.47	91.14	87.58	93.98	81.88	79.39	92.92	86.90	6.24
Sample 8	85.80	79.39	78.32	85.08	83.66	87.93	84.73	90.78	89.00	85.44	85.01	3.70
Mean											**91.86**	
SD											4.94	

Figure 4.6-1. Percentage of surface hardness (mean ± SD) relative to pre-diet hardness values (100% hardness) over 2 runs.



A plot of the change in surface hardness (figure 4.6-1), relative to the pre-diet hardness values (100 % relative hardness) shows that for all experimental runs a gradual reduction in hardness from diet 1 to diet 4 occurred. The % hardness values for diet 1 and diet 2 remained very close to the pre-diet value of 100 %.

To investigate any effects of diets (diet 1, 2, 3 and 4) and experimental run (Run 1 and 2) on the surface hardness change a 2-way analysis of variance on this data was undertaken. This revealed (table 4.6-6);

- Highly significant effects of diet ($P < 0.001$)
- No effect of experimental run ($P = 0.9609$)
- No significant interaction of diet and experimental run ($P = 0.9934$).

Table 4.6-6. 2-wat ANOVA: Hardness percentage versus Diet and Experimental run.

Source of Variation	Df	Sum-of-squares	Mean square	F
Interaction	3	3.194	1.065	0.02863
Diet	3	853.6	284.5	7.651
Experimental run	1	0.09000	0.09000	0.002420
Residual	56	2083	37.19	

A follow-up analysis (Bonferroni post-test) was undertaken to determine the effect of the diet factor over all the groups (diet 1, 2, 3 and 4). Hardness percentage values of diet 1 differed significantly relative to diets 3 and 4 ($P < 0.05$) in Run 1. Similarly, hardness values of diet 1 differed significantly relative to diet 3 ($P < 0.05$) but highly significantly with diet 4 ($P < 0.01$) in Run 2. All other differences among diets were all not statistically significant ($P > 0.05$). There was thus good agreement in terms of the statistical significance between the experimental runs.

B. Surface Loss

Tables (A7-5 to A7-8)(Appendix 7) give the raw pre- and post-diet surface profile values for all human enamel specimens tested using the immediate effect method.

The effect of each diet on surface loss (i.e. diet 1-4) over 2 experimental runs is summarised in tables 4.6-7 – 4.6-10, and is illustrated graphically in figure 4.6-2. In these tables the raw profile values have been converted to surface loss values in µm relative to the pre-diet profile value (0.00 µm).

Table 4.6-7. Surface loss in µm of human enamel relative to pre-diet readings (0.00 µm) after a 5 day, single dose diet.

Readings **Diet 1**	1	2	3	4	5	Mean	SD
Sample 1	16.89	16.29	16.74	16.39	17.02	16.66	0.32
Sample 2	7.80	7.50	7.51	7.77	7.20	7.56	0.24
Sample 3	13.62	11.22	13.96	12.60	12.17	12.71	1.11
Sample 4	12.58	11.39	11.54	12.03	12.18	11.94	0.48
Sample 5	11.97	14.96	12.23	13.51	13.08	13.15	1.19
Sample 6	9.77	8.36	8.79	9.01	9.23	9.03	0.52
Sample 7	13.54	13.91	13.61	13.11	13.66	13.57	0.29
Sample 8	10.84	11.35	11.00	10.92	11.11	11.04	0.20
Mean						**11.96**	
SD						2.82	

Readings **Diet 1R**	1	2	3	4	5	Mean	SD
Sample 1	9.51	12.75	10.31	11.07	10.66	10.86	1.69
Sample 2	8.00	8.00	6.95	7.29	7.99	7.65	0.61
Sample 3	13.27	12.7	12.61	13.19	12.51	12.86	0.36
Sample 4	10.66	10.93	10.22	10.78	10.42	10.60	0.36
Sample 5	10.29	10.29	10.37	10.30	10.33	10.32	0.05
Sample 6	6.75	6.78	6.81	6.70	6.86	6.78	0.03
Sample 7	9.93	9.71	9.13	9.57	9.60	9.59	0.41
Sample 8	12.62	13.15	12.62	13.00	12.61	12.80	0.31
Mean						**10.18**	
SD						2.17	

Table 4.6-8. Surface loss in µm of human enamel relative to pre-diet readings (0.00 µm) after a 7 day, single dose diet.

Readings Diet 2	1	2	3	4		Mean	SD
Sample 1	20.54	21.53	20.87	21.13	20.85	20.98	0.51
Sample 2	15.68	15.64	15.84	15.70	15.75	15.72	0.10
Sample 3	17.22	16.87	18.25	17.11	17.82	17.45	0.72
Sample 4	16.11	15.73	15.61	15.99	15.68	15.82	0.26
Sample 5	18.38	19.82	21.23	20.12	19.90	19.89	1.18
Sample 6	9.90	9.98	9.79	9.99	9.79	9.89	0.10
Sample 7	23.89	20.44	23.52	22.99	22.28	22.62	1.90
Sample 8	17.51	17.53	17.52	17.60	17.44	17.52	0.01
Mean						**17.48**	
SD						3.93	

Readings Diet 2R	1	2	3	4	5	Mean	SD
Sample 1	7.50	7.38	7.71	7.40	7.65	7.53	0.17
Sample 2	18.01	18.09	18.56	18.20	18.23	18.22	0.30
Sample 3	13.35	14.86	14.08	14.10	14.10	14.10	0.76
Sample 4	14.13	14.23	14.11	14.09	14.22	14.16	0.06
Sample 5	17.58	16.26	16.96	17.22	16.62	16.93	0.66
Sample 6	20.33	20.89	19.45	20.12	20.32	20.22	0.73
Sample 7	9.87	9.14	8.21	9.01	9.12	9.07	0.84
Sample 8	16.65	16.48	16.38	16.64	16.36	16.50	0.13
Mean						**14.59**	
SD						4.39	

Table 4.6-9. Surface loss in µm of human enamel relative to pre-diet readings (0.00 µm) after a 5 day, double dose diet.

Readings Diet 3	1	2	3	4	5	Mean	SD
Sample 1	17.80	19.38	20.62	19.75	19.03	19.32	1.03
Sample 2	20.98	20.69	21.00	21.00	20.48	20.83	0.24
Sample 3	26.04	26.23	25.21	25.40	26.53	25.89	0.56
Sample 4	22.85	23.01	20.44	21.85	21.31	21.89	1.07
Sample 5	18.25	20.20	20.75	20.58	20.58	20.07	1.04
Sample 6	13.26	13.37	13.61	13.57	13.50	13.46	0.15
Sample 7	18.73	19.85	18.25	19.42	19.86	19.22	0.71
Sample 8	25.47	25.01	25.23	26.31	25.25	25.45	0.50
Mean						**20.77**	
SD						3.92	

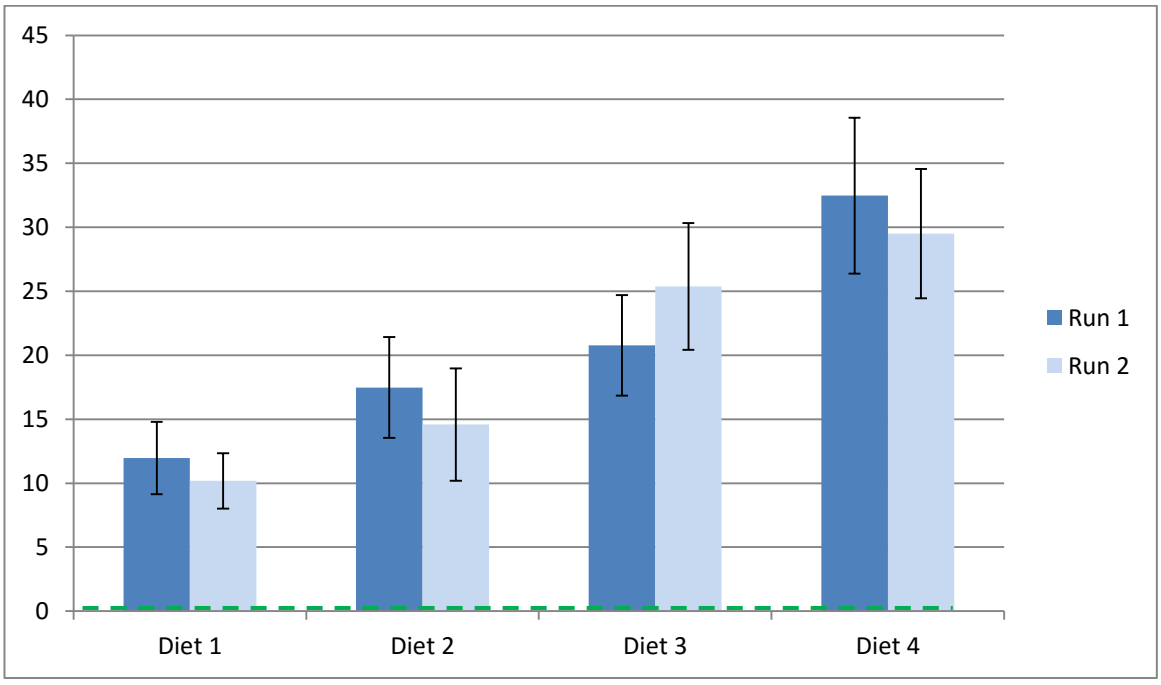
Readings Diet 3R	1	2	3			Mean	SD
Sample 1	22.38	23.07	24.47	23.52	21.63	23.01	1.08
Sample 2	31.52	31.84	30.62	30.48	31.48	31.19	0.60
Sample 3	25.85	26.22	24.35	27.16	27.24	26.16	1.18
Sample 4	28.98	28.07	28.98	28.32	28.96	28.66	0.44
Sample 5	24.71	24.08	24.08	24.34	24.77	24.40	0.33
Sample 6	16.02	16.82	16.33	16.40	15.19	16.15	0.61
Sample 7	23.48	23.13	21.09	23.28	22.74	22.74	0.96
Sample 8	30.03	31.75	30.82	30.10	30.75	30.69	0.69
Mean						**25.38**	
SD						4.96	

Table 4.6-10. Surface loss in µm of human enamel relative to pre-diet readings (0.00 µm) after a 7 day, double dose diet.

Readings Diet 4	1	2	3	4	5	Mean	SD
Sample 1	28.69	28.44	29.38	30.07	30.56	29.43	0.90
Sample 2	41.01	41.13	41.84	41.30	41.35	41.33	0.45
Sample 3	29.02	31.29	27.06	29.13	29.14	29.13	2.12
Sample 4	31.77	30.34	30.17	30.90	30.64	30.76	0.88
Sample 5	35.37	33.39	32.97	34.08	33.77	33.91	1.28
Sample 6	39.49	40.33	38.17	39.00	39.65	39.33	1.09
Sample 7	23.81	22.71	21.31	20.58	22.09	22.10	1.44
Sample 8	33.97	33.72	33.57	33.81	33.69	33.75	0.20
Mean						**32.47**	
SD						6.10	

Readings Diet 4R	1	2	3			Mean	SD
Sample 1	30.45	29.71	29.16	29.72	28.48	29.50	0.73
Sample 2	30.21	30.35	30.39	30.27	30.22	30.29	0.08
Sample 3	29.01	29.09	29.46	29.57	27.45	28.91	0.85
Sample 4	29.52	29.95	29.88	30.20	30.22	29.96	0.29
Sample 5	25.02	25.86	24.84	25.57	25.83	25.42	0.47
Sample 6	30.83	29.26	29.62	29.35	29.55	29.72	0.64
Sample 7	22.41	22.13	22.09	21.99	22.98	22.32	0.40
Sample 8	39.42	39.42	40.23	40.78	39.93	39.96	0.58
Mean						**29.51**	
SD						5.05	

Figure 4.6-2. Surface loss values in µm (mean ± SD) relative to pre-diet values (0.00 µm) over 2 runs.
Note: a positive loss value represents a loss of material.



A plot of the surface loss (figure 4.6-2), relative to pre-diet profile values shows that for all experimental runs a gradual increase in surface loss from diet 1 to diet 4 occurred. Mean surface loss ranged from 10.18 to 32.47 µm.

To investigate any effects of diets (diet 1, 2, 3 and 4) and experimental run on the amount of surface loss a 2-way analysis of variance on this data was undertaken. This revealed (table 4.6-11);

- Extremely highly significant effects of diet (P<0.0001).
- No effect of the experimental run (P=0.5852).
- No significant interaction of diet and experimental run (P=0.1776)

Table 4.6-11. 2-way ANOVA: Surface loss versus Diet and Experimental run.

Source of Variation	Df	Sum-of-squares	Mean square	F
Interaction	3	98.14	32.71	1.745
Diet	3	2254	751.2	40.07
Experimental run	1	5.700	5.700	0.3041
Residual	32	599.9	18.75	

A follow-up analysis (Bonferroni post-test) was undertaken to determine the effect of the diet factor over all the groups (diet 1, 2, 3 and 4). The overall comparison between diets over 2 runs is summarised in table 4.6-12.

Table 4.6-12. Overall comparison between the diets over 2 runs. Run 1 (a) and Run 2 (b).

(a)

Run 1	Diet 1	Diet 2	Diet 3	Diet 4
Diet 1				
Diet 2	NS			
Diet 3	**	NS		
Diet 4	***	***	***	

(b)

Run 2	Diet 1	Diet 2	Diet 3	Diet 4
Diet 1				
Diet 2	NS			
Diet 3	***	***		
Diet 4	***	***	NS	

NS: Non-significant (P > 0.05), *: Significant (P < 0.05), **: Highly significant (P < 0.01), ***: very highly significant (P < 0.001).

This reveals that, for the first experimental run ‘Run 1’, non-significant differences were found for (Diet 1 vs Diet 2) and (Diet 2 vs Diet 3)($P > 0.05$). While all other differences were either highly statistically significant (Diet 1 vs Diet 3)($P < 0.01$) or very highly statistically significant (Diet 4 vs Diet 1, Diet 2 and Diet 3)($P < 0.001$).

For the second experimental run ‘Run 2’, non-significant differences were found for (Diet 1 vs Diet 2) and (Diet 3 vs Diet 4)($P > 0.05$). While all other differences were very highly statistically significant ($P < 0.001$).

With the exception of the statistical findings for Diet 3 versus Diet 4 (Run 1 $P<0.001$, Run 2 NS) reproducibility between runs was good.

C. Ion loss

Tables (A7-9 to A7-12)(Appendix 7) give the reference calcium and phosphate concentrations values in artificial saliva, the test beverage and the mixture solution together with the raw post-diet ion content of the resultant solutions for all diets using the immediate effect method.

The effect of each diet on the amount of human enamel's ion loss in mmol/day (i.e. diet 1-4) for 2 ions (i.e. calcium and phosphate) is summarised in tables 4.6-13 – 4.6-16, and are illustrated graphically in figure 4.6-3. In these tables ion concentrations for the resultant solutions were subtracted from the reference values of the artificial saliva/test beverage mixture to reflect more clearly the quantity of ion loss.

Table 4.6-13. Calcium and phosphate ion loss of human enamel in mmol/l relative to pre-diet readings (0.00 mmol/l) after a 5 day, single dose diet.*

Test cycle **Diet 1**	**1**	**2**	**3**	**Sum**	**Mean**	**SD**
Calcium	0.06	0.06	0.08	**0.20**	**0.07**	0.01
Phosphate	0.12	0.11	0.11	**0.34**	**0.11**	0.01

Table 4.6-14. Calcium and phosphate ion loss of human enamel in mmol/l relative to pre-diet readings (0.00 mmol/l) after a 7 day, single dose diet.*

Test cycle **Diet 2**	**1**	**2**	**3**	**4**	**5**	**Sum 3**	**Sum 5**	**Mean**	**SD**
Calcium	0.09	0.05	0.09	0.07	0.08	**0.23**	**0.38**	**0.08**	0.02
Phosphate	0.13	0.13	0.07	0.07	0.07	**0.33**	**0.47**	**0.09**	0.03

Table 4.6-15. Calcium and phosphate ion loss of human enamel in mmol/l relative to pre-diet readings (0.00 mmol/l) after a 5 day, double dose diet.*

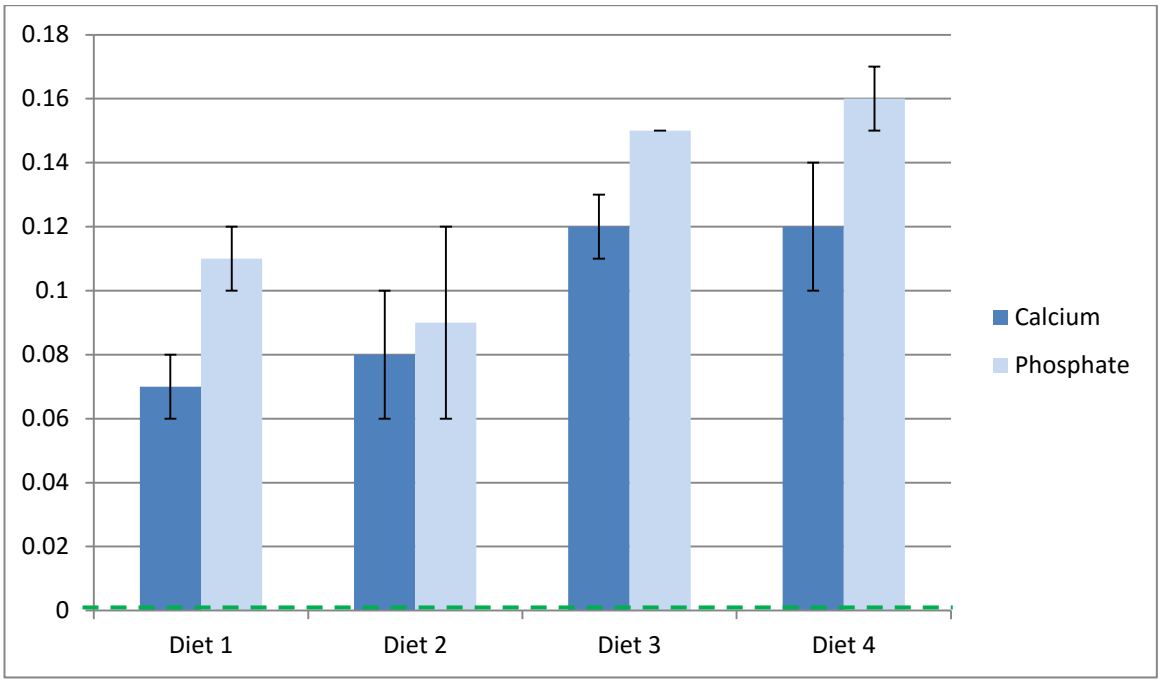
Test cycle **Diet 3**	**1**	**2**	**3**	**Sum**	**Mean**	**SD**
Calcium	0.12	0.12	0.11	**0.35**	**0.12**	0.01
Phosphate	0.15	0.15	0.15	**0.45**	**0.15**	0.00

Table 4.6-16. Calcium and phosphate ion loss of human enamel in mmol/l relative to pre-diet readings (0.00 mmol/l) after a 7 day, double dose diet.*

Test cycle **Diet 4**	**1**	**2**	**3**	**4**	**5**	**Sum 3**	**Sum 5**	**Mean**	**SD**
Calcium	0.14	0.14	0.10	0.11	0.11	**0.38**	**0.60**	**0.12**	0.02
Phosphate	0.15	0.16	0.17	0.16	0.16	**0.48**	**0.80**	**0.26**	0.01

*Each reading represents the mean of 3 readings (based on chemical analysis testing of 3 samples for each day that consists of a test cycle).

Figure 4.6-3. Ion loss values in mmol/day (mean ± SD) for both ion types, calcium and phosphate.



A plot of the ion loss (figure 4.6-3), shows that for all experimental runs a gradual increase in ion loss from diet 2 to diet 4 occurred. Calcium ion loss ranged from 0.07 to 0.12 mmol/day with a relatively higher daily loss of ions in diets 3 and 4 compared to diets 1 and 2, while phosphate ion loss ranged from 0.09 to 0.26 mmol/day with the highest daily loss of ions in diet 4.

To investigate any effects of diets (diet 1, 2, 3 and 4) and ion type on ion loss a 2-way analysis of variance on this data was undertaken. This revealed (table 4.6-17);

- Extremely highly significant effects of diet and ion type ($P<0.0001$).
- No significant interaction of diet and ion type ($P>0.05$).

Table 4.6-17. 2-way ANOVA: Ion loss versus Diet and Ion type.

Source of Variation	Df	Sum-of-squares	Mean square	F
Interaction	3	0.0001406	4.687e-005	0.1442
Diet	3	0.01739	0.005797	17.84
Ion type	1	0.007922	0.007922	24.38
Residual	24	0.0078	0.000325	

A follow-up analysis (Bonferroni post-test) was undertaken to determine the effect of the diet factor over all the groups (diet 1, 2, 3 and 4). The overall comparison between diets over the 2 ion types measured is summarised in table 4.6-18.

Table 4.6-18. Overall comparison between the diets for both ions. Calcium (a) and phosphate (b).

(a)

Calcium	Diet 1	Diet 2	Diet 3	Diet 4
Diet 1				
Diet 2	NS			
Diet 3	**	*		
Diet 4	***	***	NS	

(b)

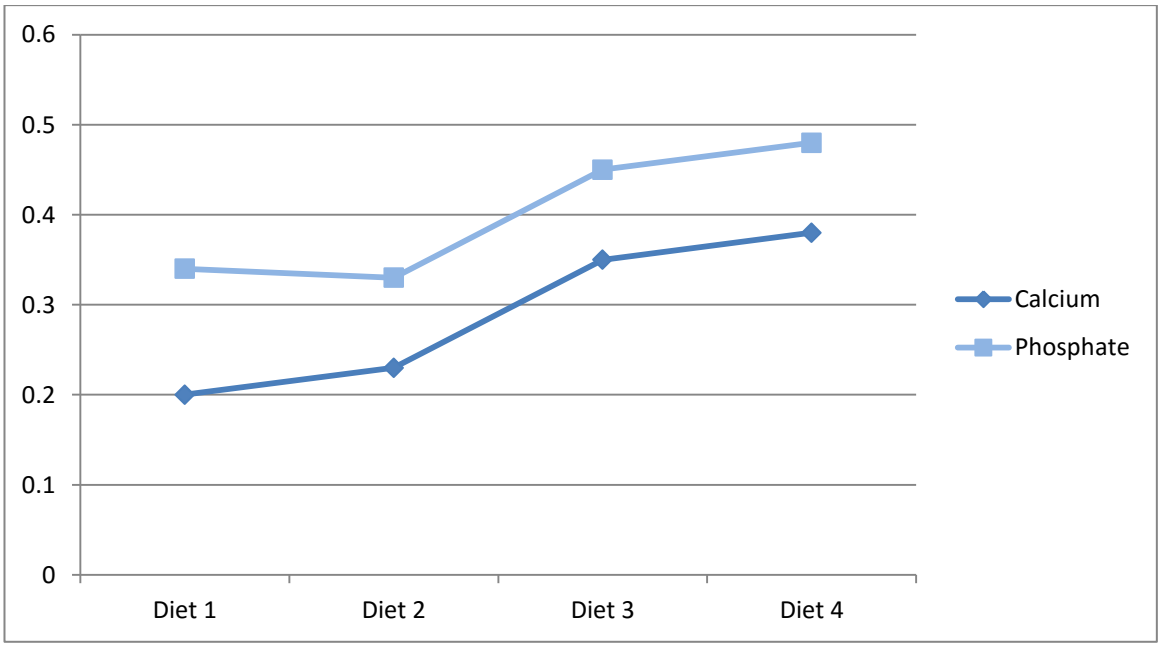
Phosphate	Diet 1	Diet 2	Diet 3	Diet 4
Diet 1				
Diet 2	NS			
Diet 3	*	*		
Diet 4	**	***	NS	

NS: Non-significant (P > 0.05), *: Significant (P < 0.05), **: Highly significant (P < 0.01), ***: very highly significant (P < 0.001).

This reveals that, for both ions, non-significant differences were found for (Diet 1 vs Diet 2) and (Diet 3 vs Diet 4)(P > 0.05). All other differences were found to be significant (Figure 4.6-18).

Figure 4.6-4 illustrates the total ion loss over 3 test cycles for all diets.

Figure 4.6-4. Total ion loss observed in mmol over 3 days for each diet (based on the values of "Sum 3").



II. Ostrich Eggshell

A. *Surface hardness*

Tables (A7-13 to A7-16)(Appendix 7) give the raw pre- and post-diet surface hardness values (Vickers Hardness) for all Ostrich eggshell specimens tested using the immediate effect method.

The effect of each diet on surface hardness (i.e. diet 1-4) over 2 experimental runs is summarised in tables 4.6-19 – 4.6-22, and is illustrated graphically in figure 4.6-5. In these tables the raw hardness values have been converted to % change in hardness relative to the pre-diet hardness value (100 %).

Table 4.6-19. Percentages of Surface hardness of Ostrich eggshells relative to the pre-diet readings (100% Hardness) after a 5 day, single dose diet.

Readings Diet 1	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	66.67	61.18	82.35	81.57	66.67	82.35	58.04	63.53	65.10	69.02	69.65	8.65
Sample 2	72.83	69.69	64.21	71.26	73.61	76.74	77.53	76.74	69.69	80.66	73.30	4.57
Sample 3	76.75	76.75	61.10	67.06	55.14	61.10	64.83	58.87	55.14	63.34	64.01	7.32
Sample 4	63.30	71.31	75.32	64.90	68.11	62.50	79.33	71.35	67.31	62.50	68.59	5.41
Sample 5	60.29	74.76	61.09	68.68	81.99	68.33	74.76	65.11	73.95	61.90	69.09	6.81
Sample 6	56.05	71.75	71.00	57.55	62.78	65.02	61.29	63.81	59.04	65.31	63.36	4.96
Sample 7	59.82	62.88	69.02	67.48	59.82	61.35	59.82	65.18	69.16	75.15	64.97	4.91
Sample 8	64.75	87.13	81.53	67.95	69.54	77.54	79.14	71.94	69.54	79.94	74.90	6.79
Mean											**68.48**	
SD											4.22	

Readings Diet 1R	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	68.40	68.40	67.66	75.84	79.55	70.63	77.32	76.58	76.58	78.07	73.90	4.35
Sample 2	64.37	71.86	66.62	59.13	67.37	60.63	62.13	69.61	65.12	62.13	64.90	3.83
Sample 3	59.12	69.94	70.66	58.40	57.68	59.12	69.94	63.45	65.61	62.73	63.66	4.87
Sample 4	71.20	68.80	71.20	72.00	68.80	70.40	73.60	74.60	71.20	71.20	71.30	1.73
Sample 5	64.07	70.18	67.12	74.75	70.18	64.84	64.84	64.84	67.89	66.36	67.51	3.19
Sample 6	74.44	76.10	77.75	71.13	62.03	75.27	77.75	70.31	81.06	71.96	73.78	5.03
Sample 7	66.02	71.96	77.89	80.12	74.93	68.25	69.73	74.93	72.70	68.99	72.55	4.23
Sample 8	65.81	73.37	70.37	68.08	70.37	72.62	68.84	66.57	68.84	65.81	69.07	2.51
Mean											**69.58**	
SD											3.96	

Table 4.6-20. Percentages of Surface hardness of Ostrich eggshells relative to the pre-diet readings (100% Hardness) after a 7 day, single dose diet.

Readings Diet 2	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	59.68	60.44	65.03	61.97	54.32	61.21	53.56	64.27	63.50	64.27	60.83	3.83
Sample 2	56.11	55.28	60.23	64.36	62.71	59.41	69.31	66.01	63.53	65.18	62.21	4.21
Sample 3	62.48	70.73	67.06	69.72	75.23	68.90	68.90	67.06	65.23	70.64	68.60	3.28
Sample 4	57.86	58.57	67.14	67.14	62.14	55.00	60.71	62.86	61.43	62.14	61.50	3.62
Sample 5	65.14	65.14	53.69	62.28	65.14	56.55	59.41	62.99	65.86	59.41	61.56	3.95
Sample 6	79.44	70.61	73.26	74.14	76.50	75.90	67.96	75.02	71.20	73.85	73.79	3.10
Sample 7	57.74	56.99	58.48	59.22	55.51	54.77	58.84	56.99	54.03	53.29	56.59	1.97
Sample 8	67.54	65.79	67.54	74.56	65.79	71.05	64.91	71.05	65.79	66.67	68.07	2.97
Mean											**64.14**	
SD											5.53	

Readings Diet 2R	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	67.95	46.36	56.75	70.34	68.75	62.35	61.55	68.75	80.72	51.96	63.55	9.46
Sample 2	55.96	63.96	73.54	51.16	81.13	74.34	53.56	66.35	65.55	57.55	64.31	9.37
Sample 3	63.95	75.94	83.13	51.16	88.73	67.15	66.35	49.56	55.16	54.36	65.55	12.87
Sample 4	57.16	66.75	68.35	57.16	62.76	61.16	61.96	61.16	65.96	58.76	62.12	3.71
Sample 5	50.36	79.14	71.94	66.35	59.95	49.56	59.15	65.92	55.96	69.12	62.75	9.01
Sample 6	59.95	59.15	66.35	63.15	56.75	57.55	63.95	61.55	59.15	57.55	60.51	2.99
Sample 7	75.94	68.35	65.96	64.76	73.95	67.95	75.16	63.96	66.33	66.36	68.87	4.23
Sample 8	65.16	74.75	76.35	65.16	70.75	69.15	69.95	69.15	73.95	66.75	70.11	3.71
Mean											**64.72**	
SD											3.31	

Table 4.6-21. Percentages of Surface hardness of Ostrich eggshells relative to the pre-diet readings (100% Hardness) after a 5 day, double dose diet.

Readings Diet 3	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	59.28	63.51	59.28	63.51	59.28	69.60	69.60	55.04	66.90	59.28	62.53	4.68
Sample 2	54.07	49.44	66.43	59.48	64.89	69.52	61.80	51.76	74.16	67.98	61.95	7.75
Sample 3	62.03	48.02	54.31	40.59	50.02	77.46	65.46	56.88	41.45	49.17	54.54	10.79
Sample 4	46.97	44.55	51.00	59.85	59.18	47.77	54.22	59.25	44.55	51.00	51.83	5.71
Sample 5	44.87	45.67	57.68	58.49	44.87	50.47	64.09	53.68	47.27	45.67	51.27	6.52
Sample 6	44.26	58.74	49.89	53.11	63.57	64.37	60.35	54.72	56.33	58.74	56.41	5.87
Sample 7	59.26	56.83	48.71	66.57	55.20	70.63	53.58	48.71	56.83	51.14	56.75	6.84
Sample 8	63.53	54.29	45.97	62.02	69.08	62.02	57.06	60.76	70.92	61.09	60.67	6.77
Mean											**56.99**	
SD											4.38	

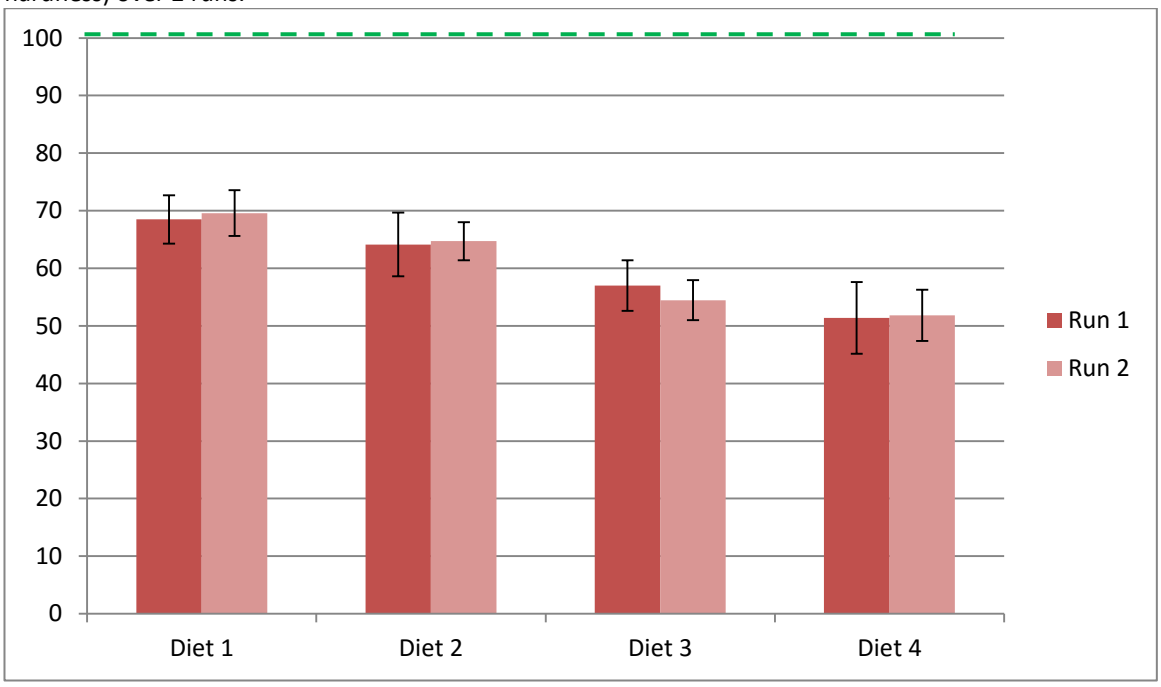
Readings Diet 3R	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	61.96	55.69	54.90	65.10	68.24	53.33	50.98	45.49	73.73	61.18	59.06	8.12
Sample 2	54.03	54.03	54.03	61.08	49.33	46.99	52.47	61.08	54.82	52.47	54.03	4.20
Sample 3	62.59	48.44	44.71	38.00	45.45	42.47	52.91	47.69	59.61	52.16	49.40	7.19
Sample 4	53.88	64.29	61.09	53.88	65.93	67.95	49.07	58.69	60.29	59.49	59.46	5.58
Sample 5	47.88	47.07	60.74	68.78	46.27	47.07	57.52	62.35	51.09	63.15	55.19	7.86
Sample 6	46.34	48.58	51.57	53.06	47.09	51.57	56.80	53.81	48.58	54.56	51.20	3.28
Sample 7	56.01	49.11	55.25	56.01	59.85	49.11	46.04	51.41	56.78	52.94	53.25	4.06
Sample 8	57.76	49.76	54.56	44.97	53.76	60.96	56.96	55.36	51.36	54.56	54.00	4.25
Mean											**54.45**	
SD											3.48	

Table 4.6-22. Percentages of Surface hardness of Ostrich eggshells relative to the pre-diet readings (100% Hardness) after a 7 day, double dose diet.

Readings Diet 4	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	53.83	50.88	53.65	49.03	49.03	50.88	51.80	47.18	54.58	49.03	50.99	2.34
Sample 2	38.77	40.39	36.35	37.96	48.47	43.62	50.08	36.35	47.66	48.47	42.81	5.20
Sample 3	59.18	53.16	50.15	59.18	50.15	48.14	49.15	52.16	47.14	51.15	51.96	3.98
Sample 4	54.30	52.49	50.68	52.49	51.54	48.87	51.58	51.58	50.63	51.58	51.57	1.35
Sample 5	46.10	46.10	46.94	36.04	52.81	51.97	36.88	41.07	63.70	37.72	45.93	8.19
Sample 6	52.38	57.23	50.44	63.05	58.20	62.08	57.23	70.81	70.81	62.08	60.43	6.46
Sample 7	39.23	47.58	45.08	58.43	51.75	45.91	41.74	45.91	59.27	40.07	47.50	6.66
Sample 8	56.72	62.21	65.87	50.32	67.70	58.55	69.53	51.24	56.72	58.55	59.74	6.21
Mean											**51.37**	
SD											6.22	

Readings Diet 4R	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	52.73	39.78	42.55	55.50	58.28	44.40	49.95	46.25	57.35	45.33	49.21	6.18
Sample 2	51.73	56.58	54.96	60.62	54.16	54.96	62.23	61.12	55.77	54.96	56.71	3.26
Sample 3	44.13	43.13	38.11	34.10	65.20	55.17	37.11	53.16	48.14	50.15	46.84	9.02
Sample 4	47.06	44.34	53.39	48.87	50.68	38.91	47.96	46.15	47.96	44.34	46.97	3.74
Sample 5	34.37	52.81	51.13	50.29	53.65	40.23	78.79	58.68	31.85	65.38	51.72	13.43
Sample 6	39.77	62.08	61.11	46.56	58.20	64.99	52.38	68.87	74.68	66.93	59.55	10.11
Sample 7	46.74	48.41	60.93	74.29	41.74	58.43	53.42	45.91	46.74	42.57	51.92	9.59
Sample 8	53.98	53.06	59.47	41.17	50.32	50.32	59.47	48.49	45.75	53.98	51.60	5.42
Mean											**51.82**	
SD											4.45	

Figure 4.6-5. Percentage of surface hardness (mean ± SD) relative to pre-diet hardness values (100% hardness) over 2 runs.



A plot of the change in surface hardness (figure 4.6-5), relative to the pre-diet hardness values (100 % relative hardness) shows that for all experimental runs a gradual reduction in hardness from diet 1 to diet 4 occurred. Furthermore, Hardness percentage values for all diets were considerably reduced relative to the pre-diet value of 100%.

To investigate any effects of diets (diet 1, 2, 3 and 4) and experimental run on the surface hardness change a 2-way analysis of variance on this data was undertaken. This revealed (table 4.6-23);

- Very highly significant effects of diet (P<0.001).
- No effect of experimental run (P=0.9283).
- No significant interaction of the diet and experimental run (P=0.6649).

Table 4.6-23. 2-way ANOVA: Hardness percentage versus Diet and Experimental run.

Source of Variation	Df	Sum-of-squares	Mean square	F
Interaction	3	32.63	10.88	0.5280
Diet	3	3040	1013	49.18
Experimental run	1	0.1681	0.1681	0.008160
Residual	56	1154	20.60	

Follow-up analysis (Bonferroni post-test) was undertaken to determine the effect of the diet factor over all the groups (diet 1, 2, 3 and 4). The overall comparison between diets over 2 runs is summarised in table 4.6-24.

Table 4.6-24. Overall comparison between the diets over 2 runs. Run 1 (a) and Run 2 (b).

(a)

Run 1	Diet 1	Diet 2	Diet 3	Diet 4
Diet 1				
Diet 2	NS			
Diet 3	***	**		
Diet 4	***	***	*	

(b)

Run 2	Diet 1	Diet 2	Diet 3	Diet 4
Diet 1				
Diet 2	NS			
Diet 3	***	***		
Diet 4	***	***	NS	

NS: Non-significant (P > 0.05), *: Significant (P < 0.05), **: Highly significant (P < 0.01), ***: very highly significant (P < 0.001).

This reveals that, for the first experimental run 'Run 1', non-significant differences were found for (Diet 1 vs Diet 2)(P > 0.05). While all other differences were either statistically significant (Diet 3 vs Diet 4)(P < 0.05), highly statistically significant (Diet 2 vs Diet 3)(P < 0.01), or very highly statistically significant (Diet 1 vs Diet 3 and 4, Diet 2 vs Diet 4)(P<0.001).

For the second experimental run 'Run 2', non-significant differences were found for (Diet 1 vs Diet 2) and (Diet 3 vs Diet 4)(P > 0.05). While all other differences were very highly statistically significant (P<0.001).

With the exception of the level of statistical significance given between Diet 3 versus Diet 4 (P < 0.05 Run 1 and NS Run 2) reproducibility of runs was good.

B. Surface Loss

Tables (A7-17 to A7-20)(Appendix 7) give the raw pre- and post-diet surface profile values for all Ostrich eggshell specimens tested using the immediate effect method.

The effect of each diet on surface loss (i.e. diet 1-4) over 2 experimental runs is summarised in tables 4.6-25 – 4.6-28, and is illustrated graphically in figure 4.6-6. In these tables the raw profile values have been converted to surface loss values in µm relative to the pre-diet profile value (0.00 µm).

Table 4.6-25. Surface loss in µm of Ostrich eggshells relative to pre-diet readings (0.00 µm) after a 5 day, single dose diet.

Readings **Diet 1**	1	2	3	4	5	Mean	SD
Sample 1	7.58	8.67	9.05	8.27	8.64	8.44	0.76
Sample 2	5.76	5.71	5.74	5.77	5.73	5.74	0.03
Sample 3	7.93	9.62	8.21	8.65	8.50	8.58	0.91
Sample 4	4.73	3.59	3.89	4.20	3.95	4.07	0.59
Sample 5	5.58	5.34	4.44	4.82	5.41	5.12	0.60
Sample 6	12.21	8.63	10.94	9.95	11.23	10.59	1.82
Sample 7	0.84	0.20	0.96	0.64	0.66	0.66	0.41
Sample 8	4.43	8.01	7.17	6.92	6.12	6.53	1.87
Mean						**6.22**	
SD						3.09	

Readings **Diet 1R**	1	2	3	4	5	Mean	SD
Sample 1	7.71	8.10	7.99	8.02	7.85	7.93	0.20
Sample 2	4.69	4.48	4.51	4.50	4.63	4.56	0.11
Sample 3	8.36	10.49	9.50	9.75	9.15	9.45	1.07
Sample 4	5.85	5.43	5.66	5.71	5.58	5.65	0.21
Sample 5	3.97	3.95	3.98	4.07	3.89	3.97	0.02
Sample 6	7.39	8.01	8.12	7.92	7.75	7.84	0.39
Sample 7	5.67	5.17	5.47	5.13	5.74	5.44	0.25
Sample 8	7.85	8.76	9.50	7.99	9.39	8.70	0.83
Mean						**6.69**	
SD						2.04	

Table 4.6-26. Surface loss in µm of Ostrich eggshells relative to pre-diet readings (0.00 µm) after a 7 day, single dose diet.

Readings Diet 2	1	2	3	4	5	Mean	SD
Sample 1	9.95	10.54	10.80	10.02	10.81	10.43	0.44
Sample 2	15.67	16.69	14.91	15.09	16.44	15.76	0.89
Sample 3	6.59	5.24	7.88	6.12	7.01	6.57	1.32
Sample 4	23.45	23.90	21.84	22.54	23.61	23.07	1.08
Sample 5	7.39	6.74	8.17	6.99	7.87	7.43	0.72
Sample 6	15.15	15.20	15.23	15.41	14.99	15.19	0.04
Sample 7	15.10	15.35	14.23	14.53	15.23	14.89	0.59
Sample 8	10.98	10.75	10.63	10.77	10.79	10.78	0.18
Mean						**13.02**	
SD						5.36	

Readings Diet 2R	1	2	3	4	5	Mean	SD
Sample 1	7.49	6.89	7.76	7.37	7.64	7.43	0.34
Sample 2	9.30	9.66	9.41	9.37	9.48	9.44	0.13
Sample 3	13.50	12.86	14.44	14.35	14.67	13.96	0.76
Sample 4	19.42	19.64	19.43	19.45	19.73	19.53	0.14
Sample 5	8.94	9.41	9.60	9.08	8.84	9.17	0.32
Sample 6	13.02	14.67	14.64	13.73	14.85	14.18	0.78
Sample 7	18.81	18.91	15.87	16.13	16.37	17.22	1.51
Sample 8	10.96	10.46	10.85	10.90	11.14	10.86	0.25
Mean						**12.73**	
SD						4.23	

Table 4.6-27. Surface loss in µm of Ostrich eggshells relative to pre-diet readings (0.00 µm) after a 5 day, double dose diet.

Readings Diet 3	1	2	3	4	5	Mean	SD
Sample 1	15.88	16.23	16.18	16.05	16.16	16.10	0.19
Sample 2	11.50	12.76	12.72	11.99	12.67	12.33	0.72
Sample 3	9.45	8.33	6.77	8.98	7.37	8.18	1.35
Sample 4	20.50	21.91	22.40	21.94	21.27	21.61	0.99
Sample 5	23.58	16.43	20.01	19.23	20.78	20.00	3.58
Sample 6	22.86	21.85	24.80	23.55	22.80	23.17	1.50
Sample 7	14.29	18.29	20.90	17.99	17.69	17.83	3.33
Sample 8	19.08	19.94	19.22	19.88	18.95	19.41	0.46
Mean						**17.33**	
SD						4.99	

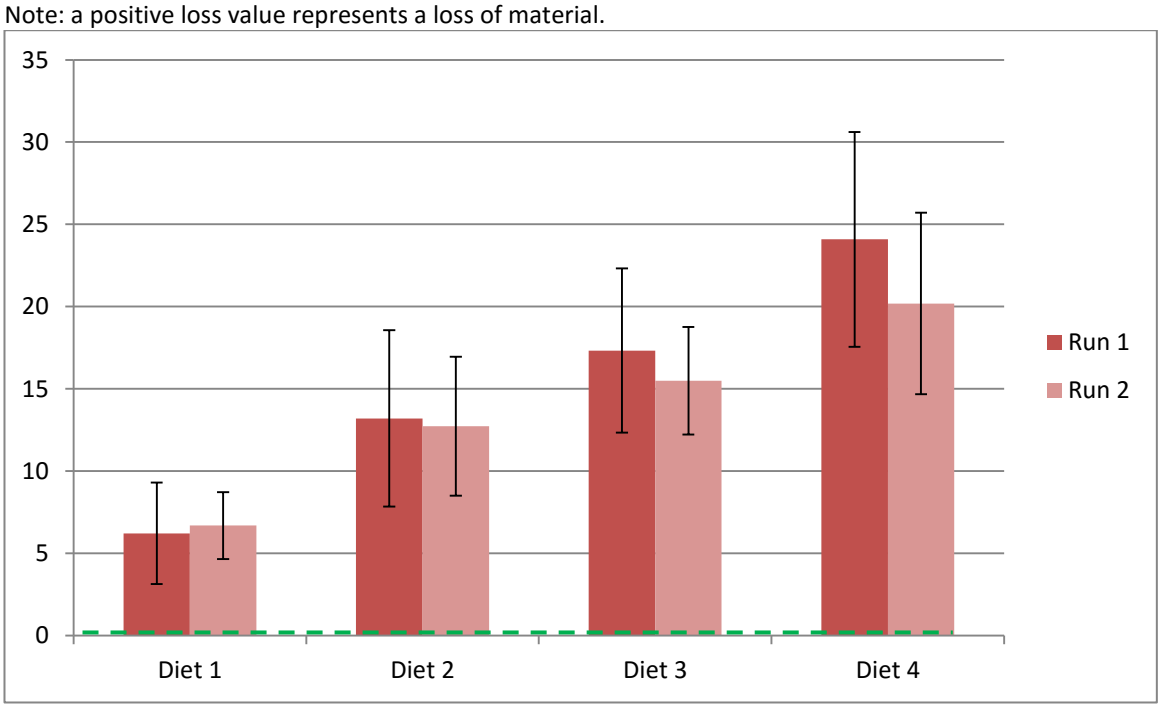
Readings Diet 3R	1	2	3	4	5	Mean	SD
Sample 1	16.58	17.67	18.05	16.99	17.89	17.44	0.76
Sample 2	14.76	14.71	14.74	14.96	14.47	14.74	0.03
Sample 3	16.93	18.62	17.21	18.03	17.10	17.58	0.91
Sample 4	12.66	12.59	12.89	12.68	12.72	12.71	0.16
Sample 5	14.58	14.34	14.45	14.35	14.58	14.46	0.12
Sample 6	21.21	20.33	19.94	20.88	20.10	20.49	0.65
Sample 7	9.84	9.90	9.96	10.02	9.81	9.90	0.06
Sample 8	16.54	17.01	16.17	16.99	16.16	16.57	0.42
Mean						**15.49**	
SD						3.27	

Table 4.6-28. Surface loss in µm of Ostrich eggshells relative to pre-diet readings (0.00 µm) after a 7 day, double dose diet.

Readings Diet 4	1	2	3	4	5	Mean	SD
Sample 1	19.68	19.72	19.34	19.56	19.08	19.48	0.27
Sample 2	29.12	31.12	26.92	28.32	29.12	28.92	1.52
Sample 3	25.56	26.96	28.36	21.56	22.56	25.00	2.88
Sample 4	33.48	35.68	31.48	33.48	34.08	33.64	1.51
Sample 5	18.84	22.24	19.04	20.04	21.84	20.40	1.57
Sample 6	21.62	19.32	20.64	20.08	21.28	20.59	0.92
Sample 7	13.65	14.65	14.62	14.31	14.25	14.30	0.40
Sample 8	29.69	30.68	30.08	33.93	27.66	30.41	2.27
Mean						**24.09**	
SD						6.53	

Readings Diet 4R	1	2	3	4	5	Mean	SD
Sample 1	21.32	21.38	23.89	18.84	18.83	20.85	2.11
Sample 2	16.90	16.78	16.56	16.50	16.97	16.74	0.21
Sample 3	15.72	18.78	16.62	16.22	18.24	17.12	1.33
Sample 4	10.80	12.42	11.85	11.58	11.88	11.71	0.59
Sample 5	28.93	29.43	26.42	28.43	28.48	28.34	1.15
Sample 6	24.16	26.30	22.34	23.69	24.80	24.26	1.46
Sample 7	17.89	16.84	17.05	16.40	17.69	17.17	0.61
Sample 8	24.97	25.92	25.94	27.51	22.50	25.37	1.84
Mean						**20.19**	
SD						5.51	

Figure 4.6-6. Surface loss values in µm (mean ± SD) relative to pre-diet values (0.00 µm) over 2 runs. Note: a positive loss value represents a loss of material.



A plot of the surface loss (figure 4.6-6), relative to pre-diet profile values shows that for all experimental runs a gradual increase in surface loss from diet 1 to diet 4 occurred. Mean surface loss ranged from 6.22 to 24.09 μm.

To investigate any effects of diets (diet 1, 2, 3 and 4) and experimental run on the amount of surface loss a 2-way analysis of variance on this data was undertaken. This revealed (table 4.6-29);

- Extremely highly significant effects of diet (P<0.0001).
- No effect of the experimental run (P=0.3460).
- No significant interaction of diet and experimental run (P=0.7253).

Table 4.6-29. 2-way ANOVA: Surface loss versus Diet and Experimental run.

Source of Variation	Df	Sum-of-squares	Mean square	F
Interaction	3	27.93	9.310	0.4409
Diet	3	1294	431.3	20.42
Experimental run	1	19.32	19.32	0.9150
Residual	32	675.7	21.12	

Follow-up analysis (Bonferroni post-test) was undertaken to determine the effect of the diet factor over all the groups (diet 1, 2, 3 and 4). The overall comparison between diets over 2 experimental runs is summarised in table 4.6-30.

Table 4.6-30. Overall comparison between the diets over 2 runs. Run 1 (a) and Run 2 (b).

(a)

Run 1	Diet 1	Diet 2	Diet 3	Diet 4
Diet 1				
Diet 2	NS			
Diet 3	**	NS		
Diet 4	***	**	NS	

(b)

Run 2	Diet 1	Diet 2	Diet 3	Diet 4
Diet 1				
Diet 2	NS			
Diet 3	**	NS		
Diet 4	***	*	NS	

NS: Non-significant (P > 0.05), *: Significant (P < 0.05), **: Highly significant (P < 0.01), ***: very highly significant (P < 0.001).

This reveals that, for the first experimental run 'Run 1', non-significant differences were found for (Diet 1 vs Diet 2), (Diet 2 vs Diet 3) and (Diet 3 vs Diet 4)(P > 0.05). While all other differences were either highly statistically significant (Diet 1 vs Diet 3) and (Diet 2 vs Diet 4)(P < 0.01) or very highly statistically significant (Diet 1 vs Diet 4)(P < 0.001).

For the second experimental run 'Run 2', non-significant differences were found for (Diet 1 vs Diet 2), (Diet 2 vs Diet 3) and (Diet 3 vs Diet 4)(P > 0.05). While all other differences were statistically significant (Diet 2 vs Diet 4)(P<0.05), highly statistically significant (Diet 1 vs Diet 3)(P<0.01) and very highly statistically significant (Diet 1 vs Diet 4)(P < 0.001).

Reproducibility of runs was thus good in terms of statistical significance.

C. Ion loss

Tables (A7-21 to A7-24)(Appendix 7) give the reference calcium and phosphate concentrations values in artificial saliva, the test beverage and the mixture solution along with the raw post-diet ion content of the resultant solutions for all diets using the immediate effect method.

The effect of each diet on the amount of human enamel's ion loss in mmol/day (i.e. diet 1-4) for 2 ions (i.e. calcium and phosphate) is summarised in tables 4.6-31 – 4.6-34, and is illustrated graphically in figure 4.6-7. In these tables ion concentrations for the resultant solutions were subtracted from the reference values of the artificial saliva/test beverage mixture.

Table 4.6-31. Calcium and phosphate ion loss of Ostrich eggshells in mmol/l relative to pre-diet readings (0.00 mmol/l) after a 5 day, single dose diet.*

Test cycle **Diet 1**	1	2	3	Sum	Mean	SD
Calcium	0.08	0.07	0.07	**0.22**	**0.07**	0.01
Phosphate	0.11	0.11	0.11	**0.33**	**0.11**	0.00

Table 4.6-32. Calcium and phosphate ion loss of Ostrich eggshells in mmol/l relative to pre-diet readings (0.00 mmol/l) after a 7 day, single dose diet.*

Test cycle **Diet 2**	1	2	3	4	5	Sum 3	Sum 5	Mean	SD
Calcium	0.09	0.10	0.09	0.12	0.12	**0.28**	**0.52**	**0.10**	0.02
Phosphate	0.09	0.13	0.13	0.13	0.16	**0.35**	**0.64**	**0.13**	0.02

Table 4.6-33. Calcium and phosphate ion loss of Ostrich eggshells in mmol/l relative to pre-diet readings (0.00 mmol/l) after a 5 day, double dose diet.*

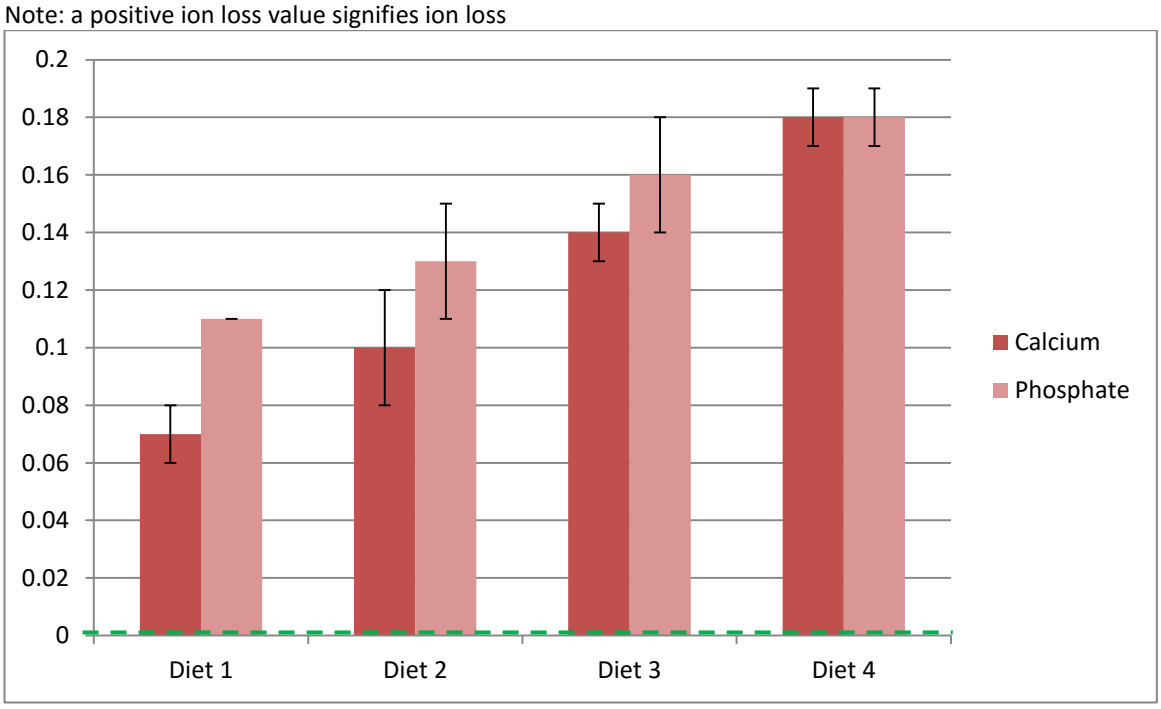
Test cycle **Diet 3**	1	2	3	Sum	Mean	SD
Calcium	0.14	0.14	0.13	**0.41**	**0.14**	0.01
Phosphate	0.14	0.18	0.15	**0.47**	**0.16**	0.02

Table 4.6-34. Calcium and phosphate ion loss of Ostrich eggshells in mmol/l relative to pre-diet readings (0.00 mmol/l) after a 7 day, double dose diet.*

Test cycle **Diet 4**	1	2	3	4	5	Sum 3	Sum 5	Mean	SD
Calcium	0.17	0.16	0.19	0.21	0.19	**0.52**	**0.92**	**0.18**	0.02
Phosphate	0.17	0.17	0.19	0.19	0.19	**0.53**	**0.91**	**0.18**	0.01

*Each reading represents the mean of 3 readings (based on chemical analysis testing of 3 samples for each day that consists of a test cycle).

Figure 4.6-7. Ion loss values in mmol/day (mean ± SD) for both ion types, calcium and phosphate.
Note: a positive ion loss value signifies ion loss



A plot of the mean ion loss (figure 4.6-7), shows that for all experimental runs a gradual increase in ion loss from diet 2 to diet 4 for both ions occurred. Mean calcium ion loss ranged from 0.07 to 0.18 mmol/day, while mean phosphate ion loss ranged from 0.11 to 0.18 mmol/day. Diet 4 resulted in equal amounts of loss of calcium and phosphate with both at 0.18 mmol.

To investigate any effects of diets (diet 1, 2, 3 and 4) and ion type on ion loss a 2-way analysis of variance on this data was undertaken. This revealed (table 4.6-35);

- Extremely highly significant effects of diet and ion type ($P<0.0001$).
- No significant interaction of the diet and ion type ($P>0.05$).

Table 4.6-35. 2-way ANOVA: Ion loss versus Diet and Ion type.

Source of Variation	Df	Sum-of-squares	Mean square	F
Interaction	3	0.001641	0.0005469	2.524
Diet	3	0.03277	0.01092	50.41
Ion type	1	0.003797	0.003797	17.52
Residual	24	0.0052	0.0002167	

A follow-up analysis (Bonferroni post-test) was undertaken to determine the effect of the diet factor over all the groups (diet 1, 2, 3 and 4). The overall comparison between diets for the 2 ion types is summarised in table 4.6-36.

Table 4.6-36. Overall comparison between the diets for both ions. Calcium (a) and phosphate (b).

(a)

Calcium	Diet 1	Diet 2	Diet 3	Diet 4
Diet 1				
Diet 2	**			
Diet 3	***	*		
Diet 4	***	***	**	

(b)

Phosphate	Diet 1	Diet 2	Diet 3	Diet 4
Diet 1				
Diet 2	*			
Diet 3	***	NS		
Diet 4	***	***	NS	

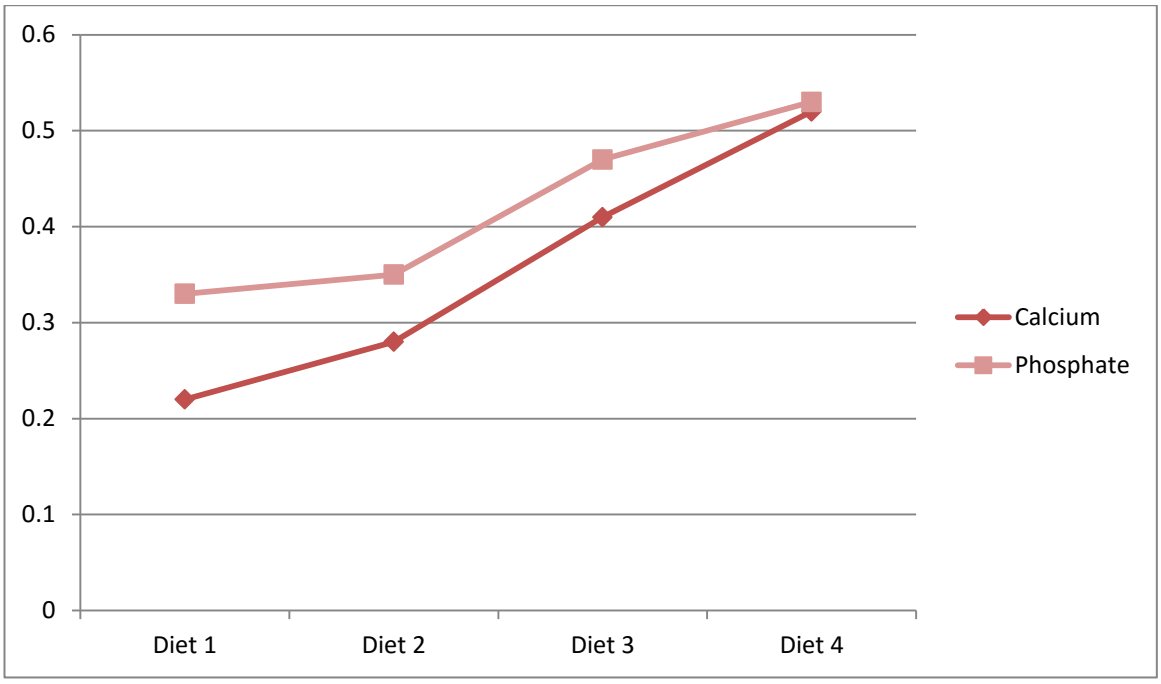
NS: Non-significant (P > 0.05), *: Significant (P < 0.05), **: Highly significant (P < 0.01), ***: very highly significant (P < 0.001).

This reveals that, for calcium ion, a significant difference was found for (Diet 2 vs Diet 3)($P < 0.05$), highly significant differences were found for (Diet 1 vs Diet 2) and (Diet 3 vs Diet 4)($P < 0.01$) and very highly significant differences for (Diet 1 vs Diet 3 and 4) and (Diet 2 vs Diet 4)($P < 0.001$).

For phosphate ion, non-significant differences were found for (Diet 2 vs Diet 3) and (Diet 3 vs Diet 4)($P > 0.05$), a significant difference was found for (Diet 1 vs Diet 2)($P < 0.05$), and all other differences were very highly significant ($P<0.001$).

Figure 4.6-4 illustrates the total ion loss over 3 test cycles for all diets.

Figure 4.6-8. Total ion loss observed in mmol over 3 days for each diet (based on the values of "Sum 3").



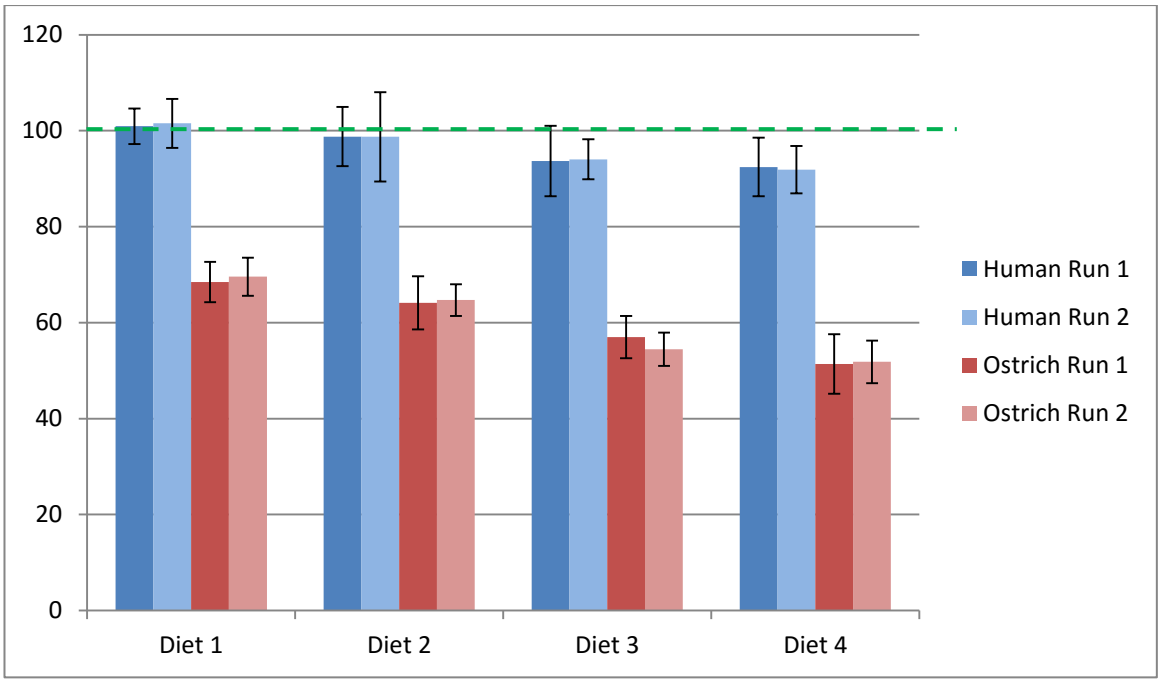
III. Human enamel versus Ostrich eggshell

This section reiterates the results previously reported for both human enamel and Ostrich eggshell so that a comparison can be made between the findings foe each substrate.

A. Surface Hardness

The effect of each diet on surface hardness percentage (i.e. diet 1-4) over 2 experimental runs for both substrates (i.e. Human enamel and Ostrich eggshell) is illustrated graphically in figure 4.6-9.

Figure 4.6-9. Percentage of surface hardness (mean ± SD) relative to pre-diet hardness values (100% hardness) over 2 runs for both substrates.



A plot of the change in surface hardness (figure 4.6-9), relative to the pre-diet hardness values (100 % relative hardness) shows that for all experimental runs Ostrich eggshell hardness percentage values were considerably less relative to Human enamel for all diets. Statistical analysis (1-way ANOVA) revealed;

For the first experimental runs of diets;

- A very highly significant effect of substrates for Diets 1, 2, 3 and 4 ($P<0.0001$).

For the second experimental runs of diets;

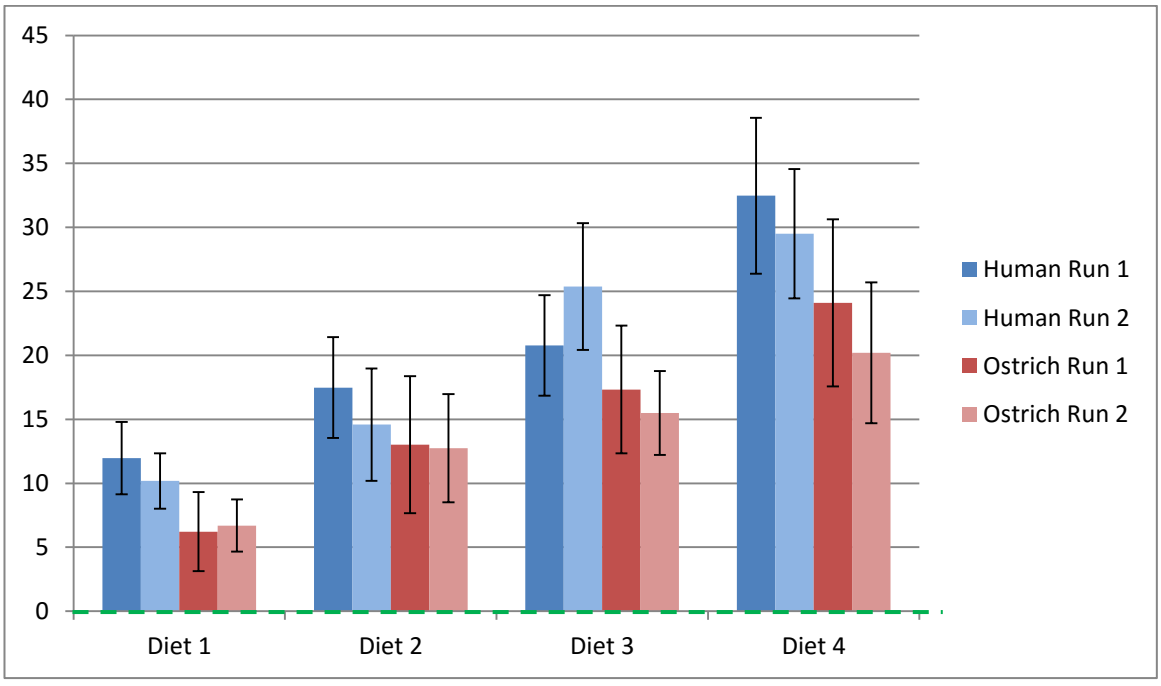
- A very highly significant effect of substrates for Diets 1, 2, 3 and 4 ($P<0.0001$).

Notwithstanding this the overall trend of surface hardness reduction is the same irrespective of the tissue.

B. Surface Loss

The effect of each diet on surface loss (i.e. diet 1-4) over 2 experimental runs for both substrates (i.e. Human enamel and Ostrich eggshell) is illustrated graphically in figure 4.6-10.

Figure 4.6-10. Surface loss in µm (mean ± SD) relative to pre-diet values (0.00 µm) over 2 runs for both substrates. Note: a positive loss value represents a loss of material.



A plot of the surface loss (figure 4.6-10), relative to pre-diet profile values shows that for all experimental runs Ostrich eggshell surface loss values were slightly less relative to Human enamel for all diets. Statistical analysis (1-way ANOVA) revealed;

For the first experimental runs of diets;

- No effect of substrates ($P>0.05$) for diets 1, 2 and 3
- A significant effect of substrates for Diet 4 ($P<0.05$).

For the second experimental runs of diets;

- No effect of substrates ($P>0.05$) for diets 1 and 2.
- Very highly significant effects of substrates for diets 3 and 4 ($P<0.0001$).

Table (4.6-37) summarises these differences between substrate groups.

Table 4.6-37. Overall comparison between both substrates for each diet.

Human vs Ostrich	Run 1	Run 2	Overall
Diet 1	NS	NS	NS
Diet 2	NS	NS	NS
Diet 3	NS	***	**
Diet 4	*	***	***

NS: Non-significant ($P > 0.05$), *: Significant ($P < 0.05$), **: Highly significant ($P < 0.01$), ***: very highly significant ($P < 0.001$).

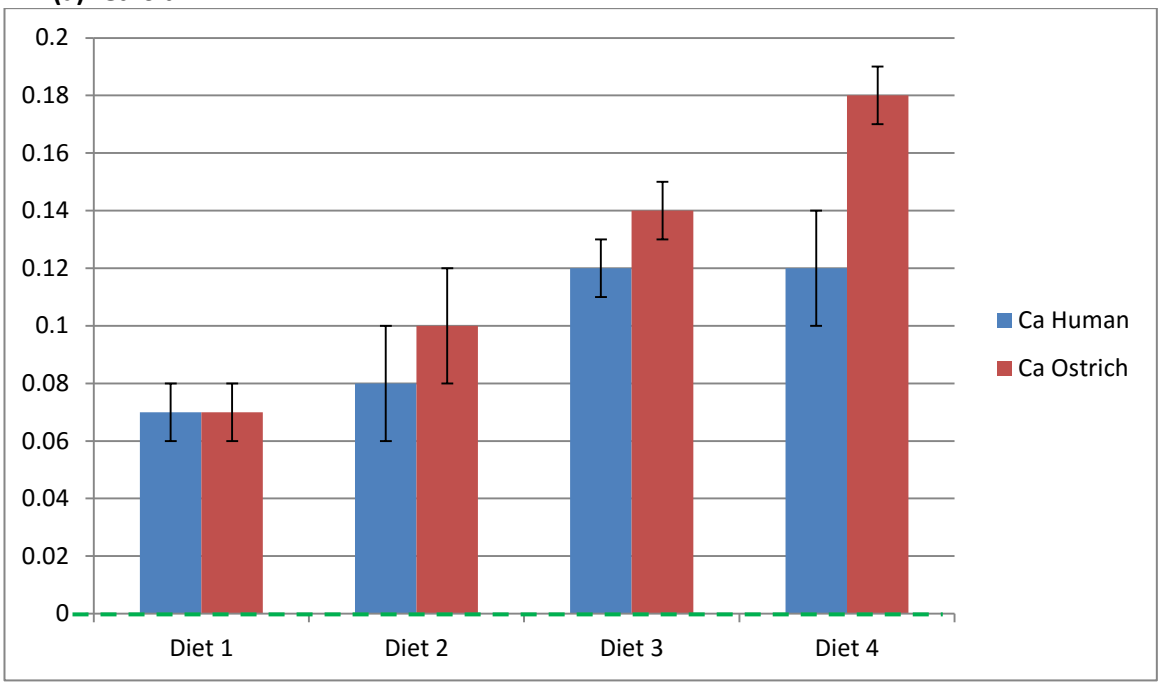
Despite of the level of statistical significance given (Diet 3 versus Diet 4), it appears that the overall trend of surface loss is comparable irrespective of the tissue.

C. Ion loss

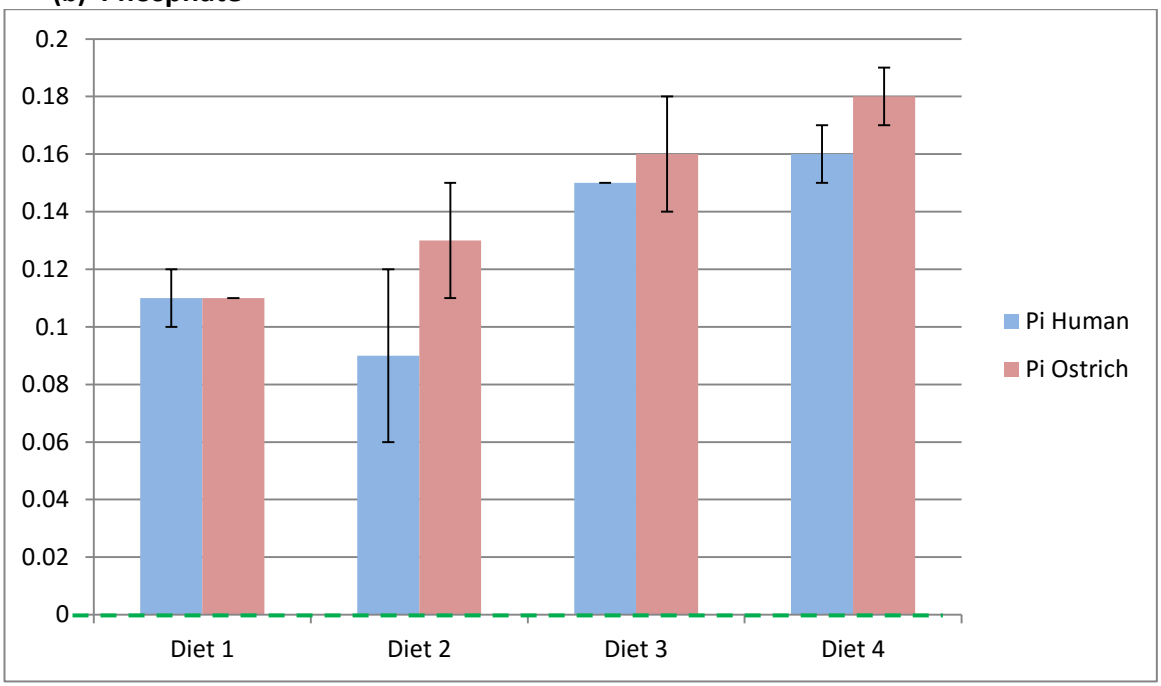
The effect of each diet on mean ion loss (calcium and phosphate) over 2 experimental runs for both substrates (i.e. Human enamel and Ostrich eggshell) is illustrated graphically in figure 4.6-11.

Figure 4.6-11. Ion loss in mmol/day (mean ± SD) over 2 runs for both substrates. (a) calcium and (b) phosphate.

(a) Calcium



(b) Phosphate



A plot of the calcium and phosphate ion loss (figure 4.6-11), shows that for all experimental runs Ostrich eggshell ion daily loss values were very close to the values of Human enamel for all diets except diet 4. In diet 4, Ostrich eggshell lost considerably more calcium and considerably less phosphate.

Statistical analysis (1-way ANOVA) revealed;

- No effect of substrate on phosphate ion loss in all diets ($P>0.05$)
- In diet 4, a very highly significant effect of substrate on calcium loss ($P<0.001$). All other differences were non-significant ($P>0.05$).

Table (4.6-38) summarises these differences.

Table 4.6-38. Overall comparison between both substrates for each diet.

Human vs Ostrich	Calcium	Phosphate
Diet 1	NS	NS
Diet 2	NS	NS
Diet 3	NS	NS
Diet 4	***	NS

NS: Non-significant ($P > 0.05$), *: Significant ($P < 0.05$), **: Highly significant ($P < 0.01$), ***: very highly significant ($P < 0.001$).

4.6.2 Accumulative effect method

In considering the results of the accumulative effect method it is helpful to summarise the testing conditions of this aspect of work that was conducted upon both samples of human enamel and Ostrich eggshell.

Table 4.6-39 summarises Diet 5

Table 4.6-39. Diet 5 summary.

Diet	Beverage	Duration	Rest cycles	Test cycles	Test cycle dose	Total number of cans per specimen	Preventive measure	Code
Extended-Single dose	*Coca-Cola*	9 days	4	5	1 can	5 cans	None	**Diet 5**

I. Human enamel and Ostrich Eggshell

A. Surface Hardness

Tables (A7-25 and A7-26)(Appendix 7) give the raw pre- and post-diet surface hardness values (Vickers Hardness) for all human enamel and Ostrich eggshell specimens tested using the accumulative effect method.

The effect of diet 5 on surface hardness of both substrates over a total period of 9 days (period 1 included 1 test cycle; period 2 included 2 additional test cycles [3 test cycles in total]; furthermore, period 3 included 2 additional test cycles [5 test cycles in total]) is summarised in tables 4.6-40 and 4.6-41, and is illustrated graphically in figure 4.6-12. In these tables the raw hardness values have been converted to % change in hardness relative to the pre-diet hardness value (100 %).

Table 4.6-40. Percentages of Surface hardness of human enamel relative to the pre-diet readings (100% Hardness) after the 1st period (a), 2nd period (b) and 3rd period (c) of an accumulative method diet.

(a)

Readings Diet 5	1	2	3	4	5	6	7	8	9	Mean	SD
Sample 1	77.33	73.79	74.85	82.30	78.04	92.23	82.66	62.43	78.40	78.01	8.01
Sample 2	77.86	87.43	87.75	66.69	83.28	78.81	87.43	82.64	80.41	81.37	6.66
Sample 3	72.66	89.86	94.07	80.73	100.39	72.66	70.55	90.21	98.64	85.53	11.65
Sample 4	77.91	61.21	91.13	99.82	94.95	69.56	90.78	88.34	101.56	86.14	13.77
Sample 5	106.18	111.04	99.29	100.91	105.37	73.35	106.99	90.78	78.62	96.95	13.26
Sample 6	101.01	98.43	84.84	89.25	98.80	110.19	97.33	80.80	98.07	95.42	8.97
Sample 7	86.83	108.94	100.50	112.56	118.99	116.58	111.35	99.69	90.85	105.15	11.30
Sample 8	81.63	99.41	77.27	93.24	81.27	85.62	95.42	77.27	107.39	88.73	10.64
Mean										**89.66**	
SD										8.95	

(b)

Readings Diet 5	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	103.94	90.46	77.33	95.07	82.30	81.59	92.94	89.40	99.69	73.43	88.62	9.83
Sample 2	72.11	76.26	76.58	81.37	83.60	82.01	85.84	81.37	91.26		81.16	5.67
Sample 3	85.30	76.17	85.30	80.38	103.90	61.43	87.75	97.23	85.65		84.79	12.09
Sample 4	67.82	74.78	84.17	87.65	88.69	77.91	83.13	65.39	77.91		78.61	8.23
Sample 5	98.48	104.96	117.93	115.90	113.07	83.89	93.61	118.33	102.12		105.37	12.01
Sample 6	108.72	66.11	80.44	110.92	92.56	107.62	94.76	88.88	116.43	94.03	96.05	15.42
Sample 7	94.47	101.70	108.94	103.71	89.24	92.06	106.53	111.35	97.28		100.59	7.76
Sample 8	100.86	90.70	83.08	80.18	92.51	88.16	98.68	88.16	86.71		89.90	6.73
Mean											**90.64**	
SD											9.40	

(c)

Readings Diet 5	1	2	3	4	5	6	7	8	9		Mean	SD
Sample 1	95.07	98.62	116.71	103.94	100.04	98.62	92.23	89.75	93.65	102.88	99.16	7.67
Sample 2	93.17	82.96	90.62	84.88	91.58	98.92	94.13	86.79	90.30	98.92	91.23	5.38
Sample 3	92.67	85.30	96.18	73.01	90.91	97.58	96.53	99.69	111.97	89.16	93.30	10.14
Sample 4	109.91	82.08	98.43	104.34	92.17	88.34	108.52	112.00	101.21	98.08	99.51	9.76
Sample 5	91.99	117.12	102.53	100.10	125.22	100.91	81.05	98.07	103.74	102.12	102.29	12.16
Sample 6	105.05	92.56	101.01	97.33	103.94	113.86	83.01	83.01	101.01	98.07	97.89	9.63
Sample 7	128.24	84.42	83.61	90.45	106.13	116.98	103.31	81.20	99.29	114.97	100.86	16.01
Sample 8	97.59	107.02	115.01	116.46	108.84	110.65	87.43	115.37	88.88	88.16	103.55	11.90
Mean											**98.47**	
SD											4.26	

Table 4.6-41. Percentages of Surface hardness of Ostrich eggshells relative to the pre-diet readings (100% Hardness) after the 1st period (a), 2nd period (b) and 3rd period (c) of an accumulative method diet.

(a)

Readings Diet 5	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	66.67	62.70	54.76	63.49	61.11	48.41	60.32	64.29	60.32		60.23	5.53
Sample 2	49.49	50.95	49.49	53.86	51.67	49.49	49.49	50.95	49.49	53.86	50.87	1.76
Sample 3	49.85	52.08	52.83	52.08	58.78	49.11	49.85	52.08	52.83	52.08	52.16	2.68
Sample 4	61.02	45.77	53.39	56.45	54.92	61.02	45.77	53.39	56.45	54.92	54.31	5.25
Sample 5	50.00	55.56	61.11	54.76	53.17	53.17	59.52				55.33	3.85
Sample 6	55.19	52.29	47.20	47.20	48.66	46.48	55.19	52.29	47.20	47.20	49.89	3.49
Sample 7	45.93	48.19	48.19	48.95	48.95	45.93	48.19	48.19	48.95	48.95	48.04	1.17
Sample 8	59.04	54.00	50.40	55.44	51.84	59.04	54.00	50.40	55.44	51.84	54.14	3.16
Mean											**53.12**	
SD											3.78	

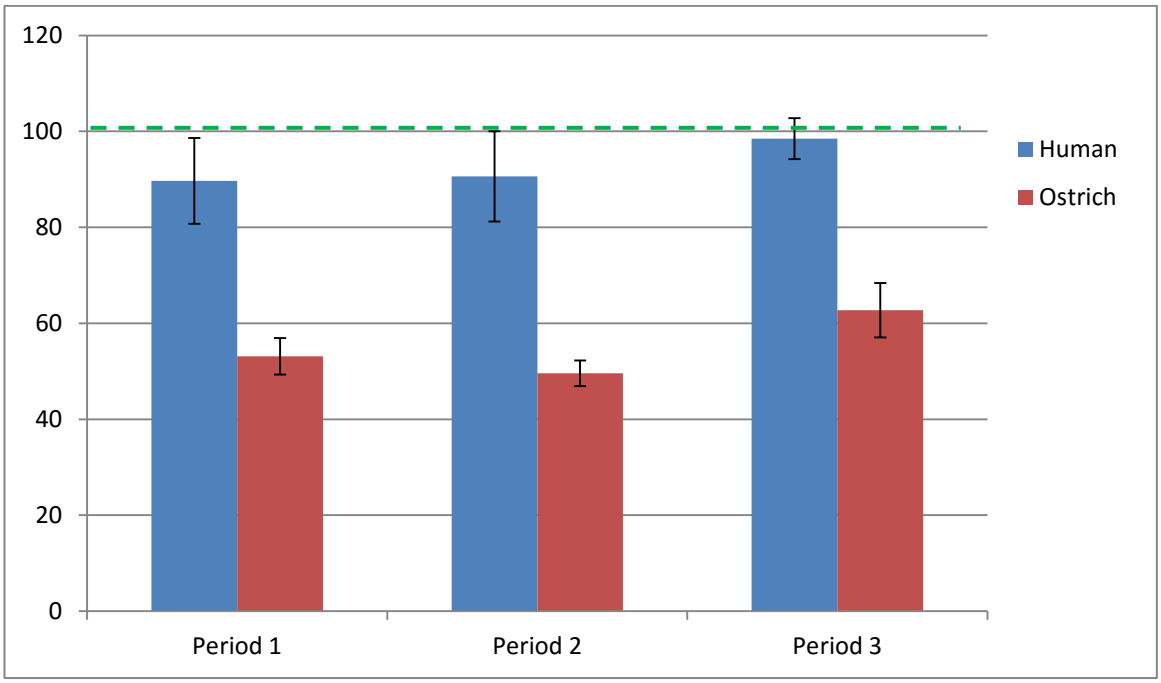
(b)

Readings Diet 5	1	2	3	4	5	6	7	8	Mean	SD
Sample 1	46.03	48.41	50.00	53.97	53.97	52.38	52.38	50.00	50.89	2.80
Sample 2	53.13	56.77	50.22	59.68	56.77	53.86	47.31	61.86	54.95	4.81
Sample 3	43.15	45.39	46.13	45.39	50.60	60.27	49.11	52.08	49.01	5.44
Sample 4	41.95	41.95	45.00	48.82	49.58	51.11	54.16	48.05	47.58	4.33
Sample 5	51.59	49.21	49.21	48.41	49.21	60.32	46.03		50.79	4.31
Sample 6	45.75	47.93	48.66	48.66	50.84	48.66	48.66	45.03	48.02	1.84
Sample 7	48.95	49.70	47.44	48.19	53.46	44.43	51.96	50.45	49.32	2.79
Sample 8	46.80	48.96	44.64	48.96	43.92	41.76	47.52	46.80	46.17	2.54
Mean									**49.59**	
SD									2.69	

(c)

Readings Diet 5	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	66.67	71.43	73.02	65.87	79.37	69.84	60.32	67.46	76.19	67.46	69.76	5.49
Sample 2	58.22	62.59	57.50	60.41	64.77	66.96	55.31	56.04	58.95	59.68	60.04	3.75
Sample 3	68.45	66.96	69.94	72.17	73.66	67.71	64.73	68.45	66.96	72.17	69.12	2.82
Sample 4	63.31	61.78	57.97	64.84	66.36	54.16	66.36	56.45	54.92	60.26	60.64	4.61
Sample 5	61.90	64.29	69.84	69.84	63.49	71.43	68.25		66.67	59.52	65.87	3.92
Sample 6	79.88	61.73	69.72	70.44	61.73	59.55	60.28	58.10	62.45	70.44	65.43	6.90
Sample 7	64.01	60.99	51.20	59.49	51.20	61.75	51.20	49.70	57.23	48.19	55.50	5.80
Sample 8	53.28	59.04	55.44	54.00	54.72	51.84	56.88	51.84	61.92	55.44	55.44	3.17
Mean											**62.73**	
SD											5.66	

Figure 4.6-12. Percentage of surface hardness (mean ± SD) relative to pre-diet hardness values (100% hardness) over 2 runs.



A plot of the change in surface hardness (figure 4.6-12), relative to the pre-diet hardness values (100 % relative hardness) shows that there was an overall reduction in hardness for both substrates. The reduction in Ostrich eggshell hardness was considerably higher relative to human enamel. Moreover, hardness values for both substrates considerably increased in period 3 compared to periods 1 and 2.

To investigate any effects of the time factor (periods 1, 2 and 3) on surface hardness percentage, statistical analysis using Repeated Measures ANOVA on this data was undertaken. This revealed (table 4.6-42);

- Very highly significant effects of time, substrate and replicates matching ($P<0.001$).
- No significant interaction of substrate and time ($P>0.05$)

Table 4.6-42. 2-way ANOVA: Hardness percentage versus Substrate and Time.

Source of Variation	Df	Sum-of-squares	Mean square	F
Interaction	2	65.28	32.64	1.636
Substrate	1	17126	17126	213.4
Time	2	1047	523.6	26.25
Matching	14	1123	80.24	4.023
Residual	28	558.5	19.95	

A follow-up analysis (Bonferroni post-test) was undertaken to determine the effect of the time factor over all the groups (periods 1, 2 and 3). Hardness percentage values of period 1 did not differ significantly from period 2 (P>0.05). Period 3, on the other hand, differed very highly significantly relative to period 1 (P<0.001) and highly significantly relative to period 2 (P < 0.01) in human enamel. Similarly, hardness values of period 1 did not differ significantly from period 2 (P > 0.05) in Ostrich eggshell, but it differed very highly significantly relative to periods 2 and 3 (P < 0.001).

Table 4.6-43. Overall comparison between time periods for both substrates. Human enamel (a) and Ostrich eggshell (b).

(a)

Human	Period 1	Period 2	Period 3
Period 1			
Period 2	NS		
Period 3	***	**	

(b)

Ostrich	Period 1	Period 2	Period 3
Period 1			
Period 2	NS		
Period 3	***	***	

NS: Non-significant (P > 0.05), *: Significant (P < 0.05), **: Highly significant (P < 0.01), ***: very highly significant (P < 0.001).

B. Surface Loss

Tables (A7-27 and A7-28)(Appendix 7) give the raw pre- and post-diet surface profile values for all human enamel and Ostrich eggshell specimens tested using the immediate effect method.

The effect of diet 5 on surface loss of both substrates over a total period of 9 days (period 1: 1 test cycle, period 2 = 3 test cycles, period 3 = 5 test cycles) is summarised in tables 4.6-44 and 4.6-45, and is illustrated graphically in figure 4.6-13. In these tables the raw profile values have been converted to surface loss values in µm relative to the pre-diet profile value (0.00 µm).

Table 4.6-44. Surface loss in µm of human enamel relative to the pre-diet readings (0.00 µm) after the 1st period (a), 2nd period (b) and 3rd period (c) of an accumulative method diet.

(a)

Readings Diet 5	1	2	3	Mean	SD
Sample 1	2.73	3.13	2.92	2.93	0.20
Sample 2	7.69	6.84	7.35	7.29	0.43
Sample 3	0.58	0.73	0.65	0.65	0.08
Sample 4	1.31	0.95	1.16	1.14	0.18
Sample 5	2.58	2.05	2.32	2.31	0.27
Sample 6	1.23	1.26	1.18	1.23	0.04
Sample 7	12.26	8.64	8.51	9.80	2.13
Sample 8	0.52	0.48	0.51	0.50	0.02
Mean				**3.23**	
SD				3.45	
Δ Loss				**3.23**	

(b)

Readings Diet 5	1	2	3	Mean	SD
Sample 1	9.25	8.93	9.62	9.27	0.35
Sample 2	11.50	9.38	13.09	11.32	1.86
Sample 3	2.98	3.40	3.20	3.19	0.21
Sample 4	3.90	2.24	2.16	2.76	0.98
Sample 5	8.67	10.27	11.08	10.00	1.23
Sample 6	3.97	5.33	4.48	4.60	0.69
Sample 7	13.37	15.83	14.00	14.40	1.28
Sample 8	1.82	1.75	1.70	1.76	0.06
Mean				**7.16**	
SD				4.68	
Δ Loss				**3.93**	

(c)

Readings Diet 5	1	2	3	Mean	SD
Sample 1	10.38	10.93	10.65	10.65	0.28
Sample 2	15.27	15.65	15.42	15.45	0.19
Sample 3	7.74	6.79	7.30	7.28	0.48
Sample 4	9.32	9.38	9.35	9.35	0.03
Sample 5	14.31	13.72	13.97	14.00	0.30
Sample 6	8.49	8.47	8.51	8.49	0.02
Sample 7	20.78	19.84	20.51	20.38	0.48
Sample 8	7.98	7.89	7.94	7.94	0.05
Mean				**11.69**	
SD				4.56	
Δ Loss				**4.53**	

Table 4.6-45. Surface loss in µm of Ostrich eggshells relative to the pre-diet readings (0.00 µm) after the 1st period (a), 2nd period (b) and 3rd period (c) of an accumulative method diet.

(a)

Readings Diet 5	1	2	3	Mean	SD
Sample 1	3.69	3.05	3.31	3.35	0.322
Sample 2	3.25	3.06	3.18	3.16	0.096
Sample 3	1.78	2.16	1.90	1.95	0.194
Sample 4	5.89	7.05	9.63	7.52	1.914
Sample 5	3.29	2.79	3.56	3.22	0.391
Sample 6	0.18	0.04	2.25	0.82	1.238
Sample 7	6.11	6.80	3.50	5.47	1.741
Sample 8	6.54	4.00	8.37	6.30	2.195
Mean				**3.97**	
SD				2.27	
Δ Loss				**3.97**	

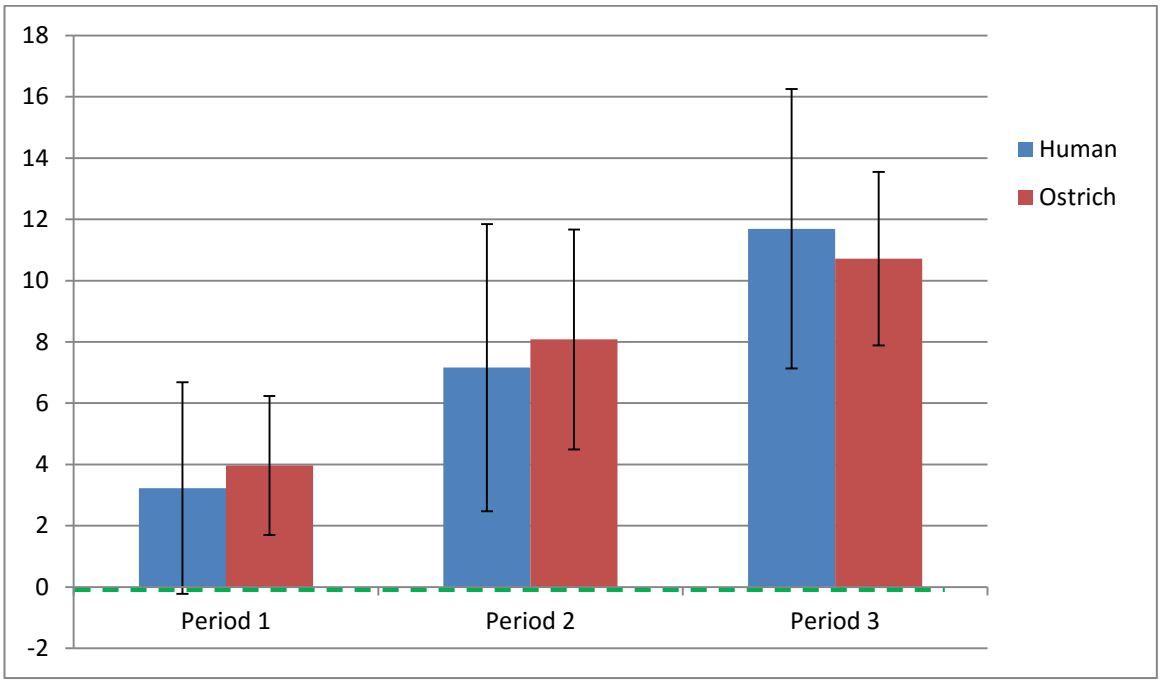
(b)

Readings Diet 5	1	2	3	Mean	SD
Sample 1	5.29	8.98	6.23	6.83	1.918
Sample 2	7.46	6.55	8.63	7.55	1.043
Sample 3	4.34	5.63	4.79	4.92	0.655
Sample 4	11.08	9.42	9.34	9.94	0.982
Sample 5	8.14	8.14	8.15	8.15	0.006
Sample 6	2.08	1.76	3.09	2.31	0.694
Sample 7	13.49	13.06	14.61	13.72	0.800
Sample 8	11.21	11.22	11.22	11.22	0.006
Mean				**8.08**	
SD				3.59	
Δ Loss				**4.11**	

(c)

Readings Diet 5	1	2	3	Mean	SD
Sample 1	10.34	10.11	10.21	10.22	0.115
Sample 2	12.03	10.95	11.56	11.51	0.542
Sample 3	7.36	6.70	6.28	6.78	0.544
Sample 4	11.79	11.78	11.27	11.61	0.297
Sample 5	11.50	11.48	11.31	11.43	0.104
Sample 6	6.82	7.32	5.28	6.47	1.063
Sample 7	12.39	15.65	15.13	14.39	1.751
Sample 8	16.58	13.06	10.33	13.32	3.133
Mean				**10.72**	
SD				2.83	
Δ Loss				**2.64**	

Figure 4.6-13. Percentage of surface hardness (mean ± SD) relative to pre-diet hardness values (100% hardness) over 2 runs. Note: a positive loss value represents a loss of material.



A plot of the surface loss (figure 4.6-13), relative to pre-diet profile values shows that there was an overall loss of structure for both substrates that gradually increased with time. Moreover, the loss in both substrates was comparable.

To investigate the effect of the time factor (periods 1, 2 and 3) on surface loss, statistical analysis using Repeated Measures ANOVA on this data was undertaken. This revealed;

- Very highly significant effects of time and replicates (matching) ($P<0.001$).
- No effect of substrate was found ($P=0.8985$).
- No significant interaction of substrate and time ($P>0.05$).

Table 4.6-46. 2-way ANOVA: Surface loss versus Substrate and Time.

Source of Variation	Df	Sum-of-squares	Mean square	F
Interaction	2	8.761	4.381	2.652
Substrate	1	0.6234	0.6234	0.01687
Time	2	462.8	231.4	140.1
Matching	14	517.3	36.95	22.37
Residual	28	46.26	1.652	

Follow-up analysis (Bonferroni post-test) was undertaken to determine the effect of the time factor over all the groups (periods 1, 2 and 3). All periods showed very highly significant differences among each other. Table (4.6-47) summarises these differences.

Table 4.6-47. Overall comparison between time periods for both substrates. Human enamel (a) and Ostrich eggshell (b).

(a)

Human	Period 1	Period 2	Period 3
Period 1			
Period 2	***		
Period 3	***	***	

(b)

Ostrich	Period 1	Period 2	Period 3
Period 1			
Period 2	***		
Period 3	***	***	

NS: Non-significant (P > 0.05), *: Significant (P < 0.05), **: Highly significant (P < 0.01), ***: very highly significant (P < 0.001).

4.6.3 Finding summary for Saltus Diets I – immediate and accumulative effects

From the ongoing results it is clear that;

- Human enamel surface hardness loss was detected only in double-dosed diets (diet 3 and 4), while single-dosed diets had no 'final' effect on hardness (diet 1 and 2).
- Human enamel tissue integrity (i.e. surface loss) and ionic composition both were negatively affected by all diets delivered by Saltus. Yet, the effects were more apparent in double-dosed diets (diet 3 and 4).
- Ostrich eggshell surface hardness, tissue integrity and ionic composition all were negatively affected by all diets delivered by Saltus.
- Overall, erosive effects increased from short to long durations and from single-dosed to double-dosed with the least effect detected in shorter/single-dosed diets and the most effects in longer/double-dosed diets.
- Ostrich eggshell's response to erosive challenges in terms of tissue integrity and ionic composition was comparable to human enamel especially in single-dosed diets.
- Although the susceptibility to erosive challenges in terms of hardness for human enamel and Ostrich eggshell differed; both followed the same pattern.
- The accumulative method revealed an intermediate phase where relative hardness drops considerably before it bounces back to expected levels of hardness loss (period 1 and 2 versus period 3).

4.7 Saltus Diets II – Applications of Saltus

4.7.1 The effect of Regenerate™ NR-5 Boosting Serum and Fluor Protector™ S

In order to assist the reader the diets used in this aspect of the work are summarised in table (4.7-1) Diet 1, Diet 6 and Diet 7. Diet 1 is the control against which the performances of Regenerate and Fluor Protector S are judged.

Table 4.7-1. Diet 1, Diet 6 and Diet 7 highlights

Diet	Beverage	Duration	Rest cycles	Test cycles	Test cycle dose	Total number of cans per specimen	Preventive measure	Code
Short-Single dose	*Coca-Cola*	5 days	2	3	1 can	3 cans	None	**Diet 1**
Short-Single dose	*Coca-Cola*	5 days	2	3	1 can	3 cans	Regenerate™	**Diet 6**
Short-Single dose	*Coca-Cola*	5 days	2	3	1 can	3 cans	Fluor Protector™ S	**Diet 7**

The results were presented according to the test substrate.

I. Human enamel

A. Surface hardness

Tables (A7-29 and A7-30)(Appendix 7) give the raw pre- and post-diet surface hardness values (Vickers Hardness) for all human enamel specimens tested using this method.

The effects of using Regenerate™ NR-5 Boosting Serum and Fluor Protector™ S on enamel surface hardness are summarised in tables 4.7-2 and 4.7-3, and illustrated graphically in figure 4.7-1 relative to the two experimental runs of Diet 1. In these tables the raw hardness values have been converted to % change in hardness relative to the pre-diet hardness value (100 %).

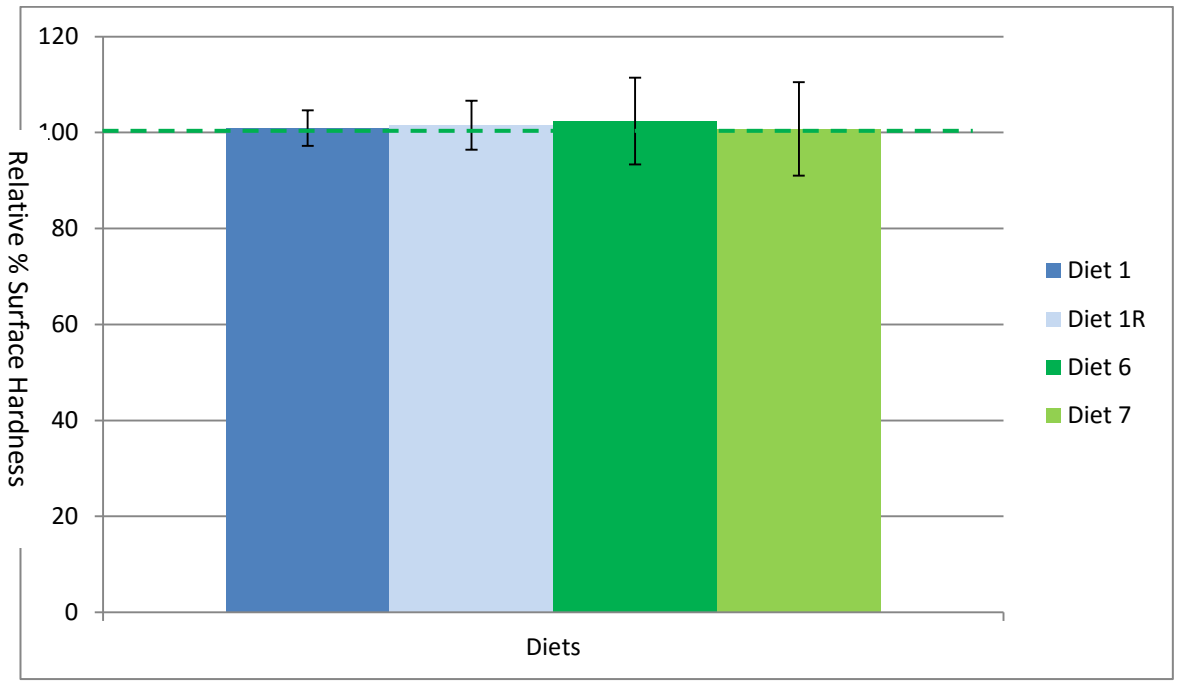
Table 4.7-2. Percentages of Surface hardness of human enamel relative to the pre-diet readings (100% Hardness) after a 3 day, single dose diet. Specimens were treated with Regenerate™.

Readings Diet 6	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	94.48	103.00	93.00	97.07	101.52	93.74	89.66	91.89	93.37	110.78	96.85	6.43
Sample 2	99.22	95.29	95.29	99.61	98.82	114.90	109.41	119.22	84.71	112.94	102.94	10.76
Sample 3	89.85	92.93	84.05	76.87	87.46	74.48	89.85	73.45	100.10	86.44	85.55	8.53
Sample 4	103.77	114.58	98.96	105.77	101.36	89.34	104.57	89.34	110.98	120.59	103.93	10.01
Sample 5	85.75	94.15	99.75	90.30	114.46	97.65	108.16	88.90	97.65	95.20	97.20	8.73
Sample 6	95.77	116.09	123.13	104.89	88.72	102.40	122.31	101.99	128.52	100.75	108.46	13.20
Sample 7	107.55	107.55	121.10	115.35	98.93	112.89	113.71	113.71	123.97	121.10	113.59	7.54
Sample 8	116.54	86.61	119.29	120.47	106.30	102.76	110.63	112.60	112.60	118.90	110.67	10.22
Mean											**102.40**	
SD											9.06	

Table 4.7-3. Percentages of Surface hardness of human enamel relative to the pre-diet readings (100% Hardness) after a 3 day, single dose diet. Specimens were treated with Fluor Protector™ S.

Readings Diet 7	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	95.28	100.35	94.65	95.92	88.64	94.97	78.19	82.62	93.38	89.59	91.36	6.71
Sample 2	116.60	104.15	102.26	116.60	101.13	101.13	116.60	102.26	104.15	116.60	108.15	7.35
Sample 3	113.34	118.43	122.84	115.71	118.43	115.37	93.99	131.66	118.09	122.84	117.07	9.63
Sample 4	94.63	103.03	104.86	100.84	92.44	90.24	86.96	107.42	96.82	104.13	98.14	6.93
Sample 5	99.18	114.29	99.84	119.21	90.97	110.67	90.97	99.18	99.84	114.29	103.84	10.04
Sample 6	88.68	94.13	86.97	81.86	90.38	88.68	94.13	86.97	81.86	90.38	88.40	4.26
Sample 7	101.40	105.56	116.16	110.86	98.37	94.97	101.40	105.56	116.16	110.86	106.13	7.27
Sample 8	103.91	97.78	88.96	92.02	82.44	82.44	92.02	88.96	97.78	103.91	93.02	7.76
Mean											**100.76**	
SD											9.76	

Figure 4.7-1. Percentage of surface hardness (mean ± SD) relative to pre-diet hardness values (100% hardness) over 2 runs.



A plot of the change in surface hardness (figure 4.7-1), relative to the pre-diet hardness values (100 % relative hardness) shows that there was a slight increase in hardness in diet 6 relative to both runs of diet 1. Overall, Hardness percentage values for all diets were very close to 100%.

To investigate any effects of the preventive measure on surface hardness percentage, statistical analysis using 1-way ANOVA was carried out. No significant effect of diet was found ($P>0.05$) on the surface hardness. Thus in this respect the preventive measures investigated (Regenerate, Fluor Protector S) had no statistical effect upon the observed surface hardness.

B. Surface Loss

Tables (A7-31 and A7-32)(Appendix 7) give the raw pre- and post-diet surface profile values for all human enamel specimens tested using this method.

The effects of using Regenerate™ NR-5 Boosting Serum and Fluor Protector™ S on surface loss are summarised in tables 4.7-4 and 4.7-5, and illustrated graphically in figure 4.7-2. In these tables the raw profile values have been converted to surface loss values in µm relative to the pre-diet profile value (0.00 µm).

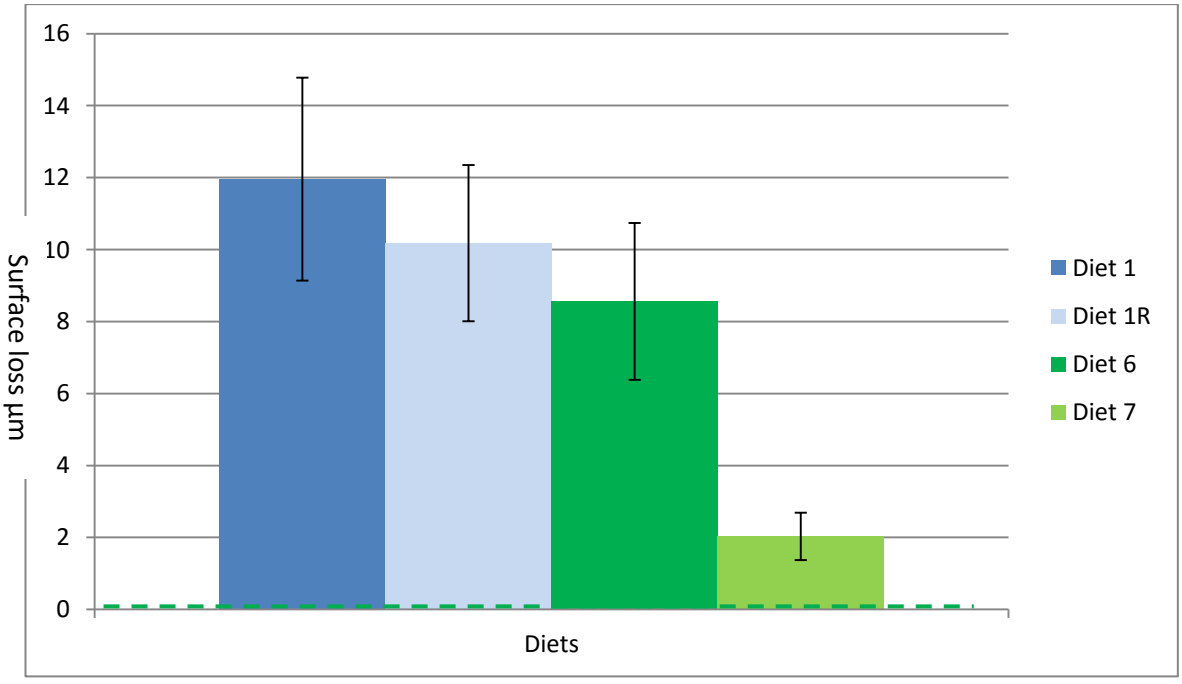
Table 4.7-4. Surface loss in µm of human enamel relative to pre-diet readings (0.00 µm) after a 3 day, single dose diet. Specimens were treated with Regenerate™.

Readings Diet 6	1	2	3	Mean	SD
Sample 1	9.01	9.41	8.30	8.91	0.56
Sample 2	10.13	10.15	10.14	10.14	0.01
Sample 3	8.55	8.14	8.31	8.34	0.21
Sample 4	12.17	11.83	12.10	12.04	0.18
Sample 5	4.86	5.29	5.09	5.08	0.22
Sample 6	10.12	9.70	9.85	9.89	0.21
Sample 7	7.04	6.60	6.89	6.84	0.22
Sample 8	6.77	7.94	7.13	7.28	0.60
Mean				**8.56**	
SD				2.18	

Table 4.7-5. Surface loss in µm of human enamel relative to pre-diet readings (0.00 µm) after a 3 day, single dose diet. Specimens were treated with Fluor Protector™ S.

Readings Diet 7	1	2	3	Mean	SD
Sample 1	2.33	2.43	2.29	2.35	0.07
Sample 2	1.97	1.92	0.97	1.62	0.56
Sample 3	1.68	2.70	1.86	2.08	0.54
Sample 4	1.36	1.55	1.41	1.44	0.10
Sample 5	1.08	1.44	1.26	1.26	0.18
Sample 6	1.56	1.90	1.76	1.74	0.17
Sample 7	2.52	2.44	2.28	2.42	0.12
Sample 8	3.70	3.13	3.07	3.30	0.35
Mean				**2.03**	
SD				0.66	

Figure 4.7-2. Surface loss values in µm (mean ± SD) relative to pre-diet values (0.00 µm). Note: a positive value of surface loss indicates a loss of material.



A plot of the mean surface loss (figure 4.7-2), relative to pre-diet profile values shows that, overall, surface loss values were reduced in diet 6 and 7 relative to the two runs of diet 1.

To investigate any effects of the preventive measure factor on the amount of surface loss, statistical analysis using 1-way ANOVA was carried out. Very highly significant effect of diet was found ($P<0.0001$) on surface loss.

These effects were localised by a (Tukey post-test) to determine the effect of the diet factor upon all the groups (diet 1, 1R, 6 and 7). The Tukey comparison between diets is summarised in table (4.7-6).

Table 4.7-6. Tukey comparison between the diets.

Diets	Diet 1	Diet 1R	Diet 6	Diet 7
Diet 1				
Diet 1R	NS			
Diet 6	*	NS		
Diet 7	***	***	***	

NS: Non-significant ($P > 0.05$), *: Significant ($P < 0.05$), **: Highly significant ($P < 0.01$), ***: very highly significant ($P < 0.001$).

This reveals that the differences found between Diet 7 and both runs of diet 1 were very highly statistically significant ($P<0.001$). Thus Fluor Protector significantly inhibited surface loss of human enamel.

II. Ostrich Eggshell

A. Surface hardness

Tables (A7-33 to A7-34)(Appendix 7) give the raw pre- and post-diet surface hardness values for all human enamel specimens tested using this method.

The effects of using Regenerate™ NR-5 Boosting Serum and Fluor Protector™ S on the surface hardness of Ostrich eggshell are summarised in tables 4.7-7 and 4.7-8, and illustrated graphically in figure 4.7-3 relative to the two runs of Diet 1.

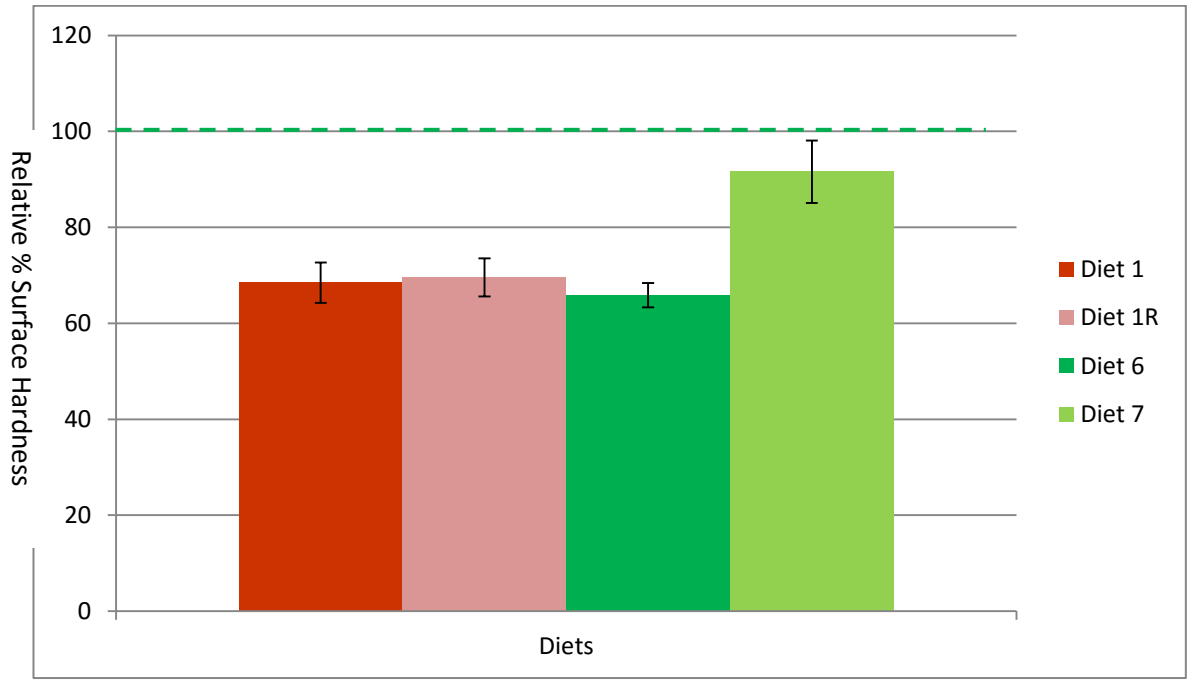
Table 4.7-7. Percentages of Surface hardness of Ostrich eggshells relative to the pre-diet readings (100% Hardness) after a 3 day, single dose diet. Specimens were treated with Regenerate™.

Readings Diet 6	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	64.54	64.54	63.75	62.15	62.95	63.75	63.75	70.12	67.73	68.53	65.18	2.65
Sample 2	64.44	65.22	62.89	69.10	61.34	69.88	61.34	64.44	63.66	62.11	64.44	2.97
Sample 3	65.49	63.17	63.17	67.03	63.94	73.19	72.42	74.73	82.43	77.81	70.34	6.77
Sample 4	60.69	66.47	62.14	61.42	64.31	57.08	61.42	55.64	64.31	64.31	61.78	3.38
Sample 5	70.04	59.14	68.48	75.49	73.15	60.70	59.92	66.15	65.37	80.93	67.94	7.16
Sample 6	68.01	68.78	62.60	61.05	69.55	65.69	69.55	61.05	66.46	65.69	65.84	3.29
Sample 7	68.97	68.18	68.18	58.78	57.99	68.18	61.13	66.61	63.48	67.40	64.89	4.21
Sample 8	67.49	68.94	70.39	60.23	63.13	70.39	67.49	61.68	62.41	71.84	66.40	4.18
Mean											**65.85**	
SD											2.53	

Table 4.7-8. Percentages of Surface hardness of Ostrich eggshells relative to the pre-diet readings (100% Hardness) after a 3 day, single dose diet. Specimens were treated with Fluor Protector™ S.

Readings Diet 7	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	86.33	91.93	95.92	91.13	83.93	95.92	96.72	103.92	96.72	104.72	94.72	6.69
Sample 2	96.67	116.17	99.92	90.17	101.54	92.61	88.55	87.73	98.29	99.92	97.16	8.37
Sample 3	114.59	91.51	108.82	93.16	91.51	108.00	89.04	93.16	112.94	91.51	99.42	10.27
Sample 4	76.16	86.82	77.68	85.30	76.16	83.02	86.06	82.25	86.82	90.63	83.09	4.99
Sample 5	96.08	92.39	85.00	93.13	79.08	99.78	107.91	105.69	85.00	96.82	94.09	9.21
Sample 6	104.59	93.32	82.86	87.69	115.04	90.91	95.74	94.93	86.08	84.47	93.56	9.91
Sample 7	80.97	84.31	89.32	81.80	78.46	75.96	90.15	75.13	75.96	80.97	81.30	5.33
Sample 8	78.35	98.57	96.88	103.62	106.15	74.14	74.14	87.62	84.25	90.14	89.39	11.74
Mean											**91.59**	
SD											6.49	

Figure 4.7-3. Percentage of surface hardness (mean ± SD) relative to pre-diet hardness values (100% hardness) over 2 runs.



A plot of the change in mean surface hardness (figure 4.7-3), relative to the pre-diet hardness values (100 % relative hardness) shows overall, there was a considerable increase in the surface hardness of the Ostrich eggshell following Diet 7 relative to all other diets.

To investigate any effects of the preventive measure on the surface hardness, statistical analysis using 1-way ANOVA was carried out. Very highly significant effects of diet were found ($P<0.0001$) on surface hardness.

These effects were localised by a Tukey post-test to determine the effect of the diet over all the groups (diet 1, 1R, 6 and 7). This is shown in table (4.7-9).

Table 4.7-9. Overall comparison between the diets.

Diets	Diet 1	Diet 1R	Diet 6	Diet 7
Diet 1				
Diet 1R	NS			
Diet 6	NS	NS		
Diet 7	***	***	***	

NS: Non-significant ($P > 0.05$), *: Significant ($P < 0.05$), **: Highly significant ($P < 0.01$), ***: very highly significant ($P < 0.001$).

This reveals that very highly statistically significant differences were found between Diet 7 and all other diets ($P<0.001$). All other differences were found to be non-significant ($P<0.05$). Thus Fluor Protector S significantly inhibited the reduction in surface hardness of Ostrich eggshell.

B. Surface Loss

Tables (A7-35 to A7-36)(Appendix 7) give the raw pre- and post-diet surface profile values for all Ostrich eggshell specimens tested using the immediate effect method.

The effects of using Regenerate™ NR-5 Boosting Serum and Fluor Protector™ S on surface loss are summarised in tables 4.7-10 and 4.7-11, and illustrated graphically in figure 4.7-4.

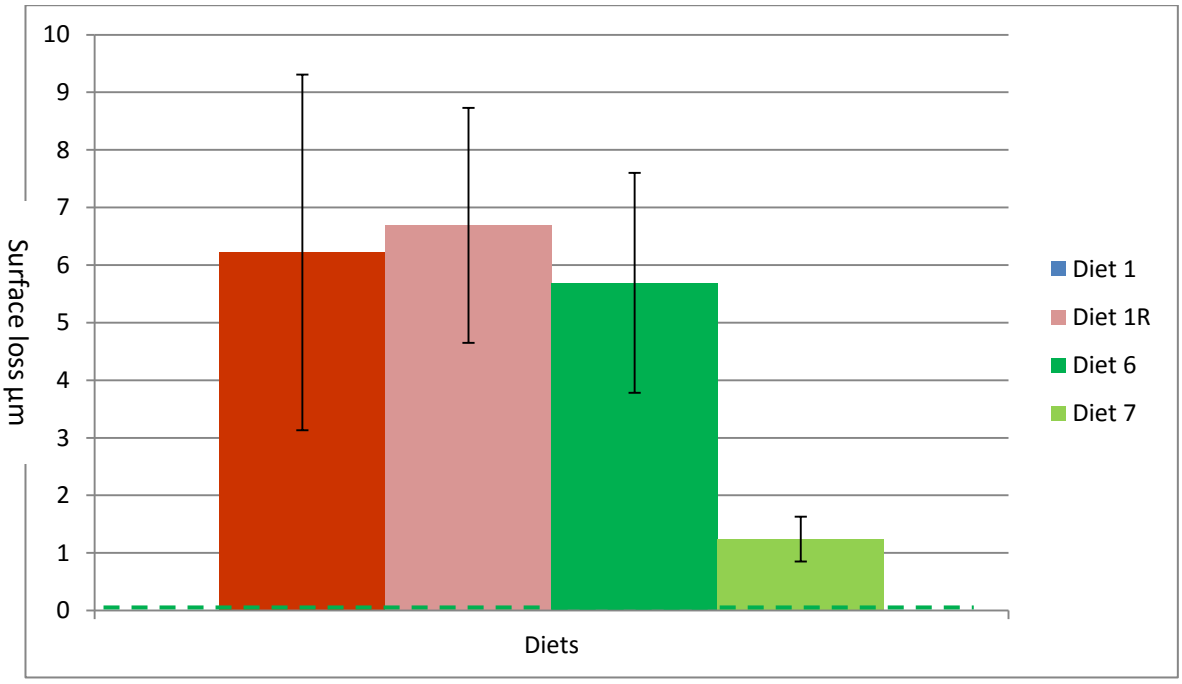
Table 4.7-10. Surface loss in µm of Ostrich eggshells relative to pre-diet readings (0.00 µm) after a 3 day, single dose diet. Specimens were treated with Regenerate™.

Readings Diet 6	1	2	3	Mean	SD
Sample 1	5.98	5.57	3.61	5.05	1.27
Sample 2	4.97	5.73	5.32	5.34	0.38
Sample 3	5.99	5.72	6.42	6.04	0.35
Sample 4	3.09	2.61	2.87	2.86	0.24
Sample 5	8.32	7.46	7.98	7.92	0.43
Sample 6	7.55	8.18	7.85	7.86	0.32
Sample 7	7.27	6.80	7.02	7.03	0.24
Sample 8	4.10	3.18	2.86	3.38	0.64
Mean				**5.69**	
SD				1.91	

Table 4.7-11. Surface loss in µm of Ostrich eggshells relative to pre-diet readings (0.00 µm) after a 5 day, single dose diet. Specimens were treated with Fluor Protector™ S.

Readings Diet 7	1	2	3	Mean	SD
Sample 1	1.13	1.26	1.46	1.28	0.17
Sample 2	0.53	0.79	0.62	0.65	0.13
Sample 3	0.59	1.16	0.83	0.86	0.29
Sample 4	1.43	0.63	1.12	1.06	0.40
Sample 5	2.48	2.10	1.04	1.87	0.75
Sample 6	1.26	1.62	1.63	1.50	0.21
Sample 7	1.65	1.52	1.37	1.51	0.14
Sample 8	1.31	1.62	0.58	1.17	0.53
Mean				**1.24**	
SD				0.39	

Figure 4.7-4. Surface loss values in µm (mean ± SD) relative to pre-diet values (0.00 µm).



A plot of the mean surface loss (figure 4.7-4), relative to the pre-diet profile values shows that overall, surface loss values were reduced in diet 6 and 7 relative to the two runs of diet 1.

To investigate any effects of the preventive measure on the amount of surface loss, statistical analysis using 1-way ANOVA was carried out. Very highly significant effects of diet was found ($P<0.0001$) on surface loss.

These effects were localised by a Tukey post-test to determine the effect of the diet over all the groups (diet 1, 1R, 6 and 7). This is shown in table (4.7-12).

Table 4.7-12. Overall comparison between the diets.

Diets	Diet 1	Diet 1R	Diet 6	Diet 7
Diet 1				
Diet 1R	NS			
Diet 6	NS	NS		
Diet 7	***	***	**	

NS: Non-significant (P > 0.05), *: Significant (P < 0.05), **: Highly significant (P < 0.01), ***: very highly significant (P < 0.001).

This reveals that very highly statistically significant differences were found between Diet 7 and both runs of Diet 1. In addition, the difference between Diet 7 and Diet 6 was found to be highly significant ($P<0.01$). All other differences were found to be non-significant ($P<0.05$). Thus upon Ostrich eggshell Fluor Protector significantly impeded surface loss.

4.7.2 Finding summary for Saltus Diets II – Applications of Saltus

From the foregoing results it is clear that;

- The effects of different preventive measures were detected in terms of both surface hardness and tissue integrity when Ostrich eggshell specimens were tested.
- The effects of different preventive measures were detected in terms of tissue integrity rather than surface hardness when human enamel specimens were tested.
- Fluor Protector™ S has shown considerable preventive effects on the erosive effects of the beverage tested. Nevertheless, Regenerate™ NR-5 Boosting Serum failed to demonstrate such an effect.

4.7.3 The effect of a different test beverage

Much of the work with Saltus used Coca-Cola. To further test the system the citric acid based drink Sprite was tested. The diets involved are summarised in table 4.7-13.

Table 4.7-13. Diet 1 and Diet 8 summary

Diet	Beverage	Duration	Rest cycles	Test cycles	Test cycle dose	Total number of cans per specimen	Preventive measure	Code
Short-Single dose	*Coca-Cola*	5 days	2	3	1 can	3 cans	None	**Diet 1**
Short-Single dose	*Sprite*	5 days	2	3	1 can	3 cans	None	**Diet 8**

The results of this work are reported according to the tissue of the substrate.

I. Human enamel

A. Surface hardness

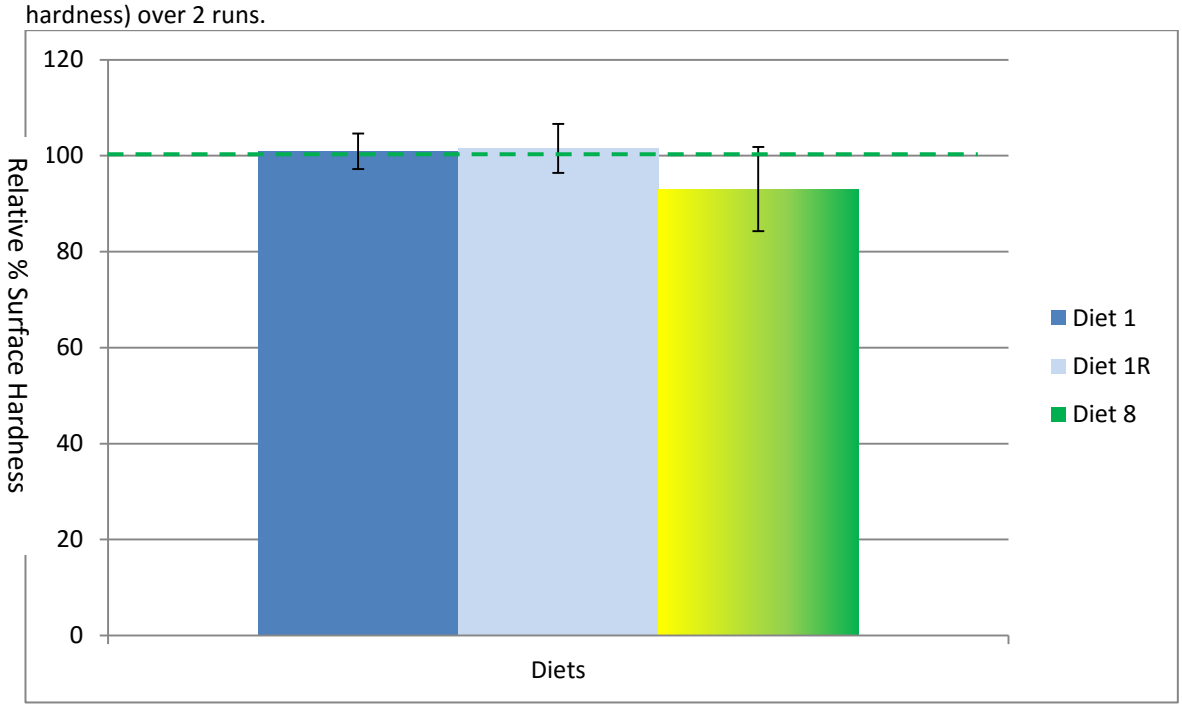
Table (A7-37)(Appendix 7) gives the raw pre- and post-diet surface hardness values (Vickers Hardness) for all human enamel specimens tested using this method.

The effects of the test beverage on surface hardness are summarised in table 4.7-14, and illustrated graphically in figure 4.7-5 relative to the two runs of Diet 1.

Table 4.7-14. Percentages of Surface hardness of human enamel relative to the pre-diet readings (100% Hardness) after a 3 day, single dose diet.

Readings **Diet 8**	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	103.07	81.31	92.01	83.10	89.87	93.79	93.79	86.66	92.72	76.32	89.27	7.65
Sample 2	86.59	67.78	94.75	80.91	79.49	92.97	90.13	95.46	80.55	73.10	84.17	9.40
Sample 3	105.23	75.21	77.33	87.57	86.51	92.16	87.22	86.51	104.52	90.75	89.30	9.78
Sample 4	101.63	99.59	93.47	106.53	112.24	99.18	104.08	100.82	115.10	101.22	103.39	6.42
Sample 5	89.51	67.05	78.10	82.74	84.88	97.00	77.75	75.96	70.26	88.09	81.13	9.14
Sample 6	82.68	99.36	94.39	110.01	90.13	92.97	99.01	92.62	92.26	113.20	96.66	9.17
Sample 7	94.99	106.99	112.64	94.99	96.75	94.28	88.98	93.93	86.51	75.92	94.60	10.17
Sample 8	88.16	118.78	112.24	109.39	100.41	124.90	102.45	83.27	109.39	110.20	105.92	12.82
Mean											**93.06**	
SD											8.76	

Figure 4.7-5. Percentage of surface hardness (mean ± SD) relative to pre-diet hardness values (100% hardness) over 2 runs.



A plot of the change in surface hardness (figure 4.7-5), relative to the pre-diet hardness values (100 % relative hardness) shows that overall, there was a noticeable reduction in hardness in diet 8 relative to both runs of diet 1.

To investigate any effects of the different test beverage factors on the relative surface hardness percentage change, statistical analysis using 1-way ANOVA was carried out. A significant effect of diet was found ($P<0.05$) on surface hardness.

A Tukey posthoc analysis was undertaken to determine the effect of the diet factor over all the groups (diet 1, diet 1R and diet 8). Diet 8 differed significantly from both diet 1 and diet 1R ($P<0.05$) demonstrating a significant reduction in surface hardness. Thus in this respect Sprite was more erosive than Coca-Cola (Diet 1 and 1R).

B. Surface Loss

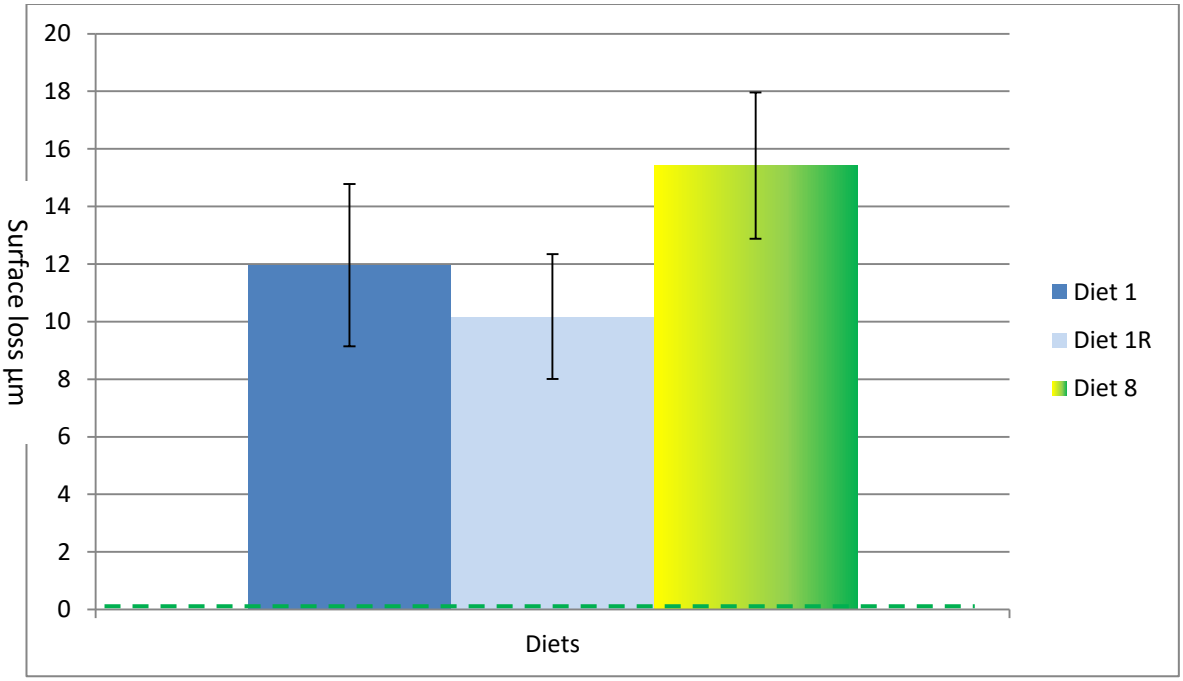
Table (A7-38)(Appendix 7) gives the raw pre- and post-diet surface profile values for all human enamel specimens tested using the immediate effect method.

The effects of the test beverage on surface loss are summarised in table 4.7-15, and are illustrated in figure 4.7-6.

Table 4.7-15. Surface loss in µm of human enamel relative to pre-diet readings (0.00 µm) after a 3 day, single dose diet.

Readings Diet 8	1	2	3	Mean	SD
Sample 1	16.58	16.05	16.30	16.31	0.27
Sample 2	11.99	11.63	11.65	11.75	0.20
Sample 3	17.17	16.76	17.02	16.99	0.21
Sample 4	15.67	14.55	16.64	15.62	1.05
Sample 5	18.73	18.55	18.61	18.63	0.09
Sample 6	11.77	11.57	11.48	11.61	0.15
Sample 7	17.19	17.44	17.34	17.33	0.13
Sample 8	14.59	15.60	15.09	15.10	0.51
Mean				**15.42**	
SD				2.54	

Figure 4.7-6. Surface loss values in µm (mean ± SD) relative to pre-diet values (0.00 µm). Note: a positive value of surface loss represents a loss of tissue.



A plot of the surface loss (figure 4.7-6), relative to the pre-diet profile values shows that overall, a higher surface loss value was noticed in diet 8 relative to the two runs of diet 1.

To investigate any effects of the different test beverage factor on the amount of surface loss, statistical analysis using a 1-way ANOVA was carried out. A highly significant effect of diet was found ($P<0.01$) on surface loss. This was localised using a Tukey post-test to determine the effect of the diet factor over the groups (diet 1, diet 1R and diet 8). Diet 8 differed significantly from diet 1 ($P<0.05$) and highly significantly from diet 1R ($P<0.01$) producing higher surface loss. Thus in this respect Sprite (Diet 8) was more erosive than Coca-Cola (Diet 1 and 1R).

II. Ostrich Eggshell

A. *Surface hardness*

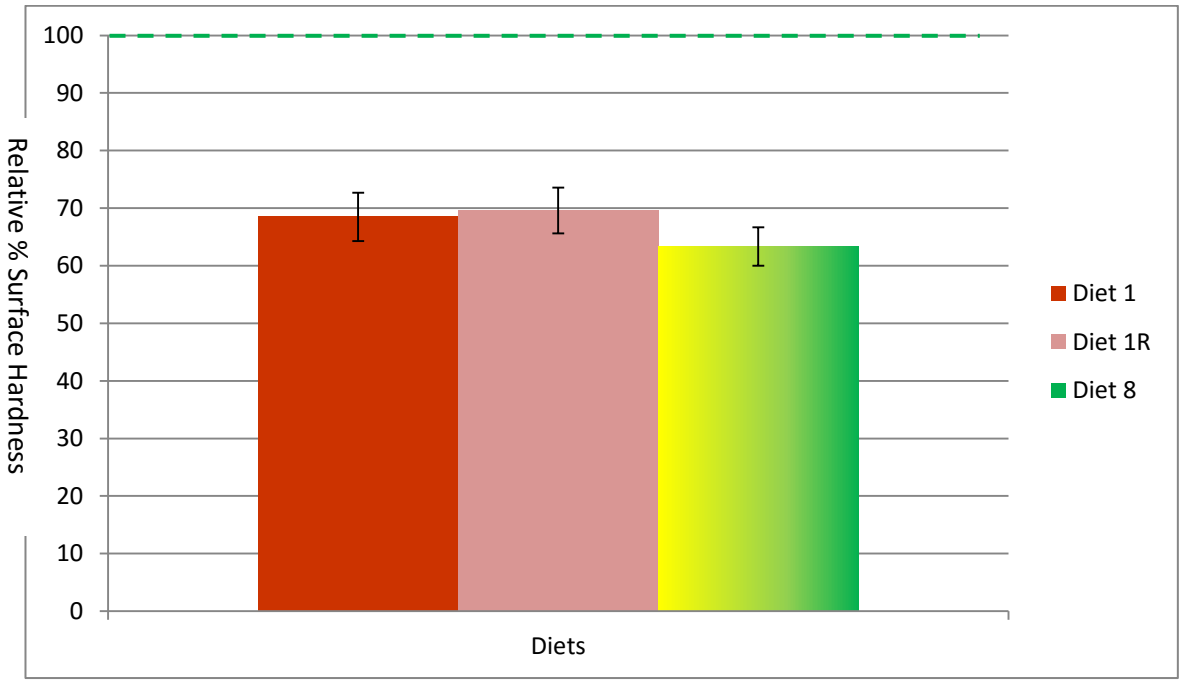
Table (A7-39)(Appendix 7) gives the raw pre- and post-diet surface hardness values (Vickers Hardness) for all human enamel specimens tested using the immediate effect method.

The effects of the test beverage on surface hardness are summarised in table 4.7-16, and illustrated graphically in figure 4.7-7 relative to the two runs of Diet 1.

Table 4.7-16. Percentages of Surface hardness of Ostrich eggshells relative to the pre-diet readings (100% Hardness) after a 3 day, single dose diet.

Readings Diet 8	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	59.66	69.24	69.98	62.61	51.56	68.51	72.19	68.51	69.98	69.98	66.22	6.40
Sample 2	66.12	82.03	57.37	52.60	56.57	47.82	67.71	66.92	62.94	66.12	62.62	9.60
Sample 3	59.52	58.00	59.52	71.73	62.57	74.01	67.15	81.64	61.05	86.22	68.14	9.95
Sample 4	61.15	58.71	57.90	57.90	57.90	55.46	60.34	50.57	55.46	67.66	58.30	4.43
Sample 5	70.72	61.88	66.30	65.56	69.98	59.66	58.19	45.66	72.19	56.72	62.69	8.06
Sample 6	61.35	75.67	65.33	62.94	63.73	68.51	58.96	72.49	66.12	64.53	65.96	5.06
Sample 7	60.28	65.62	54.18	61.05	52.66	70.96	67.15	67.15	69.44	63.34	63.18	6.16
Sample 8	61.97	55.46	58.71	56.27	56.27	62.78	56.27	67.66	57.08	63.59	59.61	4.14
Mean											**63.34**	
SD											3.35	

Figure 4.7-7. Percentage of surface hardness (mean ± SD) relative to pre-diet hardness values (100% hardness) over 2 runs.



A plot of the change in surface hardness (figure 4.7-7), relative to the pre-diet hardness values (100 % relative hardness) shows that overall, there was a noticeable reduction in hardness in diet 8 relative to both runs of diet 1.

To investigate any effects of the different test beverage factor on the relative surface hardness percentage change, statistical analysis using 1-way ANOVA was carried out. A highly significant effect of diet was found ($P<0.01$) on surface hardness.

A Tukey posthoc was undertaken to localise these and to determine the effect of the diet factor over all the groups (diet 1, diet 1R and diet 8). Diet 8 differed significantly from both diet 1 and diet 1R ($P<0.05$). Thus in this respect Sprite (Diet 8) was more erosive than Coca-Cola (Diets 1 and 1R).

B. Surface Loss

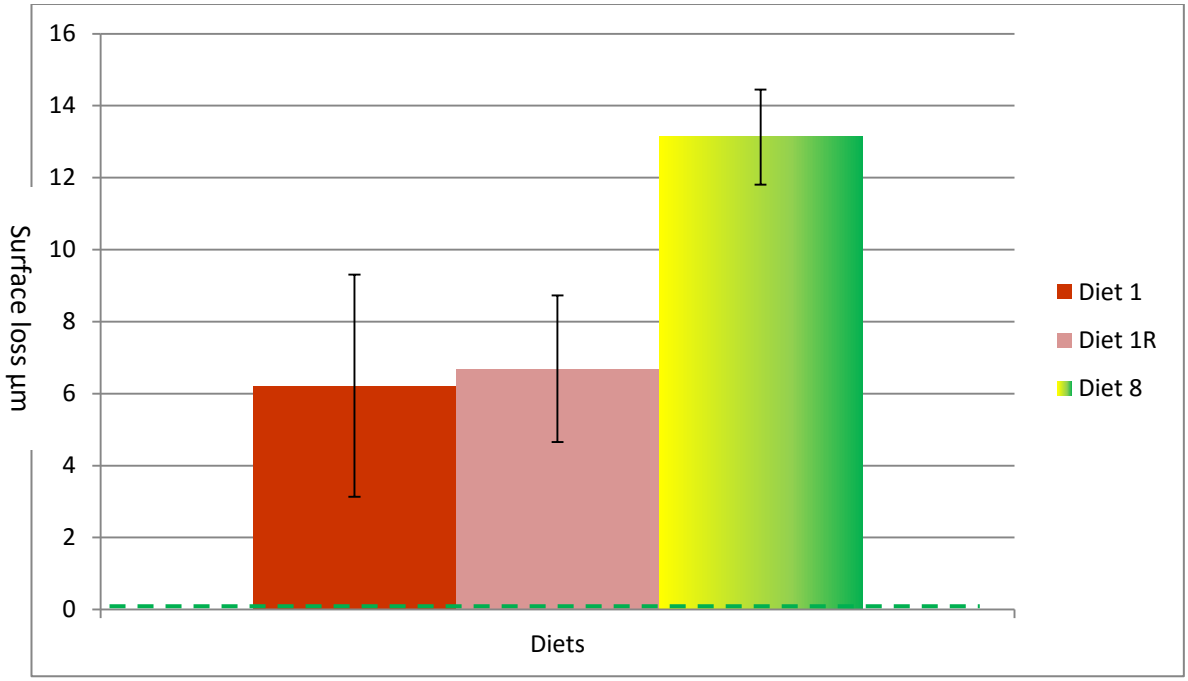
Table (A7-40)(Appendix 7) gives the raw pre- and post-diet surface profile values for all human enamel specimens tested using this method.

The effects of the test beverage on surface loss are summarised in table 4.7-17, and is illustrated graphically in figure 4.7-8.

Table 4.7-17. Surface loss in µm of Ostrich eggshells relative to pre-diet readings (0.00 µm) after a 3 day, single dose diet.

Readings Diet 8	1	2	3	Mean	SD
Sample 1	10.49	11.71	11.42	11.21	0.64
Sample 2	13.64	13.88	13.88	13.80	0.14
Sample 3	11.19	11.23	11.22	11.22	0.02
Sample 4	13.99	14.20	14.06	14.09	0.11
Sample 5	14.89	13.35	15.16	14.47	0.98
Sample 6	12.84	15.25	13.82	13.97	1.21
Sample 7	12.41	12.40	12.44	12.42	0.02
Sample 8	13.52	14.27	13.75	13.84	0.38
Mean				**13.13**	
SD				1.32	

Figure 4.7-8. Surface loss values in µm (mean ± SD) relative to pre-diet values (0.00 µm). Note: a positive value of surface loss represents a loss of tissue.



A plot of the surface loss (figure 4.7-8), relative to pre-diet profile values shows that overall, a considerably higher surface loss value was noticed in diet 8 relative to the two runs of diet 1.

To investigate any effects of the different test beverage factors on the amount of surface loss, statistical analysis using 1-way ANOVA was carried out. Very highly significant effect of diet was found ($P<0.0001$) on surface loss.

A Tukey posthoc analysis was undertaken to determine the effect of the diet factor over the groups (diet 1, diet 1R and diet 8). Diet 8 differed very highly significantly from diet 1 and diet 1R ($P<0.0001$). Thus in this respect Sprite (Diet 8) was more erosive than Coca-Cola (Diets 1 and 1R).

4.7.4 Finding summary for Saltus Diets II - the different test beverage

From the foregoing results, it is clear that;

- The test-beverage (Sprite) had a more erosive effect than the default test-beverage (Coca-Cola).
- This effect was detected in both substrates tested.

4.8 Validation of Saltus

4.8.1 The consistency of Saltus diets

A. Surface hardness

The ability of Saltus to deliver consistent diets was assessed by investigating the differences between relative surface hardness percentage values over the two experimental runs of diets 1 -4 (i.e. Run 1 vs Run 2).

Statistical analysis (2-way random consistency Intra-class Correlation Coefficient) was conducted to assess the reliability of the results. Excellent correlation was found between the readings of run 1 and 2 regardless of the substrate species (ICC = 0.973). Table (4.8-1) summarises this finding.

Table 4.8-1. Intra-class correlation coefficient calculation

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.947[a]	.914	.967	36.777	63	63	.000
Average Measures	.973	.955	.983	36.777	63	63	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

B. Surface Loss

The ability of Saltus to deliver consistent diets was assessed by investigating the differences between surface loss values over the two experimental runs of diets 1-4 (i.e. Run 1 vs Run 2).

Statistical analysis (2-way random consistency Intra-class Correlation Coefficient) was conducted to assess the reliability of the results. Excellent correlation was found between the readings of runs 1 and 2 regardless of the substrate species (ICC = 0.857). Table (4.8-2) summarises this finding.

Table 4.8-2. Intra class correlation coefficient calculation

Intraclass Correlation Coefficient

	Intraclass Correlation[b]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.749[a]	.618	.840	6.971	63	63	.000
Average Measures	.857	.764	.913	6.971	63	63	.000

Two-way random effects model where both people effects and measures effects are random.

a. The estimator is the same, whether the interaction effect is present or not.

b. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance.

From the foregoing results, it is clear that;

- Saltus is able to deliver diets that are consistent, reliable and reproducible.

4.8.2 The Reliability of Saltus components

I. Beverage-free diets

The effects of running a beverage-free diet for 5 days on the Surface hardness raw values of substrates are presented in table 4.8-3 and 4.8-4. The overall means of post-diet hardness values for both substrates were very close to the pre-diet values. Statistical analysis (Paired t-test) showed no significant differences between the groups (P>0.05).

Table 4.8-3. Raw hardness readings (HV) of human enamel before (a) and after (b) running a beverage-free diet for 5 days.

(a)

Readings Human	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	235	309	283	303	269	303	244	243	292	226	270.70	31.47
Sample 2	307	290	324	222	220	274	237	299	277	302	275.20	36.87
Sample 3	303	302	252	257	246	272	253	262	269	225	264.10	24.08
Sample 4	291	325	259	277	278	276	286	306	280	295	287.30	18.32
Sample 5	205	267	324	320	252	318	242	280	286	280	277.40	37.96
Sample 6	312	333	310	336	291	216	230	226	285	300	283.90	44.44
Sample 7	246	318	279	279	218	237	240	280	300	306	270.30	33.38
Sample 8	316	363	248	306	200	282	258	309	309	260	285.10	45.45
Mean											**276.75**	
SD											8.22	

(b)

Readings Human	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	259	282	278	258	261	249	264	277	273	264	266.45	10.60
Sample 2	270	241	270	282	281	291	300	294	278	344	285.20	26.42
Sample 3	276	267	283	212	285	321	261	284	257	277	272.25	27.49
Sample 4	314	306	306	265	311	301	280	304	283	276	294.46	17.00
Sample 5	273	294	277	252	294	261	246	244	253	237	263.22	20.46
Sample 6	305	302	294	239	232	295	262	286	338	288	284.32	31.83
Sample 7	310	261	299	323	281	254	318	290	299	267	290.25	23.81
Sample 8	270	261	255	272	250	252	275	288	275	293	269.15	14.76
Mean											**278.16**	
SD											11.81	

Table 4.8-4. Raw hardness readings (HV) of Ostrich eggshells before (a) and after (b) running a beverage-free diet for 5 days.

(a)

Readings Ostrich	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	127	113	116	130	133	118	124	120	132	119	123.2	7.04
Sample 2	113	119	117	124	116	117	126	127	118	117	119.4	4.65
Sample 3	114	113	108	104	135	125	107	123	118	120	116.7	9.48
Sample 4	122	119	129	124	126	113	123	121	123	119	121.9	4.36
Sample 5	111	133	131	130	134	118	164	140	108	148	131.7	16.89
Sample 6	111	134	133	118	130	137	124	141	147	139	131.4	10.99
Sample 7	126	128	143	159	120	140	134	125	126	121	132.2	12.11
Sample 8	129	128	135	115	125	125	135	123	120	129	126.4	6.24
Mean											**125.36**	
SD											6.00	

(b)

Readings Ostrich	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	129	125	128	123	123	125	126	121	129	123	125.2	2.78
Sample 2	118	120	115	117	130	124	132	115	129	130	123	6.78
Sample 3	129	123	120	129	120	118	119	122	117	121	121.8	4.18
Sample 4	130	128	126	128	138	124	127	127	137	127	129.2	4.64
Sample 5	125	125	126	113	133	132	114	119	146	115	124.8	10.30
Sample 6	124	129	122	135	130	134	129	143	143	134	132.3	7.02
Sample 7	117	127	124	140	132	125	120	125	141	118	126.9	8.41
Sample 8	132	138	142	125	144	134	146	126	132	134	135.3	7.15
Mean											**127.31**	
SD											4.66	

II. Remineralisation

To ascertain the ability of artificial saliva to remineralise a previously eroded substrate; human enamel and Ostrich eggshell specimens were soaked in artificial saliva for 48 hours.

The effects of artificial saliva (recipe 5) on the remineralisation of eroded human enamel and Ostrich eggshell specimens are presented in tables 4.8-5 and 4.8-6. Surface hardness increased by 28.36% in human enamel and 27.03% in Ostrich eggshell specimens. Statistical analysis (Paired t-test) showed this difference to be very highly significant ($P<0.0001$).

Table 4.8-5. The change in surface hardness values in (HV) of eroded enamel specimens (a) after soaking the specimens in artificial saliva recipe 5 for 48 hours (b). The percentage given represents hardness **gain** relative to the pre-diet overall mean value.

(a)

Readings Human	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	183	211	203	179	192	172	172	187	198	203	190.00	13.64
Sample 2	221	184	218	189	206	229	175	206	187	208	202.30	17.83
Sample 3	182	171	204	171	186	177	184	188	184	179	182.60	9.52
Sample 4	169	182	199	208	160	176	191	149	166	169	176.90	18.22
Sample 5	200	188	185	234	197	145	223	260	172	185	198.90	32.80
Sample 6	172	183	216	191	169	193	174	160	221	172	185.10	20.28
Sample 7	206	190	189	168	204	194	195	183	186	206	192.10	11.81
Sample 8	170	152	160	183	151	169	211	197	206	236	183.50	28.27
Mean											**188.93**	
SD											10.73	

(b)

Readings Human	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	231	292	286	233	238	256	254	235	254	231	251.0	22.36
Sample 2	223	248	259	230	275	275	277	231	240	246	250.4	20.19
Sample 3	288	253	263	288	255	249	265	245	261	283	265.0	15.99
Sample 4	275	277	330	267	359	238	254	304	263	273	284.0	36.72
Sample 5	263	275	217	297	226	206	273	330	314	241	264.2	41.76
Sample 6	261	279	311	272	264	247	285	284	242	320	276.5	25.15
Sample 7	243	247	271	267	247	247	219	253	291	255	254.0	19.21
Sample 8	256	263	291	249	232	222	266	294	306	269	264.8	26.86
Mean											**263.74**	
SD											12.02	
Gain											**28.36 %**	

Table 4.8-6. The change in surface hardness values in (HV) of eroded Ostrich eggshell specimens (a) after soaking the specimens in artificial saliva recipe 5 for 48 hours (b). The percentage given represents hardness **gain** relative to the pre-diet overall mean value.

(a)

Readings Ostrich	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	75	65	73	66	60	70	74	77	64	85	70.90	7.40
Sample 2	67	78	57	53	79	68	77	57	55	82	67.30	11.21
Sample 3	59	66	61	56	79	55	53	62	59	53	60.30	7.76
Sample 4	57	61	44	57	57	62	47	72	65	61	58.30	8.15
Sample 5	54	64	125	63	62	66	68	114	58	70	74.40	24.35
Sample 6	52	63	42	50	62	51	51	61	54	47	53.30	6.83
Sample 7	63	54	97	61	78	55	95	72	89	71	73.50	15.89
Sample 8	52	58	75	56	55	61	49	51	57	54	56.80	7.30
Mean											**64.35**	
SD											8.18	

(b)

Readings Ostrich	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	85	78	105	104	85	105	74	81	83	88	88.80	11.62
Sample 2	93	89	82	91	94	98	99	98	89	103	93.60	6.15
Sample 3	103	103	82	90	74	82	87	79	74	85	85.90	10.35
Sample 4	79	89	94	81	85	78	99	114	84	78	88.10	11.49
Sample 5	75	96	95	77	84	87	82	72	79	74	82.10	8.44
Sample 6	78	82	90	88	78	80	78	85	128	98	88.50	15.31
Sample 7	81	109	102	85	87	97	99	90	87	100	93.70	8.96
Sample 8	75	93	76	73	102	85	93	81	92	77	84.70	9.83
Mean											**88.18**	
SD											4.05	
Gain											**27.03 %**	

III. Calcium-deprived Artificial Saliva

This section reports the results of replacing the saliva used in Diet 1 with a calcium-deprived saliva (Diet 0). This regime is summarised in table 4.8-9 and is identical to Diet 1 on all other respects.

Table 4.8-9. Diet Ø summary.

Calcium-deprived

Diet	Beverage	Duration	Rest cycles	Test cycles	Test cycle dose	Total number of cans per specimen	Preventive measure	Code
Short-Single dose	*Coca-Cola*	5 days	2	3	1 can	3 cans	None	**Diet Ø**

A. Surface Hardness

Tables (A7-41 to A7-42)(Appendix 7) give the raw pre- and post-diet surface hardness values for all human enamel and Ostrich eggshell specimens tested in Diet Ø.

The effect of using calcium-deprived artificial saliva in a diet (Diet Ø) that is identical, in terms of dose and duration, to Diet 1 (immediate effect method) on surface hardness is summarised in tables 4.8-7 and 4.8-8, and is illustrated graphically in figure 4.8-1. In these tables the raw hardness values have been converted to a % change in hardness relative to the pre-diet hardness value (100 %). Both human enamel and Ostrich eggshell effects are reported in these tables.

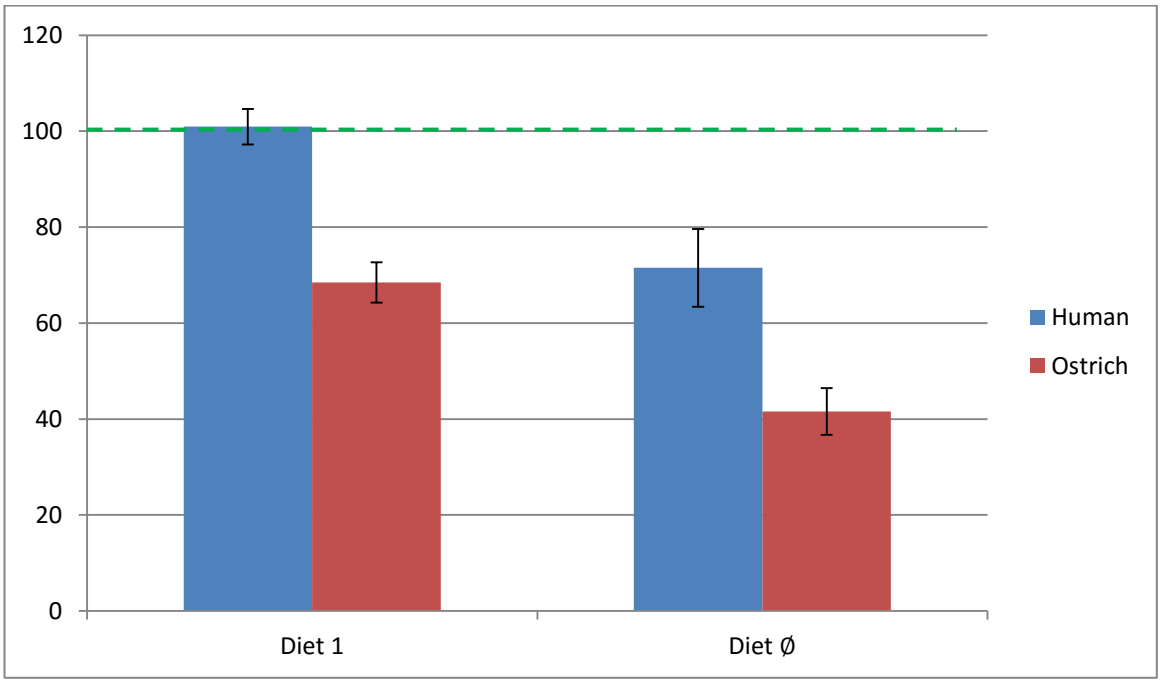
Table 4.8-7. Percentages of Surface hardness of human enamel relative to the pre-diet readings (100% Hardness) after a 5 day, single dose diet with **calcium-free** artificial saliva.

Readings Diet Ø	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	68.82	79.35	76.34	67.32	72.21	64.69	64.69	70.33	74.46	76.34	71.46	4.87
Sample 2	92.43	76.96	91.18	79.05	86.16	95.78	73.19	86.16	78.21	86.99	84.61	7.07
Sample 3	60.65	56.98	67.98	56.98	61.98	58.98	61.31	62.65	61.31	59.65	60.85	3.01
Sample 4	71.13	76.60	83.75	87.54	67.34	74.07	80.39	62.71	69.87	71.13	74.45	7.28
Sample 5	65.42	61.50	60.52	76.55	64.44	47.43	72.95	85.05	56.26	60.52	65.06	10.18
Sample 6	61.21	65.12	76.87	67.97	60.14	68.68	61.92	56.94	78.65	61.21	65.87	6.85
Sample 7	86.70	79.97	79.55	70.71	85.86	81.65	82.07	77.02	78.28	86.70	80.85	4.71
Sample 8	63.93	57.16	60.17	68.82	56.79	63.56	79.35	74.09	77.47	88.76	69.01	10.09
Mean											**71.52**	
SD											8.11	

Table 4.8-8. Percentages of Surface hardness of Ostrich eggshells relative to the pre-diet readings (100% Hardness) after a 5 day, single dose diet with **calcium-free** artificial saliva.

Readings Diet Ø	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	50.98	43.14	49.41	43.92	39.22	47.06	50.20	52.55	42.35	58.82	47.76	5.51
Sample 2	44.64	53.25	36.81	33.67	54.03	45.42	52.47	36.81	35.24	56.38	44.87	8.32
Sample 3	36.51	41.73	38.00	34.28	41.42	33.53	32.04	38.75	36.51	32.04	36.48	3.35
Sample 4	37.66	40.87	27.24	37.66	37.66	41.67	29.65	49.68	44.07	40.87	38.70	6.20
Sample 5	39.76	44.59	48.25	42.98	42.17	47.00	37.35	38.96	43.78	41.37	42.62	3.28
Sample 6	32.88	40.36	45.95	39.61	38.86	41.85	43.35	47.73	35.87	44.84	41.13	4.35
Sample 7	32.21	40.64	34.54	30.67	39.88	31.44	31.44	39.11	33.74	28.37	34.21	4.05
Sample 8	42.37	35.17	49.54	40.77	54.36	35.97	47.95	49.56	63.15	48.76	46.76	8.08
Mean											**41.57**	
SD											4.87	

Figure 4.8-1. Percentage of surface hardness (mean ± SD) relative to pre-diet hardness values (100% hardness) for Diet Ø versus Diet 1



A plot of the change in surface hardness (figure 4.8-1), relative to the pre-diet hardness values (100 % relative hardness) for both diets shows that there was a considerable reduction in hardness in Diet Ø relative to Diet 1 for both substrates. Hardness percentage reduction (Δ Hardness) (D1-DØ) was 29.41% for human enamel and 26.91% for Ostrich eggshell.

To investigate the influence of diet Ø on the surface hardness change, a 2-way analysis of variance on this data was undertaken. This revealed (table 4.8-10);

- Very highly significant effects of diet and substrate (P<0.0001).
- No significant interaction of diet and experimental run (P>0.05).

Table 4.8-10. 2-way ANOVA: Hardness percentage versus Diet and Substrate.

Source of Variation	Df	Sum-of-squares	Mean square	F
Interaction	1	12.50	12.50	0.4130
Diet	1	6344	6344	209.6
Substrate	1	7788	7788	257.3
Residual	28	847.4	30.27	

Follow-up analysis (Bonferroni post-test) was undertaken to determine the effect of the diet factor over all the groups (Diet 1 vs Diet Ø). Hardness percentage values of diet Ø differed very highly significantly relative to diet 1 (P < 0.001) for both substrates.

B. Surface Loss

Tables (A7-43 to A7-44)(Appendix 7) give the raw pre- and post-diet surface profile values for all human enamel and Ostrich eggshell specimens tested in Diet Ø.

The effect of using calcium-deprived artificial saliva in a diet (Diet Ø) that is identical, in terms of dose and duration, to Diet 1 (immediate effect method) on surface loss is summarised in tables 4.8-11 and 4.8-12, and is illustrated graphically in figure 4.8-2. In these tables the raw profile values have been converted to surface loss values in µm relative to the pre-diet profile value (0.00 µm).

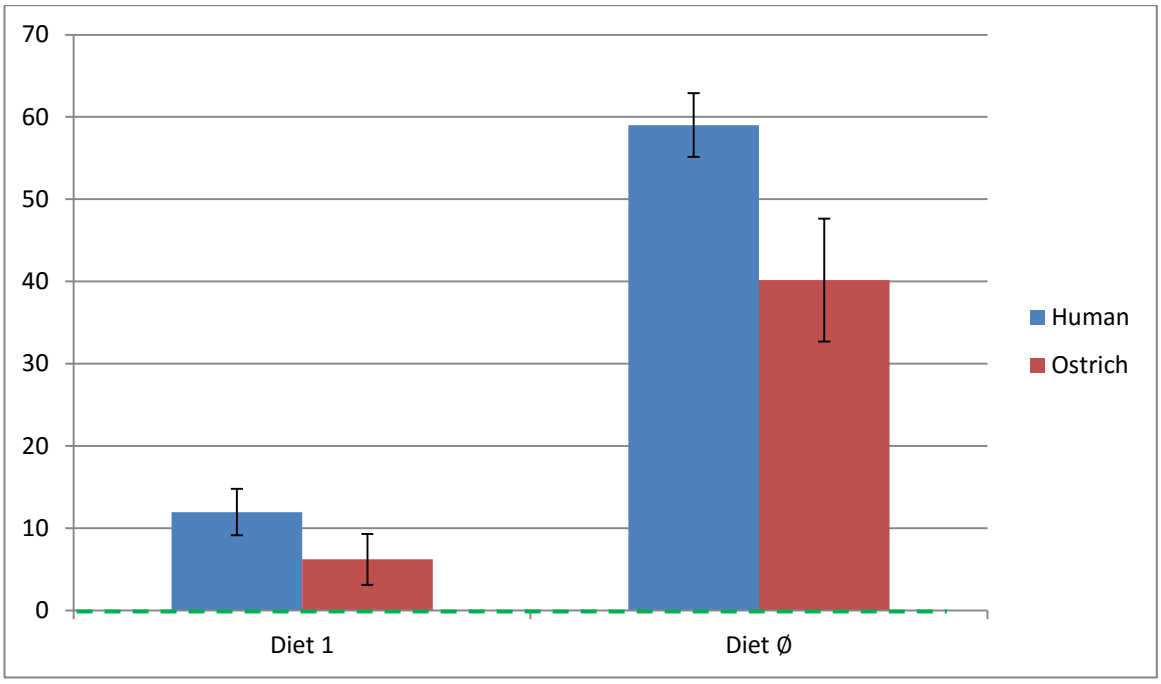
Table 4.8-11. Surface loss in µm of human enamel relative to pre-diet readings (0.00 µm) after a 5 day, single dose diet with **calcium-free** artificial saliva.

Readings Diet Ø	1	2	3	4	5	Mean	SD
Sample 1	60.90	59.43	58.32	59.44	56.97	59.01	1.46
Sample 2	60.42	60.70	60.78	60.53	60.43	60.57	0.16
Sample 3	58.01	58.18	58.91	59.13	54.90	57.83	1.70
Sample 4	59.05	59.91	59.77	60.41	60.45	59.92	0.57
Sample 5	50.04	51.71	49.68	51.14	51.65	50.84	0.93
Sample 6	61.66	58.51	59.24	58.70	59.09	59.44	1.28
Sample 7	64.82	64.25	64.17	63.97	65.96	64.64	0.81
Sample 8	58.84	58.85	60.46	61.57	59.86	59.91	1.15
Mean						**59.02**	
SD						3.86	

Table 4.8-12. Surface loss in µm of Ostrich eggshells relative to pre-diet readings (0.00 µm) after a 5 day, single dose diet with **calcium-free** artificial saliva.

Readings Diet Ø	1	2	3	4	5	Mean	SD
Sample 1	31.76	34.28	33.77	33.55	33.75	33.42	0.97
Sample 2	49.94	50.78	51.18	55.65	45.61	50.63	3.58
Sample 3	30.41	36.75	33.23	33.98	36.16	34.11	2.53
Sample 4	35.77	32.22	34.10	33.68	35.38	34.23	1.42
Sample 5	32.78	32.62	32.89	32.76	32.68	32.75	0.10
Sample 6	49.40	52.60	44.68	46.77	49.60	48.61	3.01
Sample 7	42.03	43.57	47.66	36.48	37.88	41.52	4.49
Sample 8	46.16	48.53	42.21	46.21	47.17	46.05	2.36
Mean						**40.17**	
SD						7.46	

Figure 4.8-2. Surface loss values in µm (mean ± SD) relative to pre-diet values (0.00 µm) over 2 runs.



A plot of the surface loss (figure 4.8-2), relative to pre-diet profile values shows that there was a considerable increase in surface loss in diet Ø compared to diet 1 for both substrates. Surface loss (Δ Loss) (D1-DØ) was increased by 47.06 µm for human enamel and 33.95 µm for Ostrich eggshell.

To investigate any effects of diets (diet 1, 2, 3 and 4) and substrate type on the amount of surface loss a 2-way analysis of variance on this data was undertaken. This revealed (table 4.8-13);

- Very highly significant effects of diet and substrate (P<0.0001).
- Very highly significant interaction of the diet and substrate factors (P<0.001).

Table 4.8-13. 2-way ANOVA: Surface loss versus Diet and Substrate.

Source of Variation	Df	Sum-of-squares	Mean square	F
Interaction	1	343.7	343.7	15.62
Diet	1	13125	13125	596.3
Substrate	1	1209	1209	54.94
Residual	28	616.4	22.01	

Follow-up analysis (Bonferroni post-test) was undertaken to determine the effect of the diet factor over all the groups (Diet 1 vs Diet Ø). Surface loss values of diet Ø differed very highly significantly relative to diet 1 ($P < 0.001$) for both substrates. Thus Diet 1's saliva significantly impeded dental erosion in this respect compared to Diet Ø's calcium-deprived saliva.

C. Ion Loss

Tables (A7-45 to A7-46)(Appendix 7) give the reference calcium and phosphate concentrations values in artificial saliva, the test beverage and the mixture solution along with the raw post-diet ion content of the resultant solutions for Diet Ø.

The effect of using calcium-deprived artificial saliva in a diet (Diet Ø) that is identical, in terms of dose and duration, to Diet 1 (immediate effect method) on ion loss is summarised in tables 4.8-14 and 4.8-15, and is illustrated graphically in figure 4.8-3. In these tables ion concentrations for the resultant solutions were subtracted from the reference values of the artificial saliva/test beverage mixture.

Table 4.8-14. Calcium and phosphate ion loss in mmol/l of human enamel relative to pre-diet readings (0.00 mmol/l) after a 5 day, single dose diet with **calcium-free** artificial saliva.*

Test cycle **Diet Ø**	**1**	**2**	**3**	**Sum**	**Mean**	**SD**
Calcium	0.30	0.30	0.30	**0.90**	**0.30**	0.00
Phosphate	0.35	0.34	0.34	**1.00**	**0.34**	0.01

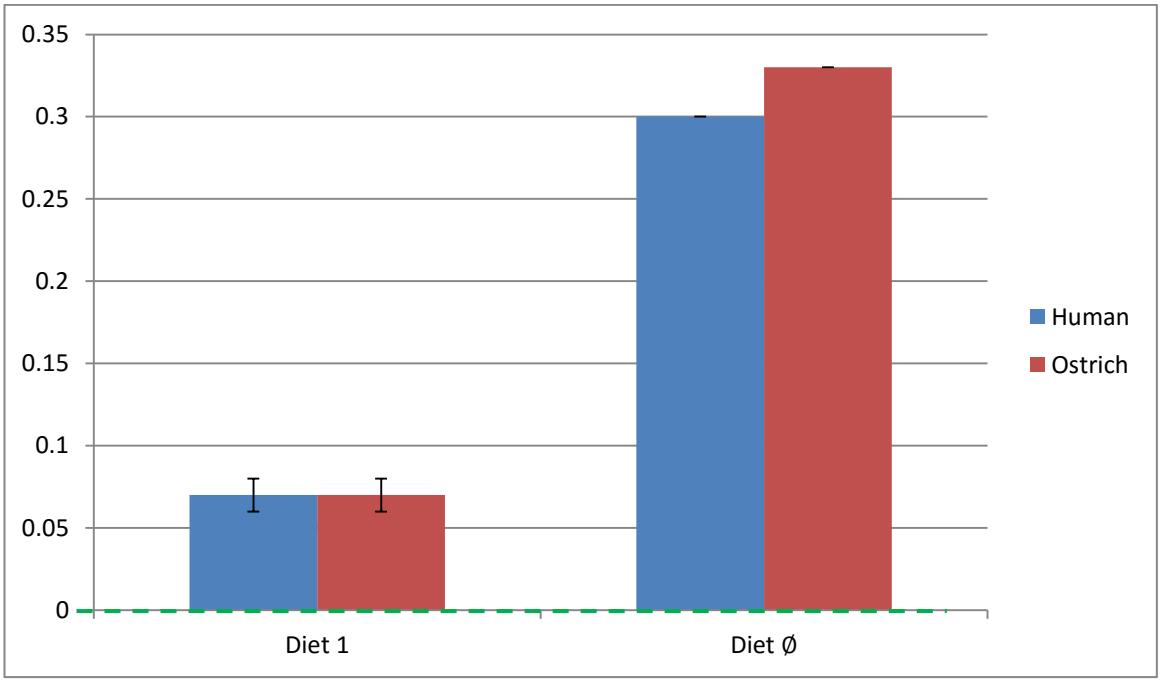
Table 4.8-15. Calcium and phosphate ion loss of Ostrich eggshells in mmol/l relative to pre-diet readings (0.00 mmol/l) after a 5 day, single dose diet with **calcium-free** artificial saliva.*

Test cycle **Diet Ø**	**1**	**2**	**3**	**Sum**	**Mean**	**SD**
Calcium	0.33	0.33	0.33	**0.99**	**0.33**	0.00
Phosphate	0.24	0.24	0.22	**0.70**	**0.23**	0.01

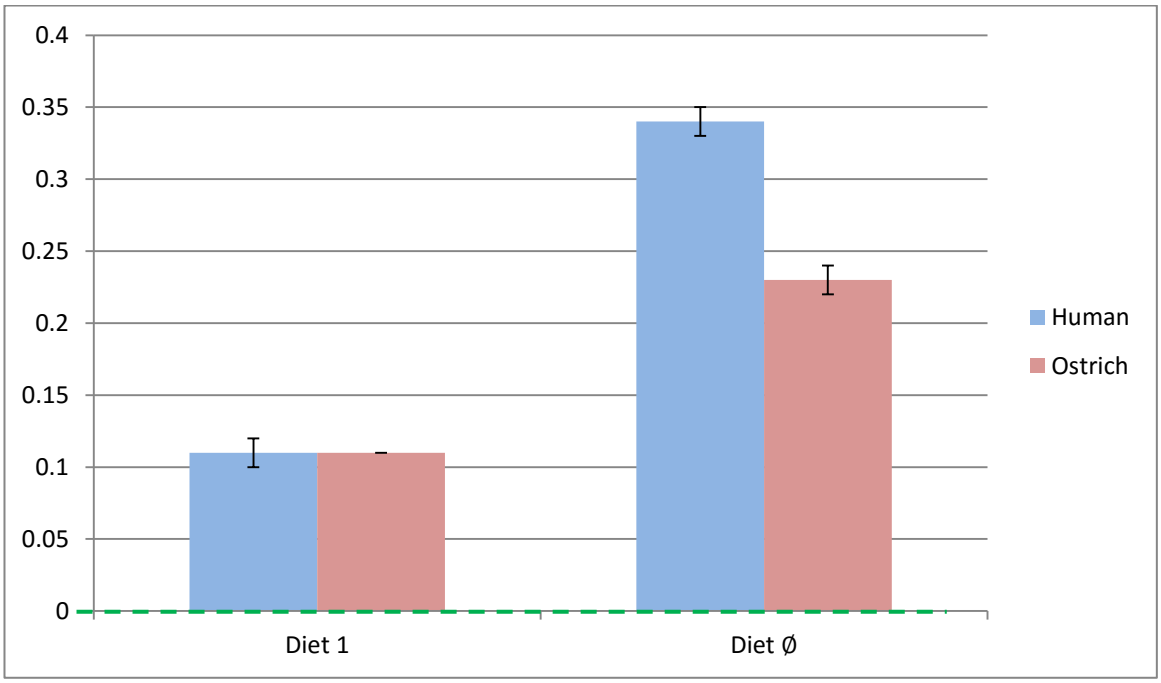
*Each reading represents the mean of 3 readings (based on chemical analysis testing of 3 samples for each day that consists of a test cycle).

Figure 4.8-3. Ion loss values in mmol/day (mean ± SD) for both substrates. (a) Calcium (b) phosphate.

(a)



(b)



A plot of the ion loss (figure 4.8-3), shows that, overall, there was a considerable increase in ion loss during diet Ø compared to diet 1 for both substrates. Calcium ion loss (Δ Loss) (D1-DØ) was increased by 0.23 mmol for human enamel and 0.26 mmol for Ostrich eggshell, while phosphate ion loss (Δ Loss) (D1-DØ) was also increased by 0.23 mmol for human enamel and 0.12 mmol for Ostrich eggshell.

To investigate the influence of diet Ø on ion loss a 2-way analysis of variance on this data was undertaken. This revealed (table 4.8-16);

For the calcium ion;

- A very highly significant effect of diet ($P<0.0001$).
- A highly significant effect of substrate ($P<0.01$).
- A highly significant interaction of the diet and substrate factors ($P<0.01$).

and for the phosphate ion;

- A very highly significant effects of diet and substrate ($P<0.0001$).
- A very highly significant interaction of the diet and substrate factors ($P<0.0001$).

Table 4.8-16. 2-way ANOVA: (a) Calcium ion loss versus Diet and Substrate (b). Phosphate ion loss versus Diet and Substrate.

(a)

Source of Variation	Df	Sum-of-squares	Mean square	F
Interaction	1	0.0006750	0.0006750	13.50
Diet	1	0.1801	0.1801	3602
Substrate	1	0.0006750	0.0006750	13.50
Residual	8	0.0004	5.000e-005	

(b)

Source of Variation	Df	Sum-of-squares	Mean square	F
Interaction	1	0.009075	0.009075	121.0
Diet	1	0.09188	0.09188	1225
Substrate	1	0.009075	0.009075	121.0
Residual	8	0.0006	7.500e-005	

Follow-up analysis (Bonferroni post-test) was undertaken to determine the effect of the diet factor over all the groups (Diet 1 vs Diet Ø). Ion loss values of diet Ø differed very highly significantly relative to diet 1 ($P < 0.001$) for both substrates and for both ions.

From the foregoing results, it is clear that the Diet 1 saliva impedes mineral loss from the test substrates compared to the Diet Ø calcium-deprived saliva.

4.8.3 The typical indentations of the hardness tester

Typical indentations made by the hardness tester for Diets 1-4 before and after are shown in figures 4.8-4 – 4.8-8 for human enamel and figures 4.8-9 – 4.8-13 for Ostrich eggshell. The figures show that the indentation size increases in the ascending order of 'Pre-Diet', 'Diet 1', 'Diet 2', 'Diet 3' and 'Diet 4'.

Figure. 4.8-4 Typical indentations made by the hardness tester upon human enamel specimen surfaces before going through any of the diets.

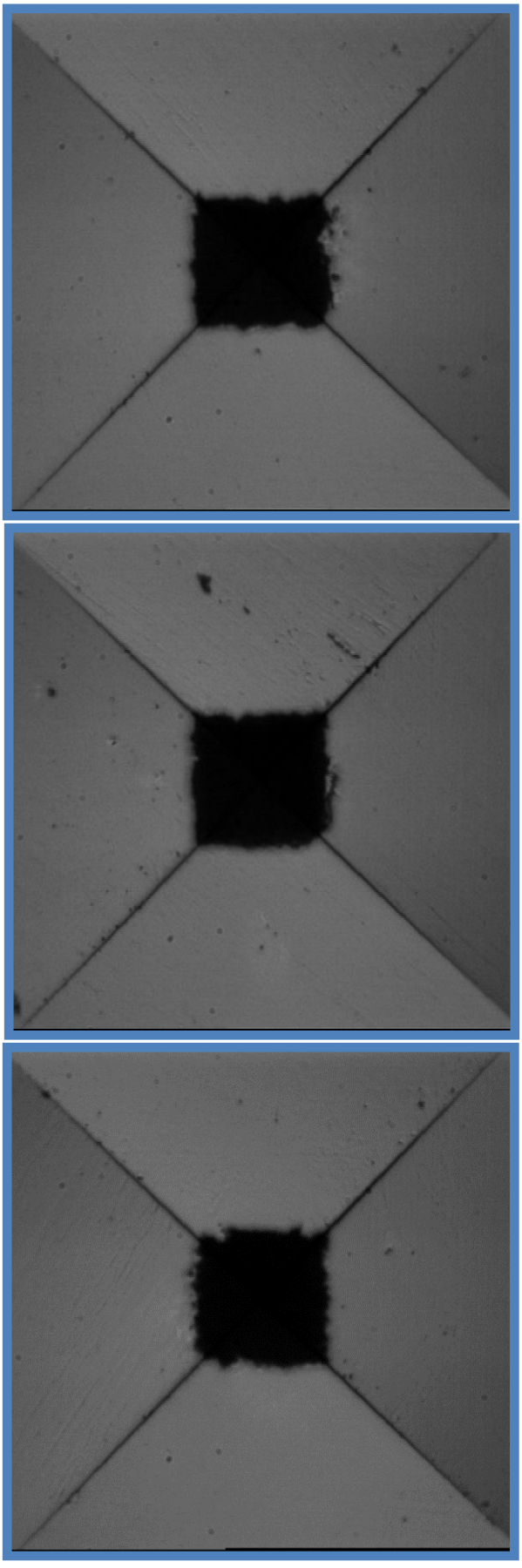


Figure 4.8-5. Typical indentations made by the hardness tester upon human enamel specimen surfaces after going through Diet 1.

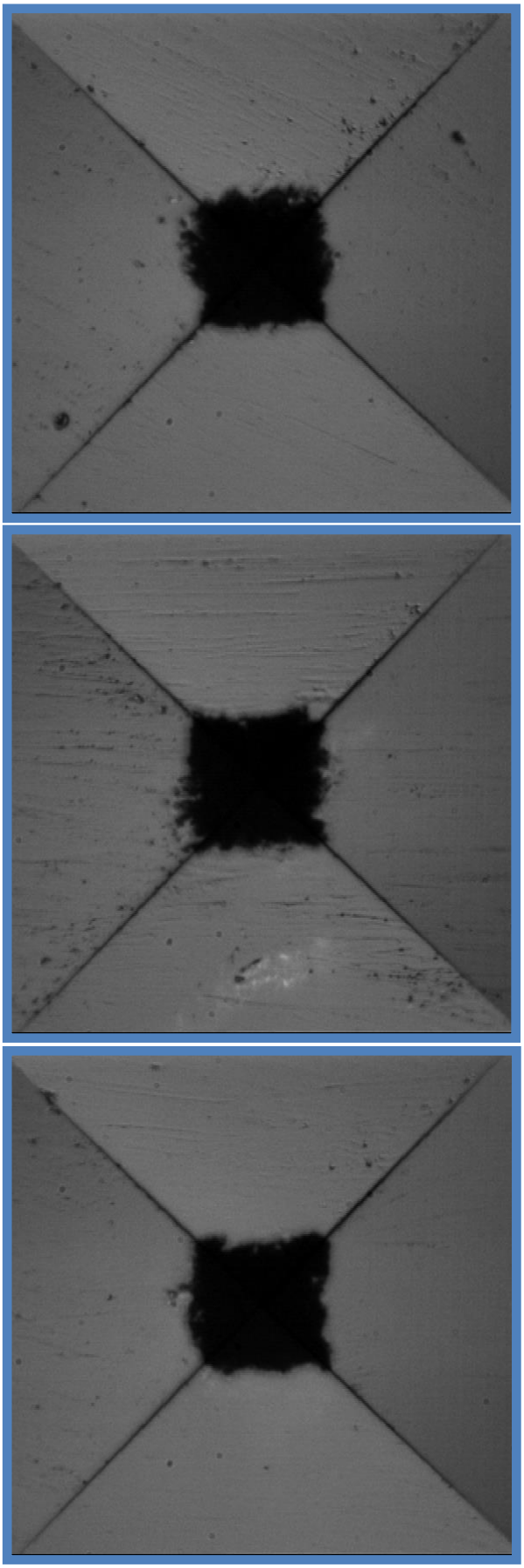


Figure 4.8-6. Typical indentations made by the hardness tester upon human enamel specimen surfaces after going through Diet 2.

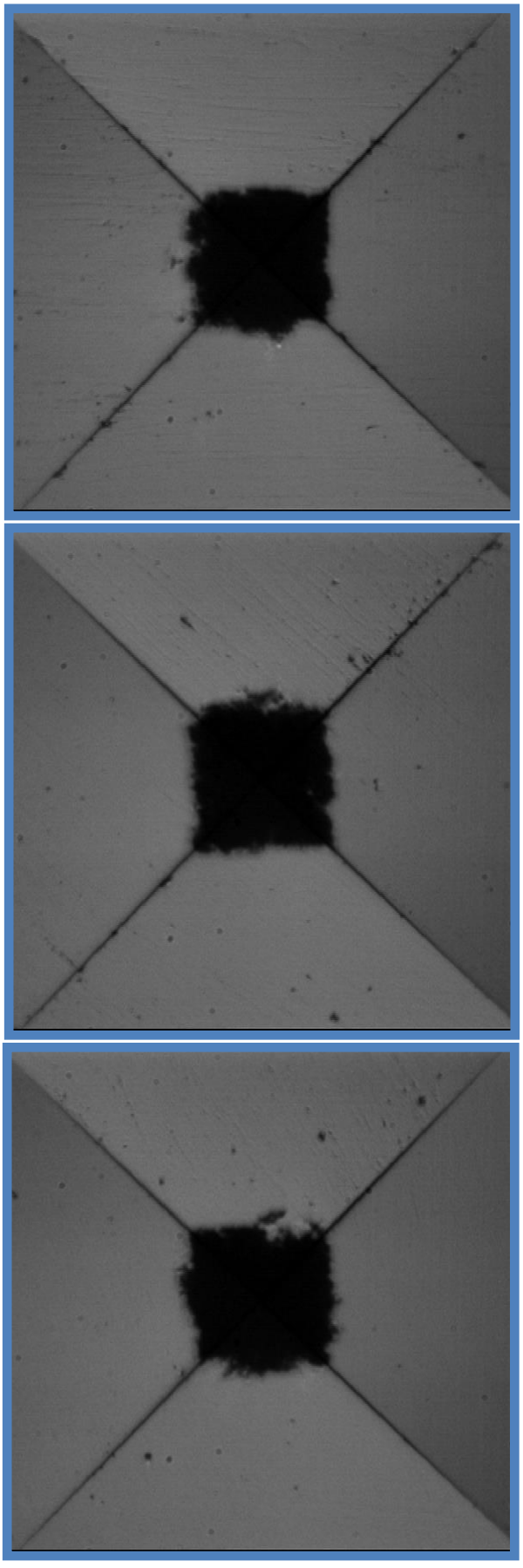


Figure 4.8-7. Typical indentations made by the hardness tester upon human enamel specimen surfaces after going through Diet 3.

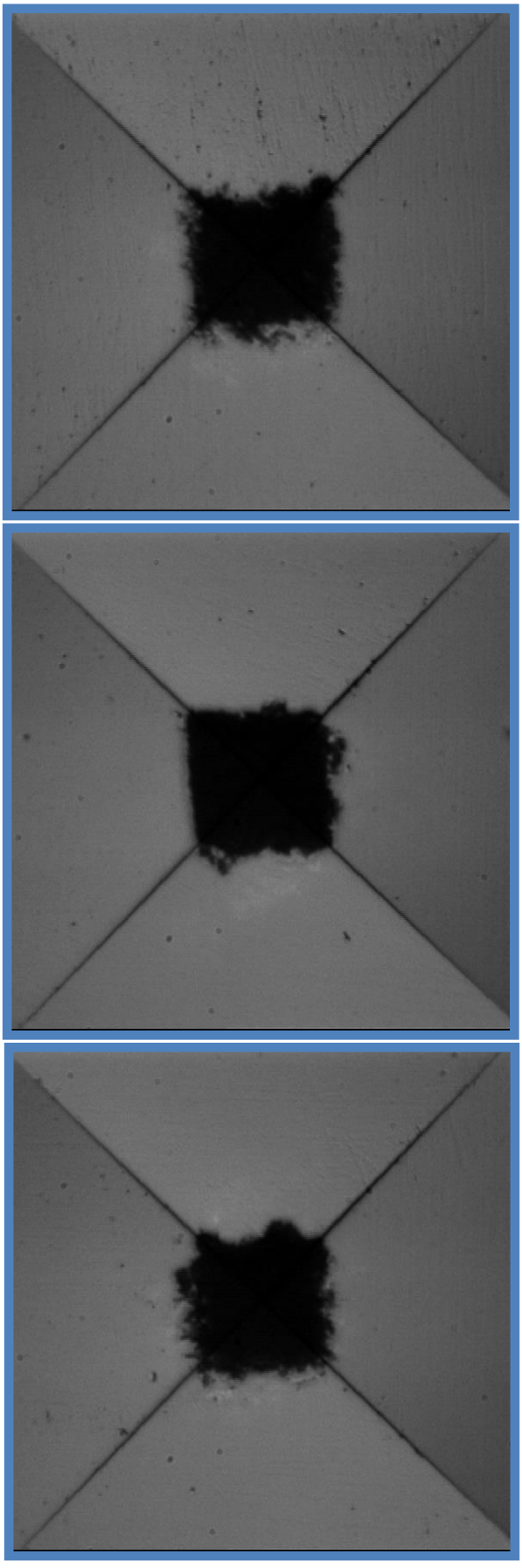


Figure 4.8-8. Typical indentations made by the hardness tester upon human enamel specimen surfaces after going through Diet 4.

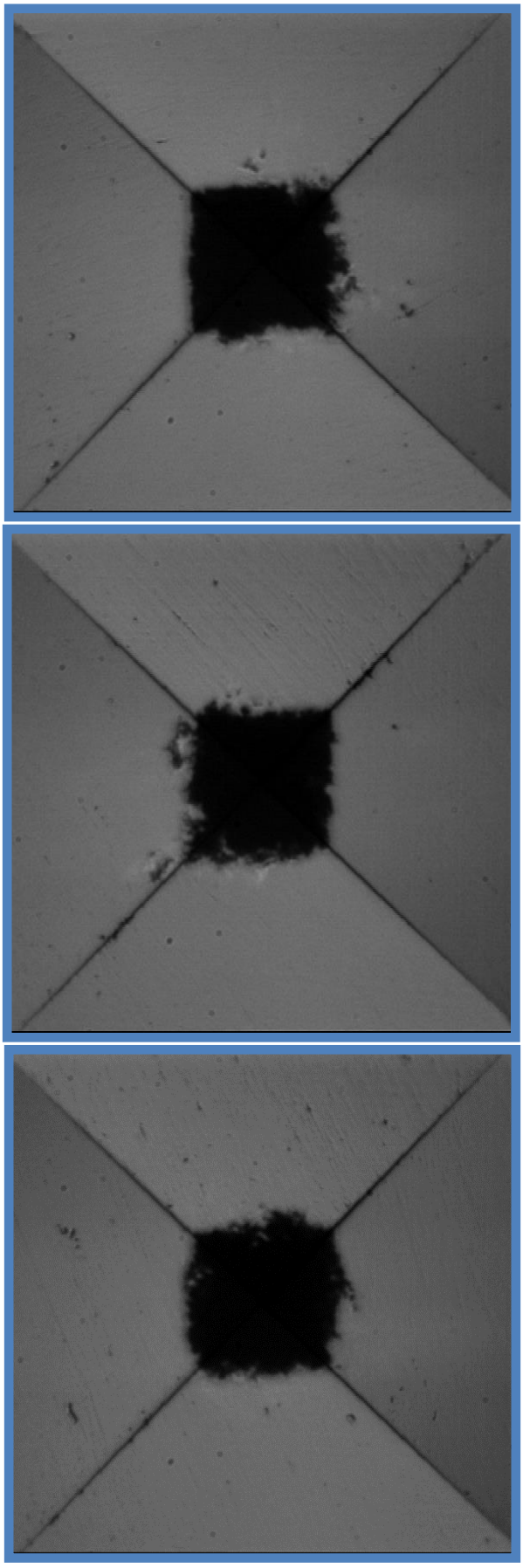


Figure 4.8-9. Typical indentations made by the hardness tester upon Ostrich eggshel specimen surfaces before going through any of the diets.

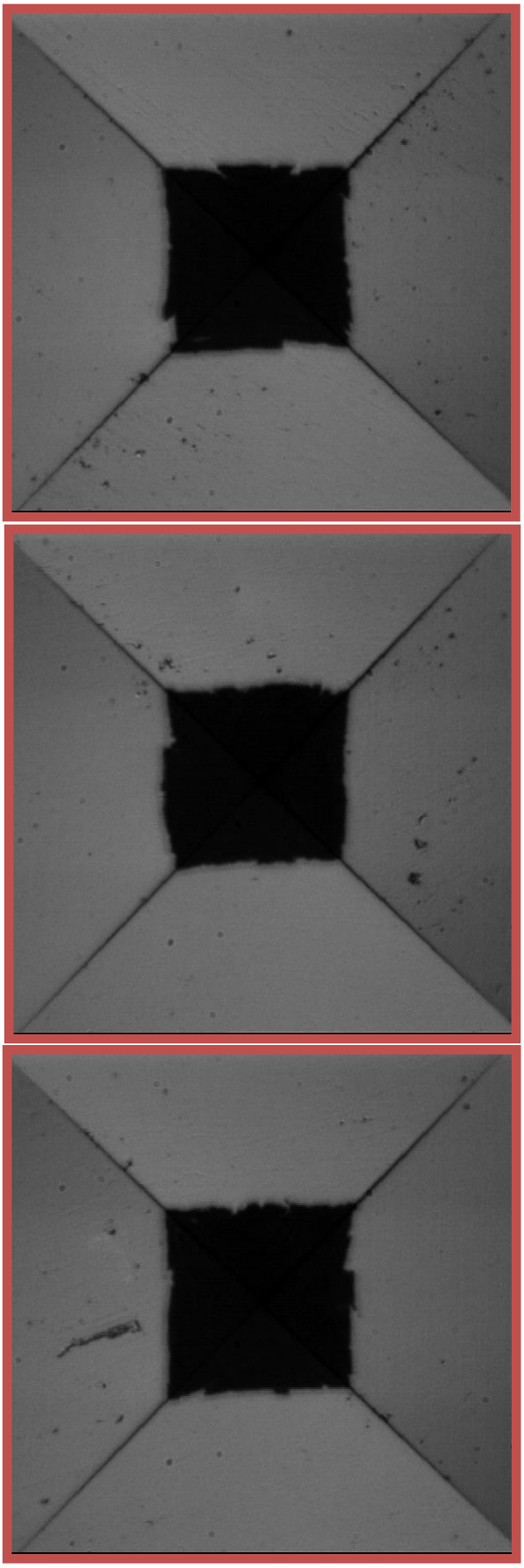


Figure 4.8-10. Typical indentations made by the hardness tester upon Ostrich eggshell specimen surfaces after going through Diet 1.

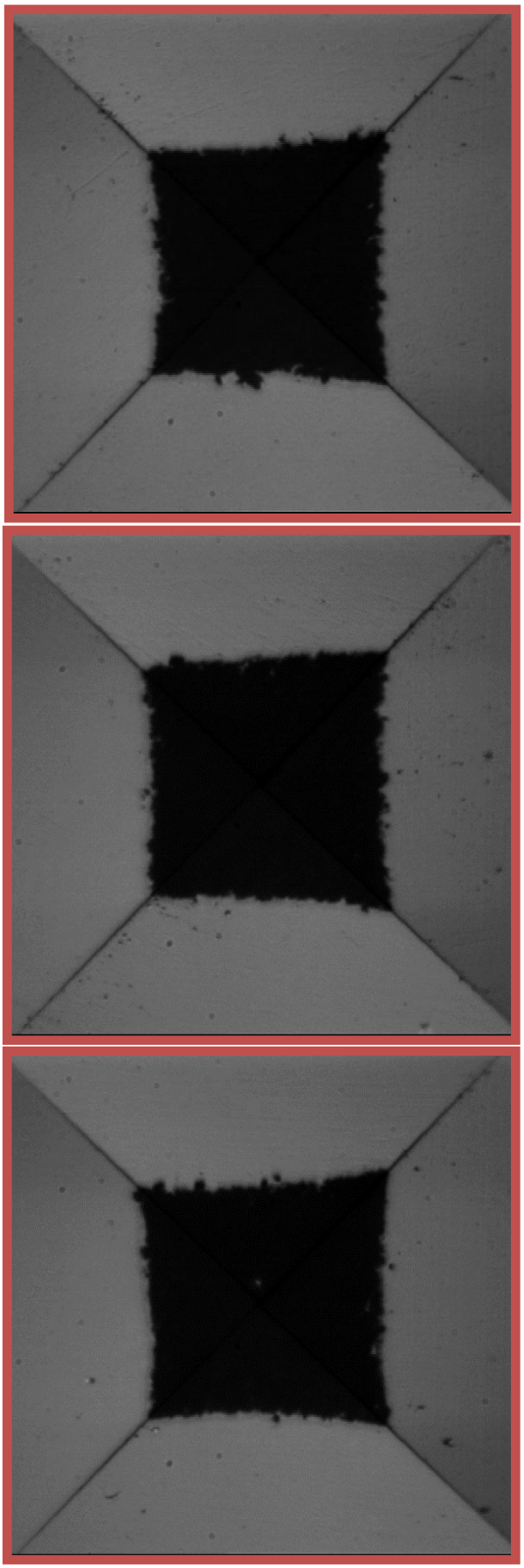


Figure 4.8-11. Typical indentations made by the hardness tester upon Ostrich eggshell specimen surfaces after going through Diet 2.

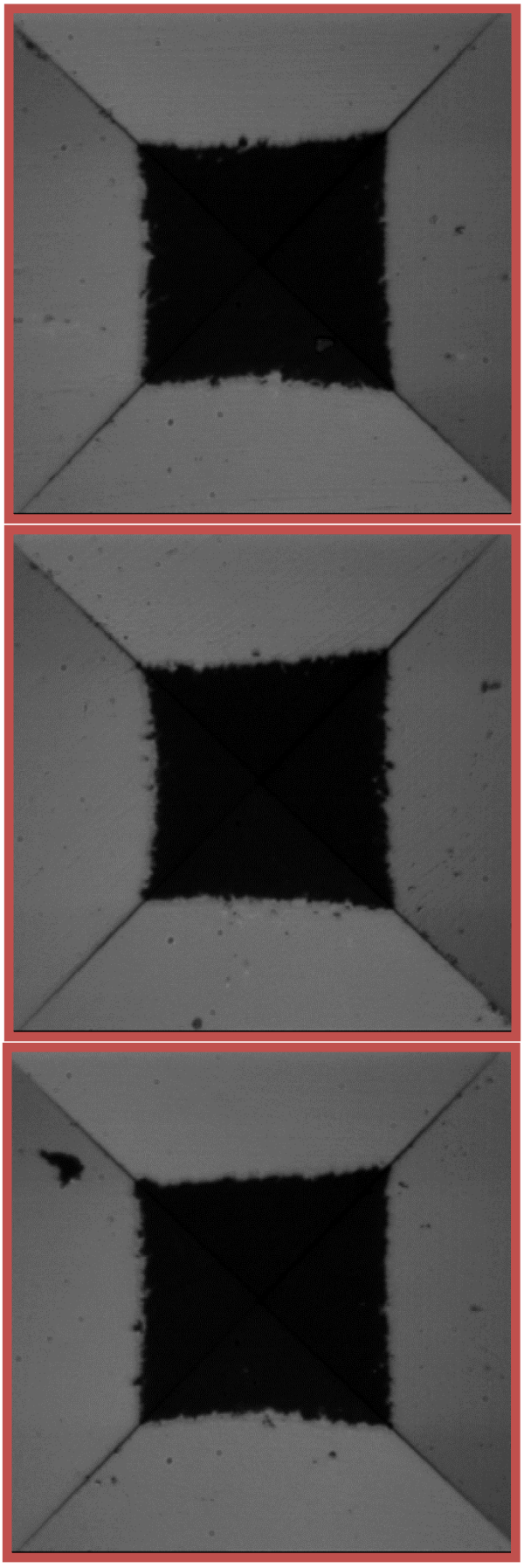


Figure 4.8-12. Typical indentations made by the hardness tester upon Ostrich eggshell specimen surfaces after going through Diet 3.

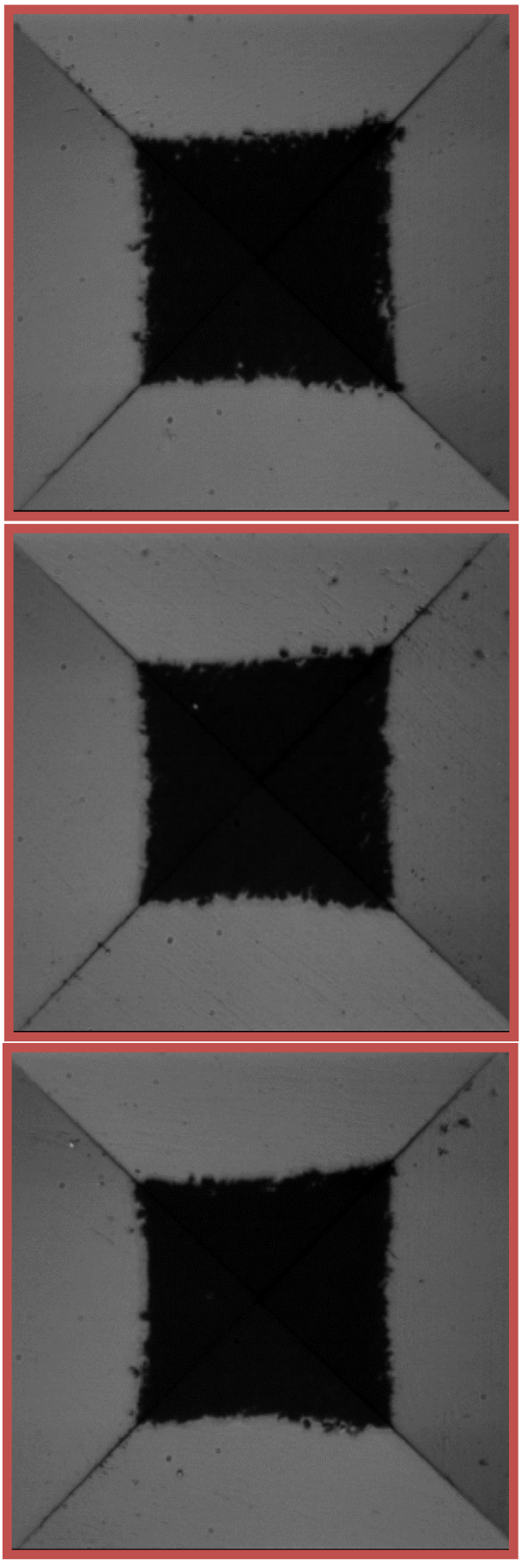
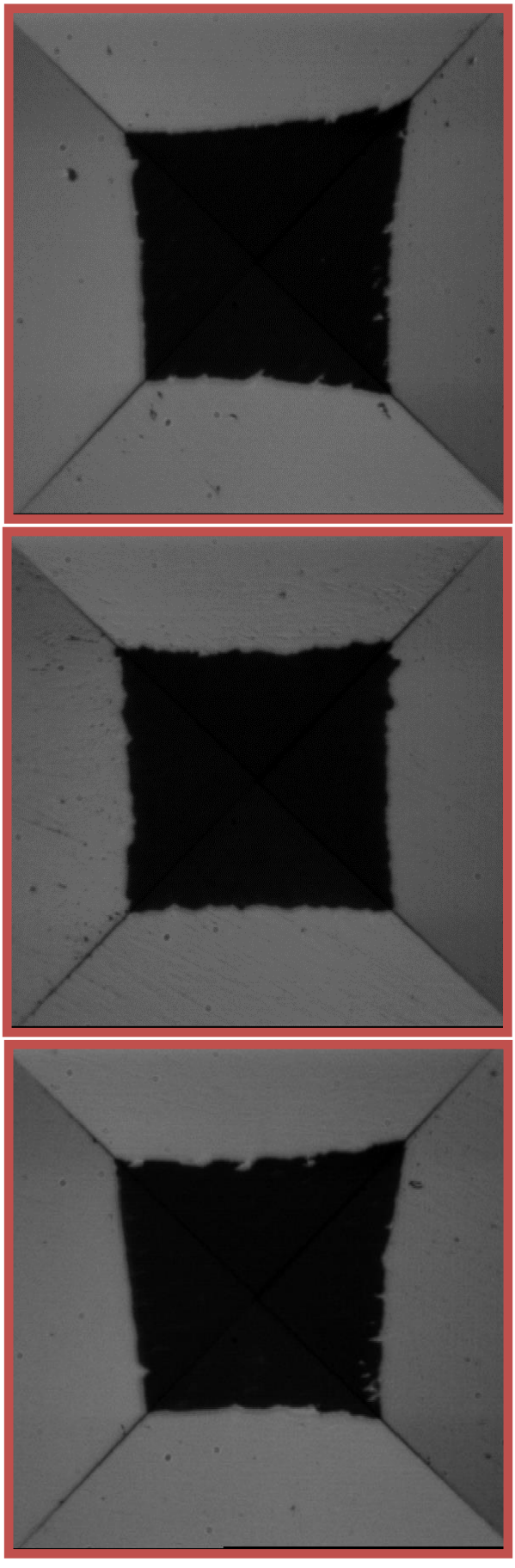


Figure 4.8-13. Typical indentations made by the hardness tester upon Ostrich eggshell specimen surfaces after going through Diet 4.



4.9 Summary of principal findings

- There is a misunderstanding about the Human Tissue Act among dentists.
- A considerable number of dentists have ceased to collect extracted teeth after the year 2006.
- Dentists in the UK nowadays tend to be too cautious with regard to the collection of teeth regardless of the reason.
- One tenth of the respondents are consuming soft drinks at least once a day, among which one third consume at least two cans per drinking episode.
- The way drinks are presented seems to be of great significance to females.
- Several human drinking behaviour values were reported in this work such as sip volume and consumption rate.
- There are differences in the drinking behaviour of males and females with respect to sip volume and count.
- The values derived from video observation agree with those measured directly validating this technique for use in further studies.
- Sipped beverages attain a temperature of only 14.9 °C in the mouth from chilled (4 °C).
- The environment in which the pizza and soft drink party experiment was held was perceived as more normal than artificial.
- Human enamel surface hardness loss was detected only in double-dosed diets (diet 3 and 4), while single-dosed diets had no ‘final’ effect on hardness (diet 1 and 2).

- Human enamel tissue integrity (i.e. surface loss) and ionic composition both were negatively affected by all diets delivered by Saltus. Yet, the effects were more apparent in double-dosed diets (diet 3 and 4).
- Ostrich eggshell surface hardness, tissue integrity and ionic composition all were negatively affected by all diets delivered by Saltus.
- Overall, erosive effects increased from short to long durations and from single-dosed to double-dosed with the least effect detected in shorter/single-dosed diets and the most effects in longer/double-dosed diets.
- Ostrich eggshell's response to erosive challenges in terms of tissue integrity and ionic composition was comparable to human enamel especially in single-dosed diets.
- Although the susceptibility to erosive challenges in terms of hardness for human enamel and Ostrich eggshell differed; both followed the same pattern.
- The accumulative method revealed an intermediate phase where relative hardness drops considerably before it bounces back to expected levels of hardness loss (period 1 and 2 versus period 3).
- The effects of different preventive measures were detected in terms of both surface hardness and tissue integrity when Ostrich eggshell specimens were tested.
- The effects of different preventive measures were detected in terms of tissue integrity rather than surface hardness when human enamel specimens were tested.
- Fluor Protector™ S has shown considerable preventive effects on the erosive effects of the beverage tested. Nevertheless, Regenerate™ NR-5 Boosting Serum failed to demonstrate such an effect.

- The test-beverage (Sprite) had a more erosive effect than the default test-beverage (Coca-Cola). This effect was detected in both substrates tested.
- Saltus is able to deliver diets that are consistent, reliable and reproducible.

CHAPTER 5

5. Discussion

5.1 The human tissue act questionnaire

In discussing the outcomes of the questionnaire it is important to clarify why this was undertaken. The questionnaire sought to assay, amongst general dental practitioners, their knowledge of the Human Tissue Act, as it relates to the collection of teeth for dental research and teaching. A secondary aim of the questionnaire was to compare and contrast the collection of extracted teeth before and after the Human Tissue Act (HTA) 2004 was validated (i.e. the 1st of Sep. 2006).

The questionnaire was designed so as to assess both the knowledge and understanding level of the participants; ensuring the intra-respondent reliability is therefore impractical to achieve as a result of the learning effect bias introduced by re-administration of the same questionnaire. Essentially we could not examine the reliability of the questionnaire for its responses were dependent on knowledge of the act. Nevertheless, the questionnaire was piloted upon a convenience sample of relevant respondents.

The respondents were given the option to return the questionnaire either by mail or via an online link. Interestingly, online responses accounted for only 12.9 % of the total responses received. Such a finding agrees with the percentage of online responses (9.3 %) reported when the response preference among healthcare professionals was assessed (Lusk et al., 2007). This might be attributed to demographic terms where

older age and female gender were found to choose mail over online as a method for completing and responding to a questionnaire (Lusk et al., 2007).

Despite the low return rate via the online link, a satisfactory overall return rate was obtained (50.8%). Geographically wide and random samples usually result in lower return rates (Hox and De Leeuw, 1994). As a result of this and the fact that there is a downward trend in returning questionnaires (Sahlqvist et al., 2011); the return rate achieved was very pleasing to see. Follow-up letters are said to enhance response rates (Roose et al., 2007, Linsky, 1975) but were not practicable here, if adopted the respondents would have been identifiable necessitating full ethical review. The then necessary ethical approval would have added to the bureaucratic workload of the participants and would have been counterproductive in actually diminishing return rates. This effect has been observed by others (Syed, 2007). Also in view of the sensitive issues of the survey the author wished the respondents to have anonymity to obtain a true impression of the state of knowledge of the Act. Perhaps making respondents identifiable would have hampered responses. It is worth mentioning, however, that the return rate by mail achieved agrees with the one reported by a meta-analysis of 45 studies that explicitly compare response rates of mail surveys (Hox and De Leeuw, 1994).

Twenty one years was the mean number of years each dentist had been practicing which is comparable to the mean number of years reported in a recent questionnaire from our research group that was distributed in the UK (Salem, 2014) for assessing attitudes and beliefs concerning root caries. The selection of participants was by the same method and perhaps the similar mean age illustrates how this technique provides a representative cross section of registrants.

Although the percentage of GDC registered dentists who qualified outside the UK was reported in 2010 to be 28.0% (Patel et al., 2011), only 15.4% of the present questionnaire respondents had qualified outside the UK. This might reflect a lower return rate from non-UK qualified dentists compared to the ones who qualified in the UK.

Sodium hypochlorite was the most popular storage medium of extracted teeth which accounted for just under half (44.4%) of all dentists who collect extracted teeth in their practice. This might jeopardise the suitability of such teeth to undergo certain *in vitro* experiments such as bond strength assessments for sodium hypochlorite was found to significantly weaken composite-to-dentine bond strength in bovine teeth (Lee et al., 2007).

The majority of respondents would agree to collect teeth for dental education (82.9%) and research (79.7%) if approached by an institution. However, among the dentists who would refuse; all of them qualified before 2006. This could be that as such dentists were at dental school at the time the act came into being they are more aware of its content and ramifications and thus, through lack of a working knowledge, more sensitised to the potential legal risks tooth collection entails.

Although relevant material (i.e. extracted teeth) obtained for education do not need to be stored under a Human Tissue Act license (providing the teeth are not to be involved in research)(Human Tissue Authority, 2014); more than 90% of respondents believed that consent was required for use in both dental education and research. Among those who believed so, their views were divided almost equally between 3 consent types namely ‘verbal consent’, ‘record in notes’ and ‘written consent’. This might suggest that dental clinicians tend to be cautious with regard to collecting teeth due to the lack

of a working knowledge of the Human Tissue Act. On the other hand, most of the respondents admitted that practicing upon human teeth was very helpful during their undergraduate training indicating that they would collect teeth more readily for such a purpose if they understood the Act better.

Most of the respondents correctly identified that freshly extracted teeth are owned by the patient and not the dentist but 13.4% of the respondents believe otherwise. It is noteworthy that among the respondents who believed that the dentist owned the freshly extracted teeth immediately after their extraction, the majority stated that consent was not required at all for collecting teeth for neither the purposes of dental education nor the purposes of dental research. This further illustrates widespread confusion concerning the Act. The correct position is that the freshly extracted tooth is the property of the patient unless consent is given for its retention irrespective of the purpose of use (Human Tissue Authority, 2014)

Only 28.3% of respondents identified the need for a human tissue authority license if the teeth collected are to be used for research purposes and to provide a further disincentive to this process, 21.7% of respondents stated that teeth collected for research purposes must be traceable back to the donor which clearly would add additional bureaucracy to the whole process.

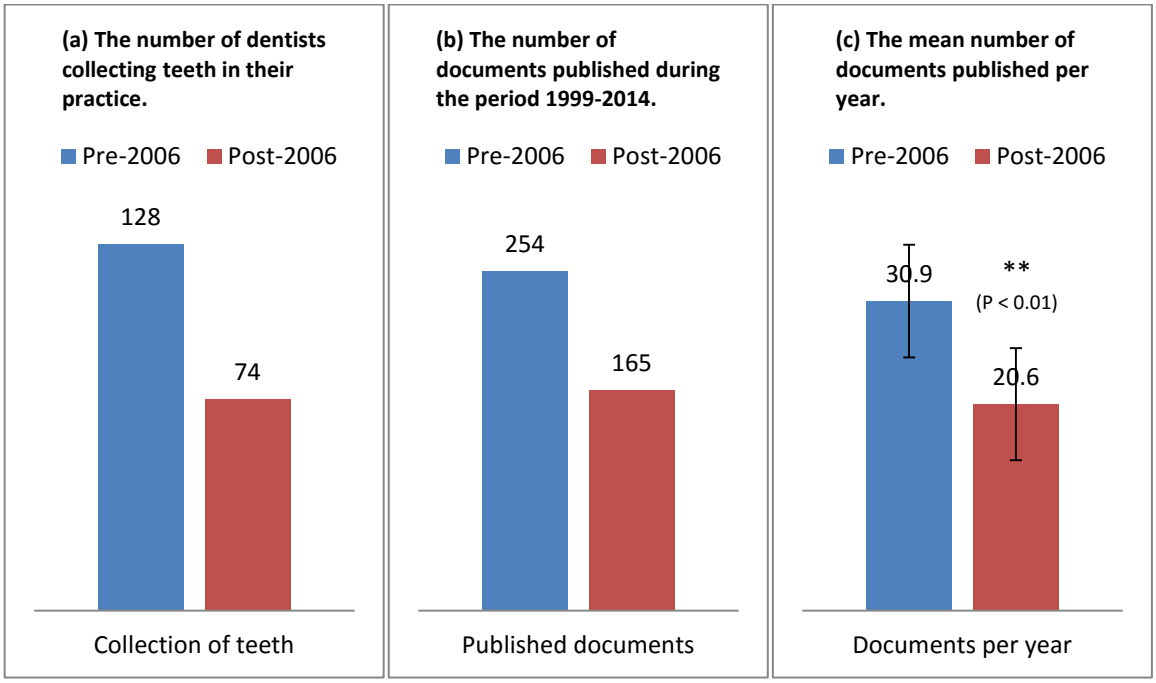
For years, extracted human teeth have made an invaluable contribution to the noticeable success of dental research. However, further breakthroughs are potentially under threat particularly in the UK after the Human Tissue Act (HTA) came into force in 2006. At the time of legislation the outlines of the Human Tissue Act relating to the deceased were agreeable; the inclusion of the living was widely controversial. Thus according to some, when the Human Tissue Act outlines were first published, it was

considered as a superfluous response to the main issues of the organ retention scandals that brought about its creation (Forsyth and Woof, 2006).

If the dentist is to use extracted teeth for training, education, clinical audit or diagnostic archiving; storing extracted teeth is permissible unless otherwise is expressly stated by the patient (Human Tissue Authority, 2014). If the extracted teeth are to be used for research purposes; they must be stored on Human Tissue Act-licensed premises.

In view of this, it was perhaps surprising to find that only 27.8% of all respondents, ceased to collect extracted teeth after the year 2006 but unsurprising that only 6% of respondents claimed to be collecting teeth for research purposes on behalf of dental institutions. This latter finding may account for the findings of the present study's literature review (See section 2.6.1 [Literature review: Human teeth]) where the number of published dental research papers as a result of work undertaken in the United Kingdom utilising human teeth under in vitro conditions dropped by more than 35%. Furthermore, the number of published documents in 2014 has gone down to its lowest since 1995, and the total number of publications in 2013 and 2014 combined is lower than the yearly mean number of publications in any single year over the period from 1996 to 2006 (Figure 5-1). Although the reasons for such a drop are not certain, they definitely need further investigation. One possible factor might be that extracted human teeth stocks are depleting rapidly, especially the ones that were collected prior to the validation of the Human Tissue Act in 2006 thus preventing such work or that such work in a focussed research environment is less popular.

Figure 5-1. The implications of the Human Tissue Act 2004 for dentistry.



Source: HTA Questionnaire (See section 4.1)

Source: HTA literature review (See section 3.1)

Source: HTA literature review (See section 3.1)

Figure 5-1 shows that the overall trends of the number of dentists collecting teeth in their practice (a) and the number of published papers in the UK (b) pre- and post-2006 were comparable. Over an 18-year period, the mean number of published papers per year during the 'post-2006' period (c) was considerably less relative to the 'pre-2006' period and this difference was highly statistically significant (Unpaired T-test)($P<0.01$).

Ideally, the implications of the Human Tissue Act 2004 for dental clinicians are minimal but from the results discussed here this is not the case. The declared intention of the Human Tissue Act 2004 legislation that "it should in no way hinder research" by Lord Warner (2004) is thus not being met (Hansard, 2004).

[Lord Warner (UK Health Minister). Lords, Hansard, 25 October 2004, Column 1073.]

In summary, this work has identified a degree of ambivalence among clinicians as to whether collecting and storing teeth for the purposes of dental education and/or

research requires patient consent and/or HTA licensing. It has also identified that dental research has potentially reduced due to the Act. It is acknowledged that the 2004 act comes short in providing dental professionals with the required assurances of legality. There is thus a need for better education concerning the act but as this would inevitably lead to increased administrative load the success in increasing research tooth donations is unknown. An alternative strategy may therefore be to explore different sources of tissue, outwith the act, upon which dental investigations may be conducted. Thus, reserving teeth to final testing of 'promising formulations' as assessed by testing on widely available alternatives.

5.2 Informing a realistic laboratory erosion-testing regime

This section sought to measure aspects of fizzy drink consumption in a social environment to inform the development of a laboratory testing regime.

5.2.1 Pre-experimental questionnaire

Those who consented to participate in the study were asked to complete a pre-visit questionnaire that assessed their beliefs concerning their personal fizzy drink consumption and preferences (beverage choice, method of drinking, serving temperature, quantity and rate of drinking). Although soft drink type has a potential impact on drinking behaviour; researchers have rarely offered subjects a selection of soft drinks to choose from (Kidorf et al., 1990). Throughout the current experiment participants were served their drink of choice which was made possible via the pre-experimental questionnaire. The questionnaire also served as a method of checking for any food or drink allergies that would impact adversely upon the smooth running of the experiment.

Prior to administration of the actual questionnaire its reliability was assessed upon a convenience sample where a reliability of 0.961 was obtained (weighted Kappa) when administered to 6 subjects on two occasions.

Potential participants were sought from all University of Dundee students by the weekly email they receive advertising events in that institution (University of Dundee SOMiS Hermes-II email distribution system); therefore, a return rate could not be calculated. However, the invitation to participate was made and sent weekly over seven-consecutive weeks by this medium.

Respondents in excess of 300 returned the questionnaire. Such a convenient number of respondents is reflected by the fact that younger respondents (in this case: university students) tend to prefer online-based questionnaires over conventional mail ones (Suh, 2013, Ward et al., 2014). This contrasts with that found in the earlier part of this thesis when, in a questionnaire to assess the workings of the Human Tissue Act, Online responses were low. This might also be attributed to the fact that younger age groups prefer the online option over all other forms as a method for completing and responding to a questionnaire (Lusk et al., 2007).

Two products from the Coca-cola Company and one product from the A.G. Barr Company were foremost, as the most preferred drinks among respondents. This agrees with a recent market report that states that the Coca-cola Company is currently the leading soft drinks market shares in the United Kingdom with 59.3% of market's share volume while the A. G. Barr company accounts for a further 3.4%, as the third largest in the UK and the second in Scotland (MLI Profile, 2013).

Overall, 9.6% of respondents stated that they were consuming a soft drink at least once a day. The remaining 90.4% of respondents were consuming less. A questionnaire of oral health habits undertaken among Australian athletes (Sirimaharaj et al., 2002) reported a higher percentage of respondents (28.3%) who consumed a soft drink once or more a day. The percentage of 9.6 is not very high; however, it is still alarming for it has been reported that subjects who consume soft drinks on a daily basis are more likely to have dental erosion (P=0.0015)(Waterhouse et al., 2008). It is worth mentioning, that among respondents who stated that they consumed soft drinks at least once a day, 34.5% consumed at least two cans or equivalent per drinking episode.

A recent systematic review (Salas et al., 2015) concluded that the consumption of soft drinks increased the odds of an individual to develop dental erosion.

Most respondents consumed their beverages with food, in parties and in cinemas but other occasions came to light for this activity. 37% of respondents consumed soft drinks as mixers for hard drinks, this coincides with the findings reported in Scotland that people were quite commonly having soft drinks with hard drinks as mixers (Syed, 2007).

With regard to the use of a straw, 34% of respondents requested one in their response to the invitation to attend the pizza and soft drink party of this study. A comparable outcome was obtained from a survey conducted among adolescents where 42% claimed to drink with a straw (Tokumbo et al., 2014).

5.2.2 Pizza and soft drink party

In the present study, food was served to relax people and stimulate the desire to drink in a comfortable atmosphere; perhaps mimicking a group lunch break or a social gathering atmosphere. Generally, fast food triggers thirstiness more than other food types and is preferred more by the targeted age group of the subjects of this study (Patterson et al., 2012). It was therefore why pizza was selected as the food to be served as well as a reflection of its ease of preparation. In order to conduct this work 108 pizzas were prepared and 115 cans of drinks were opened. In order to fully observe the subjects it was important therefore that food and beverages were easily prepared so the researchers were not distracted by preparation duties.

Although soft drink type has a potential impact on drinking behaviour; researchers have rarely offered subjects a selection of soft drinks to choose from (Kidorf et al., 1990). In this work however the subjects were able to select their preferred drink as the researchers wished to observe as normal a behaviour as possible. Drinking an unpreferred drink could result in atypical consumption values. Throughout the current experiment participants were served with their drink of choice with no imposed time or quantity limit.

In the present work participant observation data was obtained by monitoring subjects as they drank. Drinking might be held under either experimental or natural settings. Limited research has been undertaken exploring patterns of consumption in social environments; with their focus being on hard rather than soft drinks (Pennay and Lubman, 2012, Babor et al., 1980). In the present study, normal drinking behaviour, we believe, was successfully simulated in a social atmosphere. To the author's knowledge, this is the first study to report drinking behaviour values for carbonated beverages in such an environment.

It is generally accepted that temperature can significantly affect dental erosion. In the present study the overall expectorated sip temperature was found to be 14.9 ± 2.0 ˚C. In light of previous recommendations concerning the temperature at which to conduct *in vitro* erosion studies (body temperature 37 °C/ oral cavity temperature 36 °C/ room temperature 25 °C) (Shellis et al., 2011) this was surprising but such recommendations could of course be accounted for by the desire to accelerate the erosive process in the laboratory. In the author's view it is reasonable to suggest that a more physiological temperature at which to conduct such studies is around 14.9 °C based upon our observation that a carbonated beverage stored at 4˚C is found to have reached this

temperature upon expectoration having been in the mouth only for a few seconds. On an anatomical basis the oral cavity, unlike the nasal cavity with its turbinate anatomical structure, is not designed to heat (Keck et al., 2011).

Sip volume

Several researchers report liquid sip volume values that can be of use as comparators to the results reported here. It is however important to note that some use liquids other than carbonated beverages. Some have shown that there is no significant difference in sip volumes between water and carbonated beverages (Adnerhill et al.,1989), a more recent study showed that water sip size differs from carbonated beverages sip size (Steele and Van Lieshout, 2004,); this difference might be attributed to differences in both flavour and density of the imbibed liquid which appears to influence sip-sizing behaviours (Adnerhill et al., 1989; Steele and Van Lieshout, 2004,). In the present study, mean calculated sip volume of carbonated beverages in a social environment was found to be 16.8 ± 5.9 ml overall among genders. In addition, the mean expectorated sip volume (non-social) was 17.2 ± 7.9 ml. Both values are in accord with a rather wide range of liquid sip volume values reported in the literature, ranging from 12 to 37.5 ml (Jones and Work, 1961; Halpern, 1985; Speirs et al., 1988; Adnerhill et al., 1989; Nilsson et al., 1996; Hughes and Wiles, 1996; Lawless et al., 2003; Chee et al., 2005; Alves et al., 2007; Yang et al., 2012). The close agreement between the measured and calculated values, that utilised video observation in their derivation, gives pedigree to the method of observation used in this study. Such technique could therefore be of value in other studies.

Several studies have demonstrated that the sip volume for males is significantly larger compared with females (Adnerhill et al., 1989, Lawless et al., 2003). This difference is

in agreement with the results obtained from the current study, where both calculated sip volume and expectorated sip volume values for males (18 ± 5.9 ml and 19.1 ± 8.2 ml respectively) were significantly larger than the value for females (15.1 ± 5.5 ml and 14.8 ± 6.9 respectively) ($P < 0.05$). This difference is attributed, we believe, to anatomical differences among the sexes.

Unlike the present study, the aforementioned attempts to report sip volumes were conducted during non-social atmospheres in which subjects had to follow certain instructions. Some workers (Bennett et al., 2009) have reported sip volume in a more natural water drinking setting, in which subjects did not realise they were monitored while sipping, and compared it with values obtained under instructed conditions. Their reported sip volume mean value for the none instructed natural drinking task was 16 ml which is in agreement with the results reported in the present study (16.8 ± 5.9 ml) (Bennett et al., 2009). Thus, the experimental setting adopted in our study does not appear to have generated artificial behaviour.

Consumption Rate

Most previous attempts to measure consumption rates (i.e.: water drinking) have been aimed at determining swallowing velocities (known also as swallowing capacity or swallowing rate) rather than consumption rate *per se*. Swallowing velocity has been reported to be greater in males compared to females (Hughes and Wiles, 1996, Dantas et al., 2009).

Attempts to assess consumption rates by other workers have focused on hard rather than soft drinks. (Billings et al., 1976, Rosenbluth et al., 1978, Geller et al., 1986). In such work the consumption rate calculations depend on two factors, namely, quantity and time. In the present study, the mean consumption per person was found to be

719.9 ± 393.8 ml for males and 562.3 ± 249.9 ml for females. This difference in consumption between the genders is consistent with that reported in studies investigating the consumption of alcoholic drinks (Rosenbluth et al., 1978, Geller et al., 1986).

Generally, in the current study, the overall mean consumption per person was 654.9 ± 348.8 ml. However, it has been demonstrated that the mean consumption for beer and mixed hard drinks within 30 minutes of non-social laboratory drinking was 543 ± 240 ml and 519 ± 268 ml respectively (Kidorf et al.,1990). Although these values look different at first, understanding the effects of time-limit and a non-social atmosphere on subject performance, presumably, render them comparable. There might be a possibility that the subjects did not intend to drink that much at the outset but greater fizzy drink consumption might actually be due to drinkers feeling tempted to drink more just because it was free.

In contrast to an experiment that measured the time a beverage was kept in the mouth before swallowing (Johansson et al., 2002); the current experiment measured the total time that a beverage was being placed into the oral cavity. Both these factors place an erosive burden upon the teeth and so are important in assessing erosive risk. Subjects consumed their beverages over a period of 44.2 minutes on average which is alarming with respect to oral pH levels; for exposure to acids for periods longer than 10 minutes will have potential to cause loss of tooth structure in depth (Hara and Zero, 2008). Such a lengthy exposure to beverages results in a continuous source of acid in the oral cavity which could have a catastrophic effect on the integrity of tooth structure. This observation however needs to be treated with some caution for the work cited to support it (Hara and Zero, 2008), is a laboratory based study in which

there is no salivary buffering. Contemporary preventive advice for patients to prevent erosion stresses limiting drink to tooth contact time (Moynihan, 2002) and reinforces our belief that both the duration of drinking and holding time of a sip in the mouth before swallowing are important factors to be considered when evaluating erosive risk.

In the present study, the overall mean consumption rate value was calculated to be 13.3 ± 6.0 ml/min, with males drinking at a higher consumption rate compared with females (14.4 ± 6.4 ml/min and 11.8 ± 5.0 ml/min respectively). This is in agreement with a study by Rosenbluth et al. where males consumed beer at a higher consumption rate than did females (Rosenbluth et al., 1978). In another barroom observation study, beer drinking consumption rate for males were reported to be significantly higher than for females (26.1 ml/min and 15.9 ml/min respectively) ($P < 0.01$) (Geller et al., 1986).

A laboratory study reported mean total consumed volume within 30 minutes of non-social laboratory drinking to be 543 ± 240 ml for beer and 519 ± 268 ml for mixed hard drinks (Kidorf et al., 1990). Interpretation of the aforementioned data by simple mathematical calculations yields two consumption rate values, 18.1 ± 8 ml/min for beer and 17.3 ± 8.9 ml/min for mixed hard drinks. These values along with the ones provided by Geller et al (Geller et al., 1986) when weighed up against the values from the current study (i.e. 13.3 ± 6.0 ml/min) presumably gives a sensible explanation of the slight rate difference, bearing in mind the differences in beverage type, social atmosphere and the presence of food. In other words, when comparing two social scenarios, the first a social gathering over lunch or dinner with food and soft drinks being served, and the second a social gathering in a barroom with only hard drinks; consumption rate of drinks will be less in the former scenario compared with the latter owing to the presence of food and the distinctive social atmosphere of each.

Previous attempts to design and run demineralisation/remineralisation cycle regimes utilized acid flow rates ranging from 0.15 to 5 ml/min and durations from 1 to 10 minutes that replicated a daily acid intake of 30 ml at most (Wiegand et al., 2007, Magalhães et al., 2008, Attin et al., 2003, Attin et al., 2005, Lagerweij et al., 2006) which, does not reflect a realistic human drinking behaviour for the present study has shown that daily acid intake can exceed this.

More participant observation studies are needed in which to extend this research to look at factors that influence fizzy drinks consumption of individuals and to incorporate more age groups to include teenagers and older individuals. This would help to determine the generalizability of the reported drinking behaviour values and to reflect the observed behaviour on the atmosphere and experimental setting.

It is acknowledged that the dental erosion state of the participants was not ascertained in this study. Though potentially being related to drinking habits it has been demonstrated previously that *in vivo* erosion is not correlated significantly to the quantity of beverage intake (Chadwick et al., 2004) being more a product of individual susceptibility factors when the teeth are exposed to such risk. A recent systematic review (Salas et al., 2015) concludes that consumption of soft drinks, acidic snacks/sweets and acidic fruit juices increases the odds of an individual developing dental erosion.

It is also worth mentioning that, for technical reasons, it was not possible to record the full extent of the 3 independent values mentioned earlier for all subjects (i.e. V_T, t and S). Subject 21, for instance, was out of the camcorders' coverage area throughout the whole session, therefore, only the value of his total volume of consumption could be determined. As a result of this, and the fact that t and S could not be otherwise

determined for subject 21, the total volume of consumption for this subject was later utilized for the calculation of the total consumed volume per subject (V_T) but was excluded elsewhere.

Several human drinking behaviour values were reported in this study and these will be of value in the development of more realistic laboratory erosion-testing regimes. It is concluded, within the limitations of this work, that (1) there are differences in the drinking behaviour of males and females with respect to sip volume and count, (2) the intraoral rise in temperature of a 4 °C beverage is lower than that used in previous laboratory simulations and (3) the values derived from video observation agree with those measured directly validating this technique for use in further studies.

5.2.3 Post-experimental questionnaire

This post-experimental questionnaire assayed whether or not the participants felt they had behaved and performed in a way that reflected their normal behaviour.

After the pizza and soft drink party, participants ranked their behaviour and performance highly on the scaled questions, averaging 8.83 and 8.13 out of 10 (see section 4.2.3 [Post-experimental questionnaire: Figure (4.2-10)]. Their written comments were similarly positive. This indicates that participants were especially relaxed and acting normal, consuming almost exactly the same amount of drink they would usually consume in a similar real-life scenario.

The researcher was impressed with the outcome of the parties; social interaction seemed to build quickly, evidenced by participants' eagerness to engage in conversations while enjoying the food, drinks and background music. It has been shown that listening to music and socializing with a group of people while consuming

food and drinks would influence their overall intake (Wansink, 2004, Stroebele and de Castro, 2006). It has also been reported that providing more food, drink and time to a group of people would result in higher consumption (Rozin et al., 1998).

The application of a post-experimental questionnaire has been shown previously to be useful in identifying the realism or otherwise of the experimental setting (Stephens, 2010). It is however acknowledged that in the present study the simple questionnaire used has not been validated in any large study.

5.3 The artificial mouth model

Artificial systems cannot entirely mimic human parameters; however, every attempt should be made to close the gap and achieve a more realistic simulation of what actually happens in real-life scenarios.

This aspect of the work sought to design and build a model that had the potential to mimic the interaction of saliva and enamel during the process of consuming an erosive beverage. This last aspect has been many times overlooked in previous models and was informed by the behaviour observed in the pizza and soft drink parties. Once the parameters that are based upon human behaviour have been set; realistic experimental diets become more feasible. This approach permits several human drinking behaviour parameters to be implemented in a customisable manner such as salivary kinematic behaviour, beverage flow rate and volume of consumption.

Cardboard mock-up models are very informative in the early conceptual stages of design (Akaoka et al., 2010); therefore, a physical mock-up was constructed. This allowed the researcher to explore geometry, flaws, the mechanics of operation, and discover any impediments that might encounter the placement of tubing and connectors.

The AutoCAD software was used to make drawings of the model's component parts. These were refined and printed out to verify that construction was possible. Constructing models using computer-aided design and drafting software packages has been shown to facilitate technical design checking, assembly planning and geometric management (O'Brien et al., 2012)(Figure 5-2).

The Blender™ software was used to generate realistic model rendering. Its comprehensive array of modelling tools allowed modification of the working skeleton and creation of accurate male/female slot assemblies to anchor components in 3D. A virtual model resulted and its potential to receive fluids was assessed by means of allowing Blender™ to generate a mock fluid flow throughout the system. This benefitted from its powerful fluid kinematic behaviour simulation ability via the Blender™ virtual simulation tool. Blender™ fluid simulation can be used as a visualization technique for better understanding of kinematic flow behaviour (Naumov et al., 2014). It has been used to good effect in for example to simulate a biologically inspired underwater robot (Listak et al., 2008), an underwater vehicle (Kim et al., 2013), and the pressure gradient across aortic stenosis (Randles et al., 2014). In the present study, fluid simulations were carried out under a virtual temperature of 15 ˚C, a dynamic viscosity of 1.002 centipoise (cP) and a kinematic viscosity of 1.002×10^{-6} $m^2 s^{-1}$. Realistic rendering of model surfaces and connections plays an important role in retrieving in-depth information and simulating volumetric scenes (Naumov et al., 2014). Such a simulation verified that the design, with its associated slopes, allowed for the desired fluid flow, circulation and collection (Figure 5-3). To the author's knowledge this is the first time such a research tool has been applied in dental research.

Figure 5-2. AutoCAD software was used to generate three-dimensional (3D) modelling before assembly.

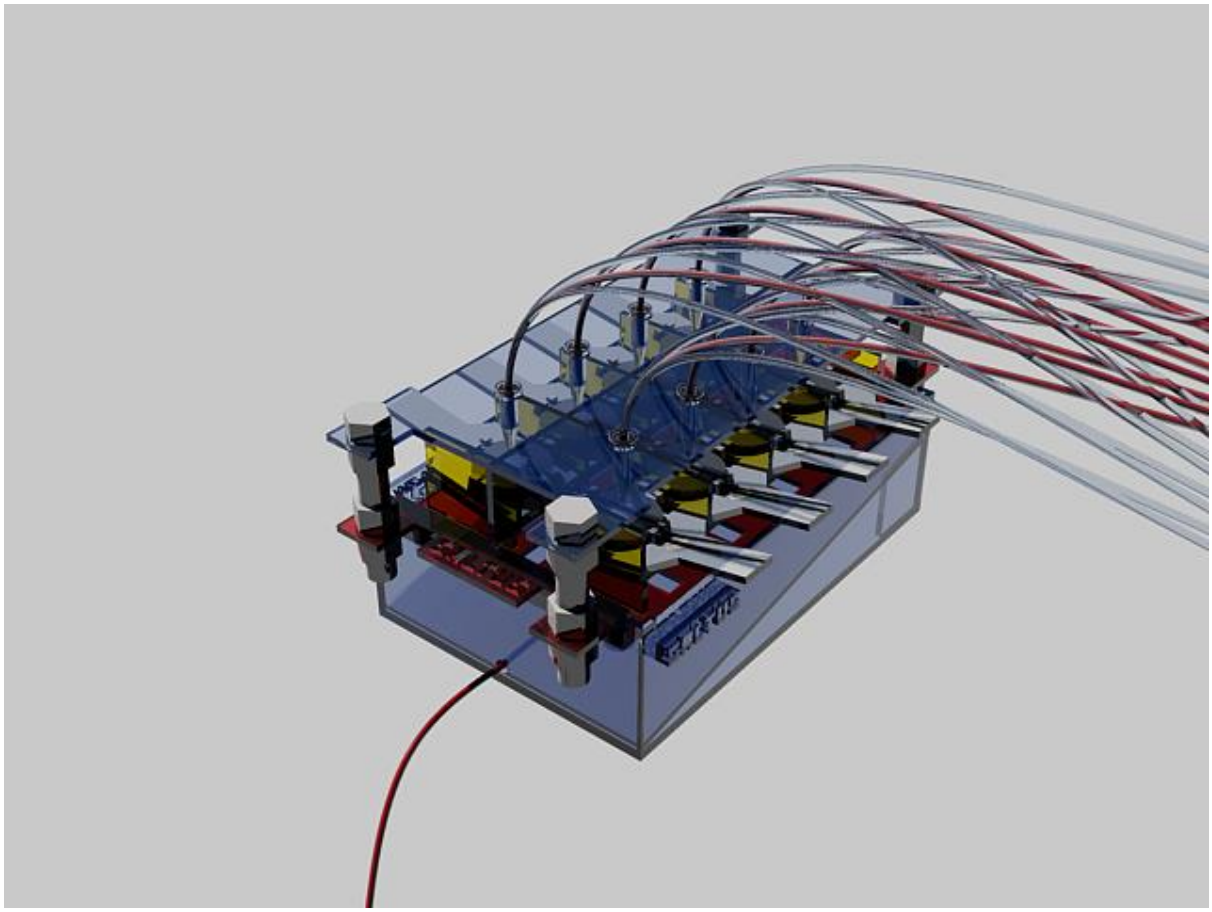


Figure 5-3. Blender™ software rendering algorithms in 'Blender Cycles' help with closing the gap between real-time virtual environments and photorealistic rendering.

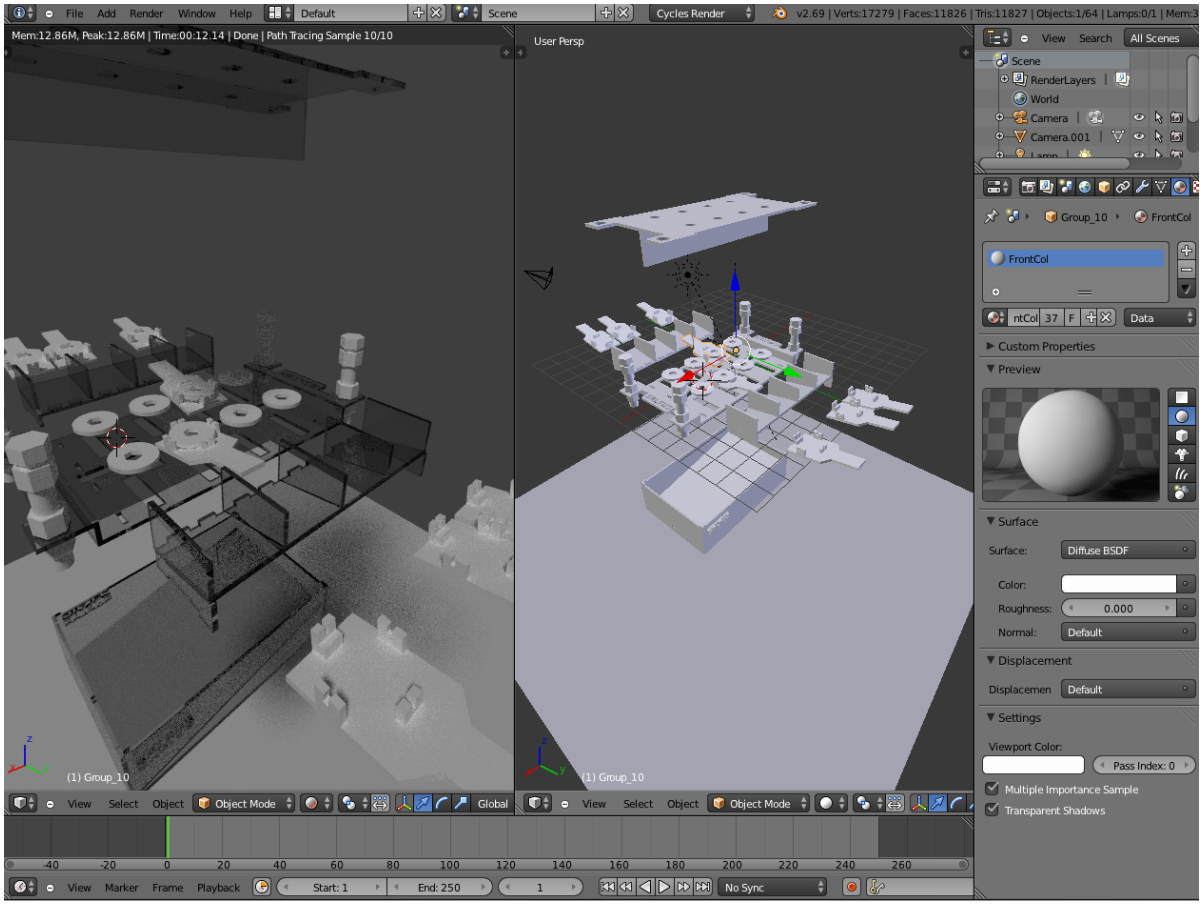


Figure 5-4. Efficient mixing of artificial saliva and the test beverage is required.

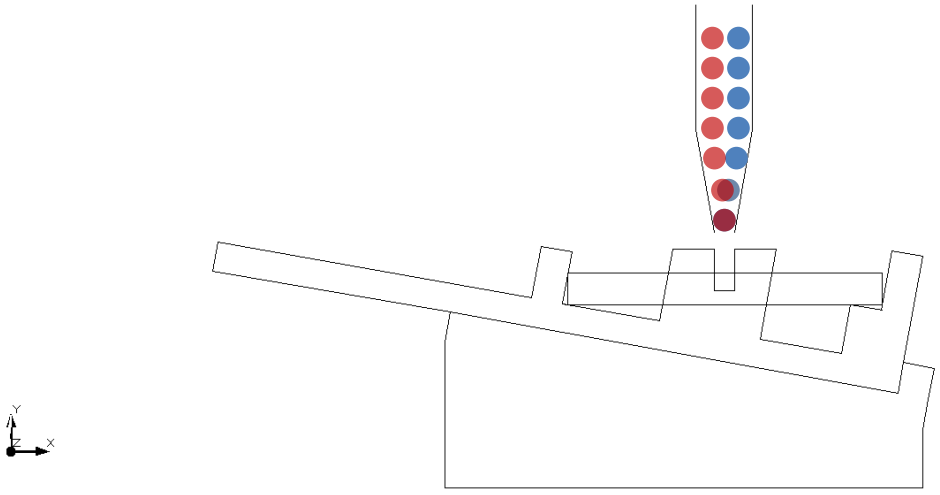
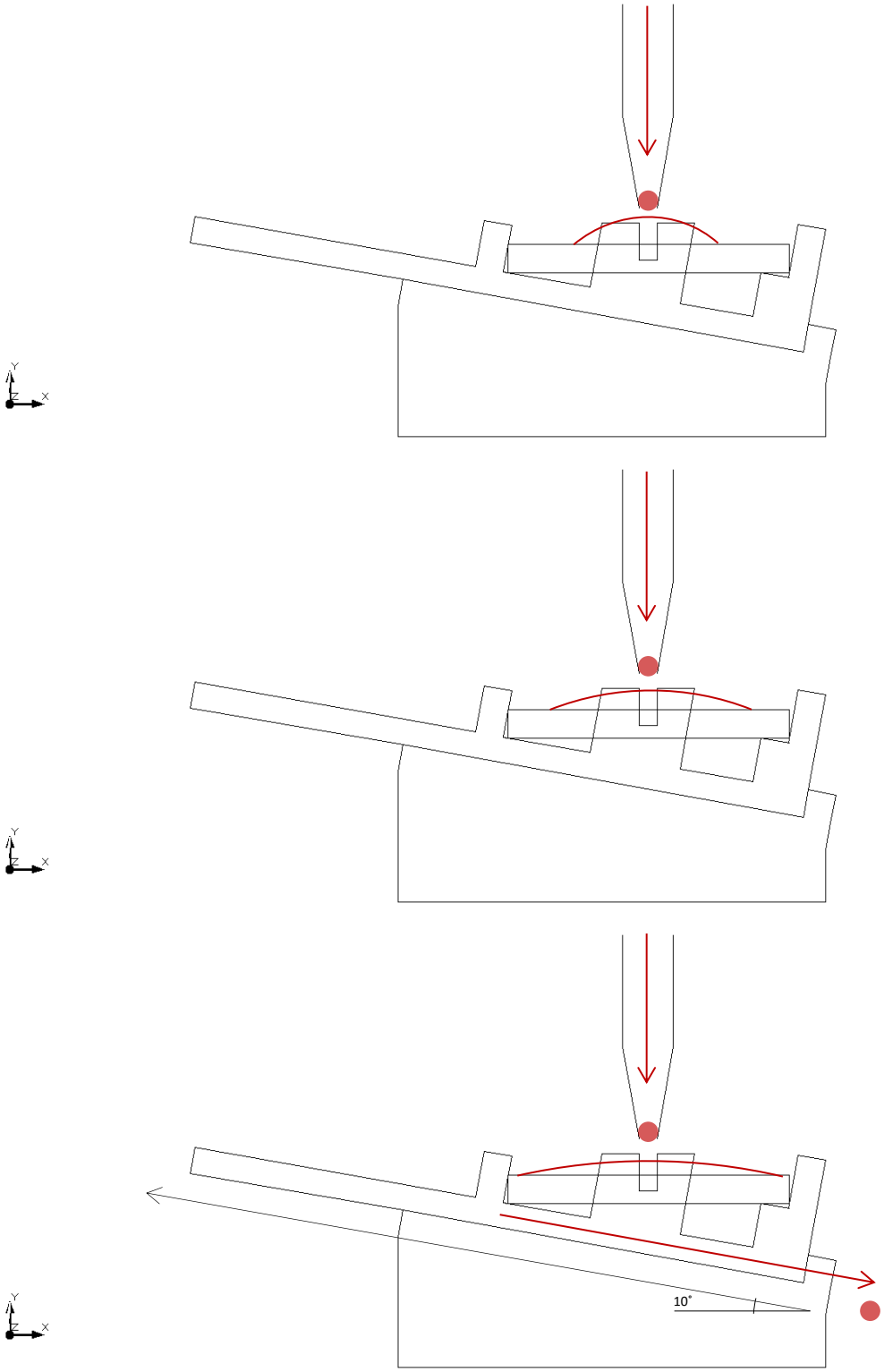


Figure 5-5. The volume of artificial saliva in contact with specimen holder's surface was around 1 ml.



Each cell in Saltus was set to represent 1 human subject, that is, each cell received a full dose of the test beverage (i.e. either single or double) as well as a volume of artificial saliva that is equal to the volume a normal individual would produce on average. This has caused the design of the shelf to be adjusted (figure 5-5) so that it can allow a volume of artificial saliva of up to 1 ml to act as 'resting saliva' and to bathe each specimen during the times of rest (i.e. minimal salivary flow). The volume of resting saliva in the oral cavity at any point of time is around 1 ml (Lagerlof and Dawes, 1984). This is perhaps most clearly understood by observing a droplet resting on an inclined surface which has a known area, similar to an artificial saliva droplet on a test substrate. Gravity together with a constant source of droplets would allow the droplet to slide down after reaching a certain size. This would occur right after the growing droplet's circular shape reaches the boundary limits of the surface (in the direction of the driving force) where the interfacial tension would break (see also (Eral and Oh, 2013)). As a result, a droplet volume limit beyond which the droplet cannot grow further will develop; which is in this case a volume of 1 ml.

The Saltus model sought to investigate the effects of realistic human drinking behaviour upon erosion susceptible substrates countered by saliva flow. To achieve this, normal physiological flow rates of saliva along with carbonated beverage drinking rate had to be calibrated for and achieved. Normal physiological flow rates were set to 5.0 ml/min for stimulated saliva (Dawes, 1987), 0.3 ml/min for the waking hours of unstimulated saliva (Thomson et al. 2011; Dorion, 2011), and 0.1 ml/min for the sleeping hours of unstimulated saliva (Dorion, 2011). A carbonated beverage drinking rate of 13.3 ml/min was adopted according to the consumption rate obtained from the pizza and soft drink party (Qutieshat et al, 2015).

The peristaltic Ismatec IPC 24 was selected for several reasons; firstly, it can produce the required flow rates simultaneously provided that the appropriate tubing is mounted (see section 3.3.2.II [Flow rate calculations]). Secondly, it is controlled by a microprocessor that offers full control over 'dispensing' and 'pause time' periods which allows the operator to pre-programme the pump to run for several cycles. Thirdly, it produces no pulsation which renders it ideal for the purpose of erosion studies. Finally, the pump's flow rate and dispensing volume can be calibrated allowing for a very accurate pumping with high repeatability on all tubing channels (see section 8.6 [Appendix 6: Pump calibration]). Such a pump has been used by other researchers (Nair and Merkel, 2015, Bento et al., 2012, Haberer et al., 2012).

5.3.1 A pilot study of Saltus mixer physics

The approach used in this section was inspired by a study that assessed the acid-base characteristics of different citrate buffer systems in in the presence of Bromophenol blue indicator dye (Li et al., 2004). In Saltus, before the fluids contact the textured substrate the fluids must be mixed. To ensure this, Saltus was equipped with mixers. Thus, at any point of time, the specimen surfaces should not be exposed to the beverage under investigation in the absence of artificial saliva (Figure 5-4). Moreover, at any given point of time, no more than 2 solutions will be dispensed through Saltus' mixer unit. Bromophenol blue dye is an accepted acid-base indicator (Kolthoff and van Berk, 1927). At low pH, the dye absorbs ultraviolet and blue light most strongly and appears yellow in solution. Thus, Bromophenol blue and citric acid were chosen to test Saltus' mixers mixing ability. Mixing solutions using both Saltus mixers and magnetic stirrers resulted in comparable spectrophotometric readings (λ 0.000 % vs λ 0.003 %). Moreover, video footage of the process (CD-ROM) has also demonstrated the efficacy

of Saltus mixers. This pilot study confirmed that Saltus' mixer units have achieved the desired efficient mixing of citric acid and Bromophenol blue by virtue of their near to λ 0.000 % value.

5.3.2 Delivering the beverage to be tested with simulated saliva flow

I. Beverage delivery

Based on the findings of the pizza and soft drink party (Qutieshat et al. 2015); the overall consumed volume per subject was 654.9 ≈ two (330 ml) cans and the overall time period of consumption was around 44 minutes. Extrapolation of this behaviour to program Saltus yields;

- Assuming a timeline of 44 minutes, at time point 0' the first can of the chilled beverage should be opened and poured in a glass.
- At time point 22', the glass should be refilled by opening and pouring the second 'chilled' can.

Saltus beverage delivery system was programed to follow this algorithm, where a micrometer timer switch controlled the flow of the chilled test-beverage into a large glass beaker (enough to provide 8 Saltus units with 1 can of test beverage each per time of operation) from which it was conveyed to the Saltus mixing tips. This set-up ensured a gassed and chilled test beverage was conveyed to specimens thus mimicking the real-life scenario of drinking from a glass. Such simulation hasn't been attempted previously.

II. Verifying the constancy of drink temperature in the time frame of an experimental run

From the pizza and soft drink party results, the temperature that we wish the drink in Saltus is to be consumed at is 14.9 ± 2.0 ˚C when it contacts the substrate (Qutieshat et al. 2015). To achieve this in day to day operation, the temperature at which the test beverage was dispensed was set to be 14 ˚C. This value was determined after conducting a pilot study to verify the constancy of beverage temperature in the time frame of one experimental run (see section 4.5 [Results: Figure (4.5-2)]). As a consequence, a temperature range of 14.0 ˚C (when the test-cycle begins) to 15.7 ˚C (when the test-cycle ends) was observed over a duration of 22 minutes. These equate to a mean of around 14.9 ˚C which matches the desired temperature sought and thus ensures the experiment will run under this desired temperature throughout. It was important that this was ascertained for the system inevitably has lead in times for the supply of the beverage to the erosion test substrate.

5.3.3 Saliva delivery

Artificial saliva tends to lose its carbon dioxide content on contact with free air (Darvell, 1978, Mandel, 1974). Therefore, in order to prevent this and to ensure stability of the prepared saliva, laboratory glass bottles' caps were modified to keep artificial saliva in a closed compartment throughout the experimental diets. In addition, the unstimulated artificial saliva was replenished on a daily basis while the stimulated saliva one was prepared fresh just before the commencement of an experimental run. If the volatility of carbon dioxide was overlooked; bicarbonate content of the prepared saliva will be readily lost which, as well as depleting one of the major buffering constituents of saliva, would cause an unpredictable rise in pH.

Generally, this phenomenon occurs in high temperatures such as 37 °C (Darvell, 1978), hence the decision of using distilled water at 4 °C to prepare the working solution of artificial saliva to prevent such a situation arising..

In the experimental runs of the study, Saltus delivered 5 litres of artificial saliva to 8 specimens. Knowing that each specimen represents one human subject; this translates into 0.626 litres per day per specimen which coincides with the range of daily amount of saliva produced in humans (0.5 - 1.5 litres) (DeAlmeida, 2008).

5.4 Formulation of artificial saliva

The work of this thesis initially relied upon the salivary formulation described by Leung and Darvel (Leung and Darvell, 1991). This contained 'typical' amounts of the major ionic constituents of human saliva (Darvell, 1978) and as such represented a firm basis upon which the development of a more complete saliva-like medium, by the addition of an appropriate calcium ion vehicle despite its lack of organic materials and some elements such as F, Br, and I. This offered the possibility of giving the artificial saliva remineralisation potential. For the purposes of the present study, proteins and other organic substances were, as argued before (Darvell, 1978), deliberately left out of the formulation due to being difficult to characterise and obtain reproducibly, despite their obvious importance in other contexts. There is limited understanding of such organic parts of saliva, and so apart from the difficulties of incorporation it is acknowledged that such an omission potentially omits from consideration surface effects due to their adsorption. It is however of note that no other system incorporates these.

To demonstrate the reliability and reproducibility of the selected artificial salivary constituents during the solution's flow in and out of the Saltus tubing, connectors and components; a comparison between artificial saliva's calcium and phosphate ion content before and after a substrate- and beverage-free single day diet was undertaken (see section 4.6 [Results: Table 4.6-I]. The good reproducibility of the experimental results in respect of Calcium (1.11 ± 0.01 vs 1.10 ± 0.01) and phosphate (4.76 ± 0.03 vs 4.75 ± 0.02) demonstrated that the method used in saliva preparation provided a satisfactory route to the desired outcome.

However, natural saliva has no definitive composition (Mandel, 1974); the composition varies among different salivary glands as well as among individuals. Thus, preparing a

solution that simulates exactly the chemical and physical characteristics of the natural salivary product is impossible (Darvell, 1978).

It has been argued that collecting a pool of natural whole saliva from human subjects for the purposes of *in vitro* studies is impractical (Higgins et al., 1973) due to several problems as discussed earlier (see section [2.4.2.III]. Furthermore, in view of the experimental settings of this work; the amount of saliva required necessitates the recruitment of a very large number of individuals from which natural whole saliva can be collected which thus further demonstrates the limitations of such approach. Not to mention the need for a large amount of natural stimulated saliva at specific times (test-cycles) throughout the diet.

In this work, it was decided to simulate both unstimulated and stimulated saliva for a more realistic outcome. Ideally, dietary regimes cannot be reliably simulated unless the distinctive characteristics of stimulated saliva are introduced, these are namely; higher flow rate, higher calcium and bicarbonate content and higher pH value (Larsen and Pearce, 2003). Therefore the use of both variants of saliva (i.e. stimulated and unstimulated) would be a necessity. On the above argument, the pH of the working solution of unstimulated saliva was fixed at 6.85 ± 0.05 and that of stimulated saliva at 7.15 ± 0.05. These values are considered appropriate for the purposes of this work and in accord with those of Larsen and Pearce (2003).

In this work, the stability of the adopted artificial saliva stock solutions were found to be satisfactory and thus permitted the smooth execution of the necessary titrations to make up the artificial saliva. Moreover, the stock solutions demonstrated indefinite stability at room temperature.

High temperature increases the tendency of CO_2 to escape from the solution when in contact with atmospheric air (Darvel, 1978). The temperature of distilled water (4 °C) rendered the working solution's effect on the erosive beverage's temperature of 14 ˚C negligible; thus highlighting another advantage of using chilled distilled water in the preparation of artificial saliva.

Although a variety of calcium vehicles were tested the only calcium vehicle tested that was able to remineralise both experimental substrates and at the same time maintain the stability of the working solution was $CaCl_2$ (see section 4.6 [Results: Table 4.6-2]. Yet, this did not seem to occur unless $CaCl_2$ had been added to the working solution at the final stage of saliva preparation just after fixing the pH value. All other vehicles including $CaCl_2$ as a stock solution component failed to remineralise both substrates and/or maintain the stability of the prepared working solution. Although this work did not seek to elucidate a mechanism for this it is possible, and worthy of exploration, that this is attributable to the equilibrium between unionised and ionised calcium in the solution.

5.5 Ostrich eggshells – why?

These were selected as potential erosion substrates, on an empirical hunch by the project's supervisor as worthy of exploration. They were both cheap and large. The latter characteristic meant comparative ease of flat sample preparation.

Their mineral matrix constitutes more than 97 % of an Ostrich eggshell's composition of which 97.4 % is calcium carbonate, 1.9 % magnesium phosphate and 0.7 % tricalcium phosphate (Yadao et al., 2004). According to Szczerbinska & Wiercinska (2010), the ostrich eggshell contains 369.6 ± 12.73 mg/g Calcium and 0.21 ± 0.06 mg/g Phosphorus (Szczerbinska and Wiercinska, 2010). Interestingly, the calcium weight percentage in Ostrich eggshell of 36.96 ± 1.27 % is comparable with that found in human enamel molars which is reported to be 34.0 ± 3.0 % (He et al., 2011, Sánchez-Quevedo et al., 2004).

The egg is characterized by its unique dense structure hence its remarkable mechanical properties. Compared to other avian species, Ostrich eggshell lacks the cuticle layer or any shell accessory material which renders the outermost layer a continuous unit of substantial thickness and uniform structure (> 1800 µm). In addition, the vertical crystal layer is characterized by an amorphous crystalline structure with no evidence of porosities. This allows immediate surface assessment without the extra burden of having to pre-prepare the surface.

This unique eggshell composition allows for better control when preparing test samples and eases their cutting into desirable shapes and sizes benefiting from its dense structure and convenient thickness (Cooper, 2001; Cooper et al., 2009).

At the post-diet stage, despite the structural differences between human enamel and Ostrich eggshell, this substrate showed surface loss values indistinguishable from that of the human counterpart for the single-dosed diets (Results: Figure 4.6-10). Although Ostrich eggshell may have been more susceptible to demineralization due to its amorphous crystalline structure compared to the more organised enamel, this structural difference may have played a role in this substrate's more predictable behaviour towards erosive challenges in terms of surface hardness.

At the pre-diet stage, raw hardness values of Ostrich eggshell specimens were, by far, lower than their human counterparts (126.1 ± 15.8 vs 278.5 ± 36.8 respectively). On the other hand, at the post-diet stage, although raw hardness values of Ostrich eggshell were more considerably affected by the diet; this substrate's relative hardness percentage values followed the same pattern as those of human enamel (i.e. from single- to double-dosed and from short to long diets), indicating that the weaker structure of Ostrich eggshell may be decisive for the progression of erosion in facilitating acid penetration. However, one should bear in mind that such a property is advantageous for it permits, compared to human enamel, earlier identification of the occurrence of dental erosion. With human enamel specimens, this could be overlooked in its early stages especially if surface profilometry is not performed for the changes in surface hardness seen in this study lack the profilometric depth loss. This difference between substrates might be also attributed to a missed transient 'hardness loss' phase in human enamel specimens. This concept will be discussed in detail in a subsequent section of this discussion.

Although the present work was run in a highly controlled fashion, especially after adopting realistic human drinking behaviour values, it can be argued that the extent of

the erosive lesion was subjected to inter-structural variations. This may have been due to the extent to which biological variations (i.e. different specimen source, location and history) come into play during a diet. However, this is unlikely to have occurred, as variability among specimens in terms of their hardness values standard deviations did not exceed 13.2 % for human enamel and 12.5 % for Ostrich eggshell (Table 5-1). It has been previously reported in the literature that hardness deviations of up to 16 % are to be expected for human enamel specimens (Turssi et al., 2010). In addition, all Ostrich eggshell specimens that were challenged by a diet in Saltus throughout this work, originated from the same Ostrich egg which renders the uncertainty caused by biological variation negligible.

Table 5-1. Mean hardness values (pre-diet) of Human enamel and Ostrich Eggshell specimens

Hardness (HV)	**Mean**	**SD**	**N**
Human Enamel	**278.5**	36.8	728
Ostrich Eggshell	**126.1**	15.8	728

5.6 Erosion testing regime

Based on previous work where natural human drinking behaviour was observed (Qutieshat et al., 2015); several drinking behaviour values were adopted for the purposes of this experiment namely: test beverage flow rate and quantity; sip volume and temperature; and consumption time period. Moreover, normal physiological stimulated and unstimulated artificial saliva flow rates were adopted.

5.6.1 Test beverage flow rate

A flow rate of 13.3 ml/min reflects the consumption rate, reported in the pizza and soft drink parties, which is believed in this study to simulate the natural human drinking rate (Qutieshat et al., 2015). In contrast, previous *in vitro* artificial mouth models (exposing dental substrates to acidic attacks via rinsing rather than immersion) have adopted several, broad ranged flow rates; 3 ml/min (Magalhaes et al., 2008; Wiegand et al., 2009; Aykut-Yetkiner et al., 2014); 3.25 ml/min (Attin et al., 2003; Attin et al., 2005); and 26, 67 and 126 ml/min (Shellis et al., 2005). All of these values do not represent what is believed by the author to be a normal human drinking consumption rate (Qutieshat et al., 2015). All other *in vitro* studies assessing erosion have adopted the approach of immersing dental substrates in the beverage or acid to be tested and this has shortcomings (see section 2.5.1 [Literature review: Table 2-3]).

Furthermore, in several *in situ* studies assessing dental erosion by 'natural' drinking, subjects were instructed to consume the test beverage at specific rates such as; 25 ml/min (Hughes et al., 2002; West et al., 2003; Hooper et al., 2003; Hunter et al., 2003; Attin et al., 2004; Hooper et al., 2004; West et al., 2004; Hooper et al., 2007a, 2007b; Turssi et al., 2010); 40 ml/min (Venables et al., 2005; Hara et al., 2006); 26, 35, 50

ml/min (Hooper et al., 2005); 60 ml/min (Hanning et al., 2009); and 75 ml/min (Fushida and Cury, 1999). These are more likely to represent a forced drinking behaviour rather than a spontaneous one. All other *in situ* studies assessing erosion have adopted the approach of immersing the intra-oral appliance, where dental substrates are embedded, into the beverage or acid to be tested (see section 2.5.1 [Table 2-3]).

Saltus' flow rate algorithm (appendix 6) was carefully set so as to generate consistent and reproducible flow rates throughout the experimental diets. The standard procedure in calculating flow rates is described in detail (see section 8.6 [Appendix 6]). It is believed that these more accurately reflected reality.

5.6.2 Test beverage quantity

A quantity of 654.9 ml, which is equivalent to 2 cans of 330 ml volume, reflects the mean consumption volume per person, reported in the pizza and soft drink parties, which is believed to simulate the natural human consumption of beverages in a social atmosphere (Qutieshat et al., 2015). Yet, there might be a possibility that the value reported might actually be affected by the fact that drinkers felt tempted to drink more just because the drinks were provided for free. This indeed still reflects natural human drinking behaviour. Therefore, and to cover this possibility, another value of 330 ml was introduced by the authors, which is equivalent to 1 can of 330 ml volume. The former value was referred to as 'Double dose' and the latter 'single dose'. This will ensure that Saltus will simulate both those who consume one can a day and those who consume two.

Previous *in vitro* artificial mouth models (exposing dental substrates to acidic attacks via rinsing rather than immersion) have allocated a volume range of 18-50 ml at most

as the total volume of test-beverage or acid (Attin et al., 2003; Attin et al., 2005; Magalhaes et al., 2008; Wiegand et al., 2009; Magalhaes et al., 2010). This volume range falls into the category of sipping rather than drinking in view of the mean sip volume calculated earlier in this work (Qutieshat et al., 2015) which is reported to be around 17 ml. Other *in vitro* artificial mouth models that exposed dental substrates to acidic attacks via immersion have also adopted several volumes ranging from 3 to 50 ml (see section 2.5.1 [Literature review: Table 2-3].

Previous *in situ* studies assessing dental erosion via natural drinking rather than immersion have utilised volumes that are more representative of drinking behaviour; apart from one study that allocated only 50 ml of the test beverage per day; other studies allocated a range of 350 – 1200 ml of the test beverage per day. This is in agreement with the consumed volume per subject values reported earlier in this work (mean 654.9 ml, min 162 ml, max 1625 ml)(Qutieshat et al., 2015).

5.6.3 Time

Daily acidic exposure time periods in previous artificial mouth models was 15 minutes at most with any single 'drinking' episode not exceeding 5 minutes (Attin et al., 2003; Attin et al., 2005; Magalhaes et al., 2008a; Wiegand et al., 2009; Magalhaes et al., 2010). In these models, the duration of the diet ranged from 1-5 days. On the other hand, daily acidic exposure time in almost all *in situ* erosion testing studies, which adopted natural drinking as the acidic exposure method, was 40 minutes; based on single drinking episodes of 10 minutes 4 times a day over the duration of 5-20 days (Hughes et al., 2002; Hooper et al., 2003; Hunter et al., 2003; West et al., 2003; Hooper et al., 2004; West et al., 2004; Venableset al., 2005; Hooper et al., 2007a; Hooper et al., 2007b; Turssi et al., 2010). In an attempt to simulate actual drinking habits *in vitro*, a

demineralisation-remineralisation cycling model was set to immerse enamel blocks in the test beverage for 1 minute and then in artificial saliva for 3 minutes over a 20 min period (Van Eygen et al., 2005). This approach however, would have limited success in simulating natural drinking for the test beverage will be allowed to solely and directly contact the surfaces of dental substrates well before artificial saliva is introduced back into the model. This contrasts with the simulation afforded by Saltus where both the beverage and saliva contact the dental substrate.

As regards time, once again, the diets delivered by Saltus, which were based upon natural human drinking behaviour, more closely resemble those of previous *in situ* erosion studies. This might be attributed to the nature of *in situ* experiments where, in order to deliver the test beverage to the specimens embedded in the intraoral appliance; subjects are often asked to drink naturally (see section 2.5.1 [Literature review: Table 2-3]).

5.6.4 Temperature

It is generally accepted that temperature can significantly affect dental erosion. Earlier in this work, the overall expectorated sip temperature was found to be 14.9 ± 2.0 ˚C (Qutieshat et al., 2015). In light of previous recommendations concerning the temperature at which to conduct in vitro erosion studies (body temperature 37 °C/ oral cavity temperature 36 °C/ room temperature 25 °C) (Shellis et al., 2011) this was surprising but such recommendations could of course be accounted for by the desire to accelerate the erosive process in the laboratory. Previous *in vitro* erosion studies utilising demineralisation-remineralisation cycling as listed in table 2-3 conducted the erosive challenges mostly at a range of 25 °C and 37 °C. In our view it is reasonable to suggest that a more physiological temperature at which to conduct such studies is

around 14.9 °C based upon our observation that a carbonated beverage stored at 4˚C is found to have reached this temperature upon expectoration having been in the mouth only for a few seconds. On an anatomical basis the oral cavity, unlike the nasal cavity with its turbinate anatomical structure, is not designed to heat (Keck et al., 2011).

5.6.5 Normal physiological salivary flow rates

To the author's knowledge, Saltus is the first artificial mouth model to convey both stimulated and unstimulated artificial saliva for erosion research under *in vitro* conditions. In addition, it was able to run the flow at 3 different physiological rates namely; stimulated flow rate of 5.0 ml/min (Dawes, 1987); waking hours unstimulated flow rate of 0.3 ml/min; and sleeping hours unstimulated flow rate of 0.1 ml/min (Thomson et al., 2011; Dorion, 2011). However, some previous *in vitro* models were set to deliver artificial saliva at a steady flow rate of 0.5 ml/min (Wiegand et al., 2009) or 1.1 ml/min (Attin et al., 2003; Attin et al., 2005; Magalhaes et al., 2008a) mainly overnight.

Thus, Saltus is the first *in vitro* artificial mouth model that simulates natural human drinking behaviour in terms of test beverage flow rate and quantity; temperature; consumption time period; as well as normal physiological stimulated and unstimulated saliva flow rates.

5.6.6 Verifying the constancy of drink temperature in the time frame of an experimental run

In the time frame of a test-cycle, either a single 22-minute period for a single-dosed diet or two consecutive 22-minute periods for a double-dosed diet are introduced. The constancy of beverage temperature was verified to ensure that the test-cycle is

conducted at the desired temperature of 14.9 °C. As test beverages are kept in a container housed in a mini-fridge at a temperature of 14 °C prior to the commencement of the test-cycle; this pilot study revealed that the temperature of the test beverage was found to rise by 1.7 °C to become 15.7 °C by the end of the 22 minute period averaging at 14.9 °C. This is the desired temperature at which the test cycle is to be conducted. Clearly, in the double-dosed diets, the second test-beverage's temperature will follow the same fate by the end of the second 22 minute period. This verifies that the test conditions are in accord with those observed in the pizza and soft drink parties.

5.7 Assessment of dental substrates

Before considering the findings revealed by the pre- and post-exposure measurements of the erosion substrates it is important to appreciate the main issues of the measurement techniques themselves. The present work was reliant upon both profilometry and hardness testing.

Profilometry: A contacting surface profilometer was used. It is worth mentioning that the stylus might be able to scratch the demineralised surface (Barbour and Rees, 2004), but this would occur in all test groups and therefore should not bias the results. Although numerous research laboratories have purchased non-contacting instruments they are said by some to give the impression of efficacy but lack the accuracy afforded by contacting instruments (MR Pintado – RG Chadwick, Personal communication).

TIV Hardness Tester: The image of the indentation is automatically processed and evaluated by determining the length of the two diagonals of the indentation simultaneously whilst determining the depth of the indentation.

The corresponding Vickers hardness value (HV) is therefore derived from the determined dimensional values according to the Vickers hardness definition. This is achieved by identifying the intersection points and/or corners of the edge lines and by applying the following formula:

$$Hardness\,Value\,(HV) = test\ load\ \div size\ of\ indentation$$

Edge lines determination and diagonals length measurements are dependent upon the analysis of the different grey-scale values in the image taken by the equipped CCD

camera. The shadows on the edges produced by the indenter have different grey-scale values and thereby picked up by the camera as demarcation lines.

The resultant value is immediately displayed on the TIV device screen along with an image of the indentation. This allows the operator to further assess and evaluate the quality of the measurement and reliability of the reading. Any surface defect, chatter mark, foreign particle or dirt can be easily identified and rejected so accuracy is not compromised by artefacts upon the surface of the specimen.

The device allows the operator to manually correct skewing. Yet, in the present work, all readings with faults were rejected and retaken rather than corrected manually. In addition, a diamond cleansing cloth (GE Measurement & Control, Groby, UK) was used to clean the indenter in between readings to prevent particles from depositing on the diamond and therefore prevent errors. To the author's knowledge this is the first time such a device has been used in dental research and its operation is far less fatiguing than the manual application of a Vickers Hardness microscope. It thus affords many more measurement possibilities in a more time efficient manner, in fact this project would not have been feasible in its extent were such an instrument not available.

5.8 Saltus Diets I

In order to more easily follow this section, it is recommended that the reader refers to (Literature review: Tables 2-2 and 2-3).

4.8.1 Surface loss

As mentioned earlier, all surface loss values reported in the literature review were converted to per hour values to allow for comparison. Table 5-2 presents the experimental per-hour values of surface loss reported in this work (figure 4.6-10) following application of the Saltus diets. These are presented here readjusted to the average of the two means (i.e. the diet and its repetition) before converting the average into per hour value. This renders them more readily comparable to those from literature review.

Table 5-2. Surface loss per hour values for both substrates in the immediate effect method. The value of each represents the average value of the diet and its repetition.

Per hour loss	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**
Human enamel	10.06 ± 1.15 µm/hr	8.75 ± 1.11 µm/hr	20.98 ± 2.96 µm/hr	16.90 ± 2.09 µm/hr
Ostrich Eggshell	5.87 ± 0.30 µm/hr	7.02 ± 0.11 µm/hr	14.91 ± 1.18 µm/hr	12.08 ± 1.14 µm/hr

The single-dosed diets (Diet 1 and 2) had a surface loss rate of 8.75 - 10.06 µm/hr in human enamel and 5.87 – 7.02 µm/hr in Ostrich eggshell. This corresponds to almost half the surface loss rate observed in double-dosed diets (Diet 3 and 4)(16.90 – 20.98 and 12.08 – 14.91 µm/hr respectively)(Table 5-2). Clearly, a dose-response relationship between the quantity of consumption and the amount of surface loss was found. A similar dose-response relationship was reported recently in a study that assessed the association between dental erosion and several dietary risk indicators such as the amount of consumption (Søvik et al., 2015).

Although the total amount of loss increased from short- to long-diets (i.e. from Diet 1 to 2 and from Diet 3 to 4); the hourly surface loss rates were comparable as was expected in view of the standardised test-cycles that Saltus was programmed to deliver.

The reported surface loss rate in this work is in agreement with the rate of 5.58 µm/hr concluded from an *in situ* study assessing the effect of the same beverage type upon human enamel over a period of time comparable to that of Diet 2 (Sales-Peres et al., 2007). Another *in situ* study assessing the effect of a citrus test beverage upon human enamel with and without a preventive measure reported a surface loss rate of 13.49 µm/hr in the group that did not receive any preventive measurement (Vieira et al., 2007). Interestingly, when this citrus beverage (Sprite) was tested using Saltus (i.e. diet 8); a surface loss rate of 14.01 µm/hr was reported in human enamel and 11.94 µm/hr in Ostrich eggshell. The comparability of the aforementioned results further demonstrates the potential of this artificial mouth model to generate reliable data.

On the other hand, several other *in situ* studies have reported a lower surface loss rate (0.88 – 2.74 µm/hr)(see section 2.5.1 [Literature review: table 2-3])(Rios et al., 2006; Honorio et al., 2008; Magalhaes et al., 2008; Rios et al., 2009). This difference might be attributed to the higher calcium ion content found in the beverages tested in these studies (i.e. 0.84 mmol/l compared to 0.22 mmol/l of calcium found in the test beverage in this work). It is worth mentioning that the same beverage might possess variable calcium ion concentrations depending on the source of water used during its manufacturing process. In a study that analysed the erosive potential of calcium containing acidic beverages, it was concluded that the higher the original calcium ion content found in the test beverage the less potential it possesses to cause dental

erosion (Hara and Zero, 2008). This also agrees with the conclusions of several other *in vitro* studies that sought to lessen the erosive effects of acidic beverages (Larsen and Nyvad, 1999; Parry et al., 2001; Jensdottir et a., 2007; Syed and Chadwick., 2009).

Among the *in vitro* artificial mouth models (i.e. utilising rinsing rather than immersion as a means of exposing dental substrates to test beverages) assessing dental erosion, one study reported a surface loss rate of 9.16 µm/hr after exposing bovine enamel to a citrus test beverage 6 min/day for 3 days (Magalhaes et al., 2008). Another study (Attin et al., 2005) reported a surface loss rate of 6.36 µm/hr after exposing human enamel to a citrus test beverage for a total time period of 15 minutes. Yet, when a regular cola drink was tested, a surface loss rate of only 0.76 µm/hr was reported. This might be attributed, once again, to the original calcium ion content of the test beverage which was reported to be 0.94 mmol/l compared to 0.22 mmol/l in the beverage tested in this study as well as the parent acids of the drinks (Hara and Zero, 2008).

As for other *in vitro* studies utilising demineralisation-remineralisation cycling and exposing dental substrates to test beverages via immersion; a range of 1.44 – 3.75 µm/hr was reported (Passos et al., 2013; Barac et al., 2015). However, these did not share a standard experimental setting where the daily test beverage exposure time period ranged from 3 to 6 minutes at most.

It can be concluded from the aforementioned studies that the surface loss rates obtained in this work are in agreement with other studies especially if the variables were, to a certain extent, comparable. Interestingly, studies held under *in situ* conditions more resembled the results obtained in this work compared to the ones held under *in vitro* conditions. This might indicate that the extra quality that Saltus possesses over other in vitro models, the ability to simulate human drinking behaviour,

might have contributed in closing the gap between *in situ* and *in vitro* models in terms of surface loss determination.

As for the accumulative effect method, the surface loss values in this work were also converted to per hour values to allow for comparison (Table 5-3).

Table 5-3. Surface loss per hour values for both substrates in the accumulative effect method. The value of each represents the average value of the diet and its repetition.

Per hour loss	Diet 5 **Period 1**	Diet 5 **Period 2**	Diet 5 **Period 3**
Human enamel	8.81 µm/hr	6.51 µm/hr	6.38 µm/hr
Ostrich Eggshell	10.83 µm/hr	7.35 µm/hr	5.85 µm/hr

The accumulative effect method demonstrated, as seen in table (5-3), that the surface loss rate was relatively high after the first test-cycle (i.e. period 1) compared to the after effect of the periods 2 and 3. This might be attributed to the lack of a convenient rest period within which the substrate surface structure can remineralise owing to the fact that test specimens were tested for surface loss right after the first acidic challenge. This was not the case with diets 1-4, used in the direct effect method, where the specimens were left in the artificial mouth system for longer time periods where they, presumably, had a better chance to re-harden the already softened enamel via the process of remineralisation.

The accumulative effect method, in fact, is an extended 'Diet 2' as seen in table 5-4. Although both Diet 2 and Diet 5 shared the same number of test-cycles and dosage, the extended diet had 2 extra rest-cycles. This difference is reflected in the hourly loss rates obtained in the extended diet for both substrates displayed marginally lower overall loss values than the ones obtained in diet 2 (Table 5-2). This might be

attributed to the fact that the test substrates in the extended diet benefitted from the extra 2 rest-cycles during which remineralisation probably occurred.

Table 5-4. Extended-single dose versus long-single dose diets

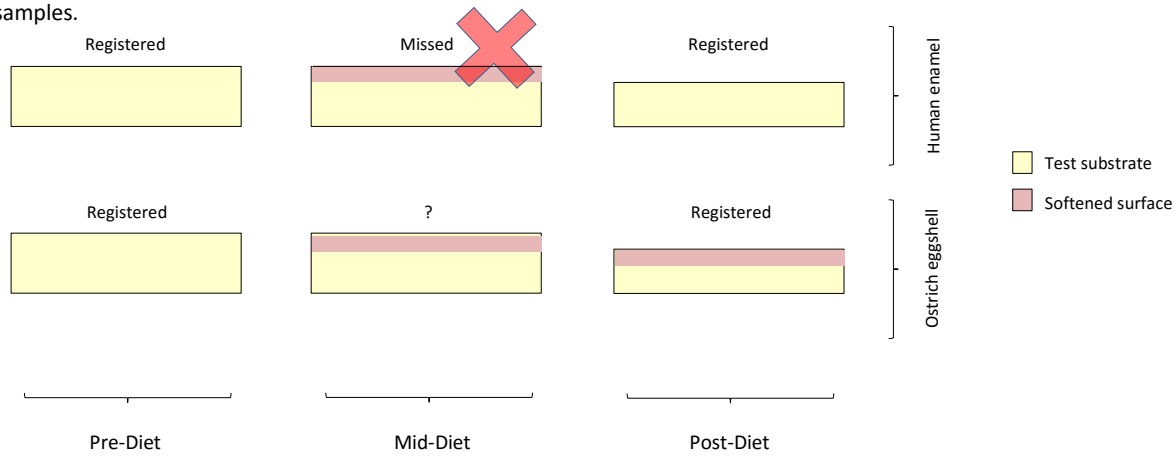
Diet	Beverage	Duration	Rest cycles	Test cycles	Test cycle dose	Total number of cans per specimen	Preventive measure	Code
Extended-Single dose	*Coca-Cola*	9 days	4	5	1 can	5 cans	None	**Diet 5**
Long-Single dose	*Coca-Cola*	7 days	2	5	1 can	5 cans	None	**Diet 2**

4.8.2 Surface hardness

In the immediate effect method, all post-diet relative hardness percentage values for human enamel were greater than 90 % despite the surface loss observed. This might not reflect the actual damage caused by the introduced acidic challenge. If surface assessment solely relied upon hardness testing in this case; it might lead to false conclusions such as assuming that the test beverage had no effect upon human enamel especially in Diets 1 and 2. Clearly, this was not the case for surface loss was, in fact, reported (i.e. a range of 10.18 ± 2.17 to 17.48 ± 3.93 µm for diets 1 and 2). Figure (5-6) illustrates this and postulates a transient 'hardness loss' phase that occurred at some point during the diet which was probably missed by the hardness tester after the diet had ended. This phase has been referred to previously in the literature; where it was believed to explain the loss of structure despite the zero change in hardness (Van Eygen et al., 2005). During the test-cycle and as the test-substrate surface softens due to erosion, hardness will drop, which is in turn associated with instant surface loss exposing a harder surface. Therefore, after the diet had ended, hardness testing will only reflect that of the newly exposed surface rather than the one that was originally associated with surface loss which has most probably gone un-noticed (Figure 5-6).

The fact that hardness loss is proportional to acidic exposure period has been disputed; theoretically correct but practically enamel loss might render this assessment method (i.e. hardness testing), if used solely, questionable in terms of assessing dental erosion *in vitro*. However, this phenomenon was not clearly demonstrated in Ostrich eggshell specimens. Surface hardness values of Ostrich eggshell registered after the experimental diets have perhaps reflected the amount of surface tissue loss and presented an overall clearer cause-and-effect image of what is perceived after an erosive attack. This might be attributed to the fact that such erosive attack affected deeper parts of Ostrich eggshell specimens, which, despite of the surface structural loss, was still able to demonstrate the expected hardness values that are more reflective of the 'process' of dental erosion compared to human enamel specimens (Figure 5-6).

Figure 5-6. an illustration that describes the missed 'surface hardness loss' phase when testing human enamel samples for surface hardness after an erosive challenge which is believed not to be the case with Ostrich eggshell samples.



Interestingly, Ostrich eggshell specimens were able to demonstrate both hardness drop and surface loss as a result of an acidic challenge. Regardless of the diet introduced, Ostrich eggshell's response in terms of hardness and structural integrity was predictable and inter-dependant (i.e. the lower hardness the higher surface loss).

Figure (5-6) shows that the transient 'hardness loss' phase in Ostrich eggshell has been probably registered by the hardness tester.

In a demineralisation-remineralisation cycling *in vitro* model assessing the effect of a regular cola beverage upon human enamel (see section 2.5.1 [Literature review: Table 2-3]), relative surface hardness percentage values of 83.8 % and 85.9 % were reported over a period of 3 and 7 days respectively. These values, the authors argued, did not represent the ultimate decrease in hardness; instead, these reflected the hardness of the harder surface formed as a result of surface loss (Van Eygen et al., 2005).

In a similar *in vitro* model, relative hardness percentage of human enamel dropped to 59.5 % after 3 days and to 53.7 % after 5 days of 25 min daily acidic exposure by immersion (Maupome et al., 1999). Other *in vitro* models reported relative hardness percentage values that fall in the narrow range of 45.0 – 63.3 % despite of the noticeable differences in their experimental settings (Wongkhantee et al., 2006; Murakami et al., 2009; Passos et al., 2013; Xavier et al., 2015). This further demonstrates the shortcomings of hardness testing in the assessment of dental erosion in human enamel where fluctuations in relative hardness values are inevitable in view of the factors discussed earlier.

On the other hand, two *in vitro* studies, one on human enamel of permanent teeth (Panich and Poolthong, 2009) where 100 seconds of acidic exposure was adopted in a demineralisation-remineralisation cycling model, and the other on human enamel of primary teeth (Torres et al., 2010) where specimens were exposed to the test beverage daily for 15 min/day over 7 days under similar conditions, have reported considerably higher relative hardness percentage values of 89.89 % and 97.65 % respectively. It can be argued that the former was able to 'register' the

aforementioned transient 'hardness loss' phase owing to the very limited acidic challenge applied of 100 seconds. As for the latter, the transient phase has most probably been missed in view of the longer daily acidic exposure over a prolonged period of time (i.e. 7 days).

Several *in situ* studies that investigated the effect of a regular cola beverage upon dental erosion in human teeth have reported relative hardness percentage values in the range of 10.37 – 21.5 % (Rios et al., 2006; Sales-Peres et al., 2007; Honorio et al., 2008; Magalhaes et al., 2008b; Rios et al, 2009). Nevertheless, another in situ study reported a 76.2 % relative hardness percentage value after 8 minutes of exposure to the test beverage in a combined *in situ / in vitro* model (Srinivasan et al., 2010).

It is worth mentioning that the number of *in situ* studies utilising natural drinking rather than immersion as means of dental substrate exposure to acidic beverages has considerably dropped since the year 2009 (see section 2.5.1 [literature review: Table 2-3]). The 8-year period from 2000-2007 witnessed 11 of these out of 15 *in situ* studies, while on the other hand, only 2 out of 15 *in situ* studies utilised natural drinking in their setting over the 8-year period from 2008-2015. To add to the confusion, it is doubtful if ethical approval would be given for prion-prone specimens to be placed in the mouth in *in situ* studies in the UK (West et al., 2011a).

Relative hardness percentage values of human enamel obtained from the accumulative method over 3 periods (i.e. period 1 (P1), period 2 (P2) and period 3(P3)) further demonstrates the impact of the transient 'hardness loss' phase where the values in P1 and P2 were significantly lower than P3. Diet 5-P3 (i.e. a total of 5 test cycles) is equivalent in terms of acidic challenges to Diet 2 in the immediate effect method. Interestingly, the relative hardness percentage values for all of these diets

(i.e. Diet 2, Diet 2R and Diet 5-P3) are remarkably comparable (98.78 %, 98.72 % and 98.47 % respectively). On the other hand, Ostrich eggshell at the end of P3 in the accumulative effect method has also demonstrated comparable relative hardness values to the ones obtained in Diet 2 and Diet 2R in the immediate effect method (62.73 %, 64.14 % and 64.72 % respectively).

In view of this transient 'hardness loss' phase, and the fact that considerable enamel 'hardening' is expected after each acidic exposure; it can be argued that certain *in vitro* experimental settings with unjustifiably prolonged acidic exposure time period (e.g. immersion of dental substrates for 14 days (Von Fraunhofer and Rogers, 2004)) are thus contraindicated for these tend to over-simplify a rather complex process.

All of the aforementioned points support the assumption that the use of Saltus in erosion-testing is valid and that it is capable of delivering consistent, reliable and reproducible diets.

4.8.3 Ion loss

Several *in vitro* erosion testing models have been conducted by others to investigate the effect of a regular cola beverage on the ionic composition of human enamel (Larsen and Richards, 2002; Willershausen and Schulz-Dobrick, 2004; Jensdottir et al., 2005; Cochrane et al., 2009). Two of which (Jensdottir et al., 2005; Cochrane et al, 2009) adopted an excessively prolonged exposure-by-immersion time period of 24 hours which; as discussed earlier, would generate an altered behaviour of surface ions. In one study it has been reported that calcium ion loss per hour of acidic exposure was 0.46 mmol/l (Larsen and Richards, 2002). Another study utilising demineralisation-remineralisation cycling has also demonstrated calcium ion loss of 0.43 mmol/l per

hour and phosphate ion loss of 0.52 mmol/l per hour (Xavier et al., 2015). The per hour values reported in this work had comparable Ca:P ratios with the ones found in literature, but the overall amount of ion loss was, however, less. In the present work, human enamel calcium loss in Diets 1 and 2 was in the range of 18.2-20.7 mmol/l and the phosphate loss was in the range of 25.6-30.9 mmol/l, while Ostrich eggshell ion loss values for calcium and phosphate were in the range of 20.0-28.4 mmol/l and 30.0-34.9 respectively. This is in line with the conclusions made in a study, which assessed human enamel mineral loss upon the exposure to a regular cola beverage, where dental substrates were found to lose a stable ratio of calcium and phosphate throughout the erosive process (Willerhausen and Schulz-Dobrick, 2004).

Once again, a dose-response relationship was reported in this work, where single-dosed diets lost significantly less ions relative to double-dosed diets. It can be seen from the aforementioned comparisons that that Ostrich eggshell was able to demonstrate a predictable behaviour in terms of all surface effects tested (i.e. surface hardness, surface loss and ion loss) while human enamel specimens were able to demonstrate this in surface and ion loss but came short in terms of surface hardness, where, although a dose-response relationship was noticed, the loss of surface hardness in single-dosed diets was missed.

This predictable Ostrich eggshell behaviour can be understood by considering that the solubility of calcium carbonate, the main composition of Ostrich eggshell, is increased by the presence of phosphate, and that of calcium phosphate is increased by the presence of bicarbonate in acidic environments (Greenwald, 1945), along with the fact that the default test-beverage used in this work contains carbonic and phosphoric acid and has a pH of 2.47.

5.9 Saltus Diets II – Applications of Saltus

These examined the efficacy of potential protective agents. The *in vitro* results obtained were in accord with published studies on the effect of fluoride varnishes and gels upon dental erosion. It was not the role of this study to evaluate effects; it was to test the capabilities of the Saltus system, and for that reason the theory underlying how these preventive measurements work has not been covered in the literature review.

Fluoride has been known to be effective in preventing mineral loss due to its ability to form a fluoride reservoir on the surface of enamel as a result of calcium fluoride deposition (Ganss et al., 2008). This will not only reduce enamel dissolution and enhance its remineralisation but also will provide an additional mineralised layer to be challenged by the acid before it reaches the underlying enamel. Therefore, regular brushing with fluoride toothpastes has been advocated to reduce the demineralisation of enamel and at the same time promote its remineralisation (Ganss et al., 2008; Hove et al., 2008).

Apart from tooth pastes, there are some other possible vehicles for fluoride such as gels, solutions and varnishes. Different fluorinated compounds have been incorporated in the composition of each including sodium-, ammonium-, stannous-, titanium-, fluoride (de Carvalho et al., 2014). All these compounds have been reported in literature to have, to a certain degree, a positive effect in preventing mineral loos upon acidic challenges (de Carvalho et al., 2014).

Fluoride varnish, however, has shown the most preferable outcome in terms of erosion protection owing to its dual-protective properties of adhering to tooth

structure via its resinous composition and releasing fluoride ions onto the tooth structure (Vieira et al., 2007). Consequently, high fluoride concentration will lead to the formation of calcium fluoride which will in turn act as a physical barrier and allow longer effect of fluoride (Vieira et al., 2008).

The role of different fluorinated compounds in protecting against erosion has been controversial in literature (de Carvalho et al., 2014). Among these, sodium fluoride is the most common and has shown more consistent positive results compared to other compounds (Magalhaes et al., 2008; Murakami et al., 2009; Magalhaes et al., 2007).

Researchers have been continuously developing and testing various experimental agents that are capable of protecting against erosion (de Carvalho et al., 2014; Wiegand et al., 2008) one of which is an ammonium fluoride based varnish (Fluor Protector S) (Composition: ethanol, water, polymer, additive, saccharin, mint flavouring, 1.5% ammonium fluoride [7700 ppm fluoride]) as used in the present work. This is considered the most recent fluoride varnish in the market and the only available ammonium fluoride-based varnish (Lendenmann and Bolis, 2013).

In this work, the fluoride varnish applied showed protective effects against dental erosion. It was able to reduce the rate of surface loss from 10.06 µm/hour to 1.85 µm/hour in human enamel and 1.13 µm/hour in Ostrich eggshell (rates were obtained by converting the amount of surface loss in µm after each diet to per hour values to allow for comparison). This is in agreement with several reports that demonstrate the protective effect of fluoride varnishes (Vieira et al., 2007; Magalhaes et al., 2008; Murakami et al., 2009).

In an *in vitro* study to assess the protective effect of a fluoride varnish on dental erosion utilising an artificial mouth model that was set to rinse the specimens with the test beverage; the varnish was able to reduce the rate of surface loss from 9.16 µm/hour to 1.73 µm/hour in bovine enamel after 3 days of 6 min/day exposure to the test-beverage (magalhaes et al., 2008a)(See section 2.5.1 [Literature review: Table 2-3). In an *in situ* study conducted upon human enamel specimens, the fluoride varnish applied was able to reduce the amount of surface loss rate from the range of 10.8 – 13.49 µm/hour to 1.55 – 4.39 µm/hour in human enamel (Vieira et al., 2007). The results in these studies support the positive preventive effects of the fluoride varnish reported in this work.

As regards hardness, an *in vitro* study utilising demineralisation and remineralisation cycling assessed the protective effect of a fluoride varnish upon the surface hardness of human enamel and reported lower surface hardness loss in fluoride varnish groups compared to the control (72.6 % vs 56.9 % respectively)(Murakami et al., 2009). A comparable effect was reported in this work upon Ostrich eggshell were specimens treated with the fluoride varnish maintained 91.59 % of their hardness while the control group had a relative hardness percentage of 69.03 % (the average of diet 1 and 1R). No hardness loss was reported in the treated group upon human enamel specimens; however, the control group against which this diet was compared (i.e. diet 1 and diet 1R) did not demonstrate hardness loss either. This is attributed, the author believes, to the unique 'realistic' experimental setting that renders the effect of dental erosion upon hardness loss in human enamel specimens insensitive to mild and/or short erosive challenges.

On the other hand, positive protective effect of fluoride varnishes have not always been demonstrated in literature. In an in vitro study utilising demineralisation-remineralisation cycling upon bovine enamel, no effect of applying a varnish was reported on surface loss or surface hardness (Magalhaes et al., 2007). This might be due to the experimental setting adopted where exposing dental substrates to the test beverage was undertaken via immersion rather than exposing by rinsing which does not reflect a realistic simulation as discussed earlier.

Recently, a newly developed dual-phase gel (Regenerate NR-5 serum) was introduced as a preventive and therapeutic gel that protects against enamel erosion (Hornby et al., 2014, Joiner et al., 2014). This gel comes in two tubes; the first contains calcium silicate and sodium phosphate salts; and the second contains sodium fluoride. In contrast to Fluor protector S that needs professional application by a dentist, this gel is to be applied by the patient at home.

This formula has been shown to form hydroxyapatite via the deposition of calcium silicate onto the enamel surface (Parker et al., 2014, Sun et al., 2014). Moreover, it has been demonstrated under *in vitro* conditions that this gel can enhance the remineralisation process of enamel (Hornby et al., 2014, Jones et al., 2014).

The effect of calcium silicate materials upon enamel remineralisation has been demonstrated in several studies (Dong et al., 2011, Wang et al., 2012). A combined use of calcium silicate and fluoride has been shown to have a protective effect against dental erosion using a demineralisation-remineralisation cycling model *in vitro* (Wang et al., 2012).

In this work, overall, the application of the gel did not show an improved resistance against dental erosion relative to the control groups. However, the overall surface loss was reduced in both test substrates but this was not of statistical significance. The relative hardness percentage, on the other hand, did not change in the human enamel group where it remained around the 100 % level while in the Ostrich eggshell group it was slightly reduced but this effect was also not statistically significant. In contrast to a number of in vitro and in situ studies that demonstrated a positive protective effect of this gel against erosive challenges (Jones et al., 2014; Hornby et al., 2014; Joiner et al., 2014). It is worth mentioning here that this effect was only demonstrated using hardness testing only and the amount of surface loss was not quantified in any way. This might indicate, as discussed earlier, that certain experimental settings might alter the final hardness levels favourably giving a 'false' sense of substrate recovery while possible surface loss might still have taken place. Thus, surface loss assessment is needed as an adjunctive tool along with hardness testing.

In an i*n vitro* artificial mouth model that assessed the erosive effect of both regular cola and citrus cola beverages upon bovine enamel (Attin et al., 2005); the citrus variant caused significantly more surface loss relative to the regular one. Another *in vitro* demineralisation-remineralisation cycling model also reached the same conclusion but with a wider argument that less bovine enamel surface hardness drop and calcium ion loss was observed in the regular cola beverage relative to the citrus variant (Zimmer et al., 2015). These results are in agreement with the ones reported in this work where surface hardness and structural integrity were significantly more affected in the citrus beverage group relative to the regular variant.

On the other hand, several *in vitro* studies reported both variants (i.e. regular cola vs citreous cola) to be associated with dental erosion but with no significantly different effect on surface hardness (Lussi et al., 2012; Xavier et al., 2015) or surface loss (Murrell et al., 2010; Aykut-Yetkiner et al., 2014) between the two variants. However, in an *in vitro* study, regular cola beverage was found to be more erosive relative to its citrus variant in terms of structural and ionic integrity (Cochrane et al., 2009).

5.9.1 Beverage-free and Calcium-deprived diets

To ensure Saltus conveys artificial saliva form source containers to erosion substrates without affecting its ionic composition; a substrate- and beverage-free single day diet was conducted to assess the effect of an experimental run on calcium and phosphate ion concentrations. Saltus imposed no effect on calcium and phosphate ion concentrations after the diet has ended relative to pre-diet concentration values. In addition, to ensure the desired remineralisation effect of artificial saliva used in this work calcium-deprived artificial saliva was used under experimental conditions similar to those of Diet 1. The considerable amount of surface and ion loss along with the large drop in hardness in such diet has reflected the role that Saltus plays in simulating a realistic behaviour in view of the ‘very high’ test-beverage dosage relative to previous artificial mouth models which, in turn, demonstrates the desired ‘protective’ effect of saliva in this model.

CHAPTER 6

6. Conclusions

Within the limitations of this study;

- Several human drinking behaviour values were reported in this study, which were the basis upon which Saltus was developed.
- Saltus is the first *in vitro* artificial mouth model that simulates natural human drinking behaviour.
- Saltus yields comparative values to those obtained in complex *in situ* studies.
- The high degree of consistency in the results of the repeated diets run in Saltus implies great reliability. Adoption of such an artificial mouth system and regime would therefore be recommended in any similar investigation.
- This work has demonstrated the suitability of using Ostrich eggshell as a substitute for human enamel in erosion models under *in vitro* conditions.

Further work;

- Assess the impact of an educational initiative to educate dentists on the collection of teeth according to the Human Tissue Act upon levels research tooth collection.
- Conduct more participant observation studies in which to extend this research to look at factors that influence fizzy drinks consumption of individuals and to incorporate more age groups to include teenagers and older individuals. This would help to determine the generalizability of the reported drinking behaviour

values and to reflect the observed behaviour on the atmosphere and experimental setting.

- Incorporate more organic components of saliva (e.g. salivary proteins) in the formulation of a stable artificial saliva that is able to simulate natural saliva, then taking that even further by simulating the acquired pellicle.
- Introduce the act of brushing into the system to assess how the substrates would behave under erosion/abrasion cycling.
- Conduct more biochemical and ultrastructural investigations on Ostrich eggshell tissue to more fully understand its ionic composition and behaviour upon exposure to acidic beverages.

CHAPTER 7

7. References

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CHAPTER 8

8. Appendices

8.1 Appendix 1 – Ethical Approvals

RE: Proposed Survey on Extracted Tooth Collection - R&D approval and U of D Sponsorship required?

Coote Liz (NHS TAYSIDE) <liz.coote@nhs.net>

Tue 7/24/2012 1:03 PM

To Catrina Forde <C.Forde@dundee.ac.uk>; Graham Chadwick <r.g.chadwick@dundee.ac.uk>;

Cc Abubaker Qutieshat <a.s.qutieshat@dundee.ac.uk>; Andrew Mason <a.g.mason@dundee.ac.uk>;

Hi Graham,

Re: Proposed Survey on Extracted Tooth Collection - R&D approval and U of D Sponsorship required?

I can confirm that the above proposal as it reads just now does not require R&D review and approval. I appreciate that your study does involve dentists, but in this case, they are the participants and not researchers or providing a support function. Therefore the sudy does not require NHS review and approval. Many thanks for checking with TASC. I wish you success with your proposal.

Kind Regards,

Liz Coote
R&D Manager
***TA**yside medical **S**cience **C**entre (TASC)*
Ninewells Hospital & Medical School
TASC Research & Development Office
Residency Block, Level 3
George Pirie Way
Dundee, United Kingdom
DD1 9SY

e: liz.coote@nhs.net
dl: +44 (0) 1382 496536
w: www.tasc-research.org.uk
Tw: http://twitter.com/TASC_research

From: Catrina Forde [C.Forde@dundee.ac.uk]
Sent: 24 April 2012 10:48
To: r.g.chadwick@dundee.ac.uk; Coote Liz (NHS TAYSIDE)
Cc: Abubaker Qutieshat; Andrew Mason
Subject: RE: Proposed Survey on Extracted Tooth Collection - R&D approval and U of D Sponsorship required?

Hi Graham,
As Abubaker will be contacting 'NHS staff' only this particular piece of work doesn't need to have a Sponsor assigned – and won't need NHS R&D approval – although notification of the activity is always welcome. I assume that this is funded internally as it forms part of a PhD? To note however, that if the PhD does involve in part some clinical research ie involving humans, NHS resources (other than staff only) or NHS data, then that would need sponsorship, possibly ethics, and R&D approval in place.
Bye.
Catrina

Catrina Forde PhD
Senior Research Governance Manager
Tayside Medical Sciences Centre
Ninewells Hospital & Medical School
Residency Block, Level 2
George Pirie Way
Dundee, United Kingdom
DD1 9SY

e: c.forde@dundee.ac.uk
t: 01382 7(40125)
f: 01382 7(40122)
m: 0775 967 0244

From: Graham Chadwick
Sent: 20 April 2012 14:59
To: liz.coote@nhs.net
Cc: Abubaker Qutieshat; Andrew Mason; Catrina Forde
Subject: Proposed Survey on Extracted Tooth Collection - R&D approval and U of D Sponsorship required?
Importance: High

Liz & Catrina

As you will see from both below and the attached we are propsing to do a postal survey of dentists in the UK on the collection of extracted teeth. This will all be administered by the University. We have been advised that Ethics is not required. Can you determine if we need NHS R&D approval. I also have copied this to Catriona Forde to see if we require University of Dundee sponsorship approval. I look forward to hearing from both of you soon.

Regards

Graham

From: Tayside Ethicshelpline (NHS TAYSIDE) [mailto:ethicshelpline.tayside@nhs.net]
Sent: 18 April 2012 08:40
To: Graham Chadwick
Subject: Proposed Survey on Extracted Tooth Collection12/GA/062
Importance: High

Hello Graham

Thank you for your enquiry.

In line with the Governance Arrangements for Research Ethics Committees (GAfREC) 2011, studies involving NHS staff no longer require ethical review by an NHS Research Ethics Committee (REC), you may however still require R&D approval.

with kind regards,

Arlene Grubb, Sent on behalf of Caroline Ackland, Scientific officer
East of Scotland Research Ethics Service

Tayside Academic Health Sciences Centre
Residency Block Level 3
George Pirie Way
Ninewells Hospital & Medical School
DUNDEE
DD1 9SY

Tel: Direct line: +44 (0)1382 425598/ **+44 (0)1382 740336**/Ninewells: **+44** (0)1382 660111 ext 35598/40336
Email: ethicshelpline@nhs.net

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From: Caroline Ackland [c.ackland@dundee.ac.uk]
Sent: 17 April 2012 14:30
To: Tayside Ethicshelpline (NHS TAYSIDE)
Subject: Fwd: Proposed Survey on Extracted Tooth Collection

Sent from my iPhone

Begin forwarded message:

> **From:** Graham Chadwick <r.g.chadwick@dundee.ac.uk>
> **Date:** 17 April 2012 11:27:38 GMT+01:00
> **To:** Caroline Ackland <c.ackland@dundee.ac.uk>
> **Cc:** Andrew Mason <a.g.mason@dundee.ac.uk>, Abubaker Qutieshat <a.s.qutieshat@dundee.ac.uk>
> **Subject: Proposed Survey on Extracted Tooth Collection**
>
> Caroline
>
> In you capacity as scientific advisor to the ethics committee I should be gratfeul if you could advise if we require ethical approval for the attached survey.
>
> We intend to write to approx 500 dentists selected at random from the Dental Register inviting them to complete and return the questionnaire. We will not know from whom the responses have come which may be either by mail or electronically. As result we will not issue reminders to none responders.
>
> with many thanks
>
> Graham
>
> R Graham Chadwick
> Clinical Senior Lecturer and Hon Consultant in Restorative Dentistry
> The Dental School
> Park Place
> Dundee
> DD1 4HN
>
> Telephone/Fax 01382 635984
> Answerphone 01382 660111 x35899

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TASC
TAyside medical Science Centre

KG/CF
Tuesday, 19 March 2013

Professor R Graham Chadwick
The Dental School
University of Dundee
Park Place
Dundee
DD1 4HN

Dear Prof. Chadwick

Sponsorship Letter

Sponsor R&D Reference Number: 2013DE04
Study Title: An in-vitro investigation of the effects of erosive beverage upon human hard tooth tissue and its restoration.

Under the requirements of the Scottish Executive Health Department Research Governance Framework for Health and Community, The University of Dundee agrees to act as Sponsor for this trial. Sponsorship is subject to you obtaining a favourable ethical opinion and NHS Tayside R&D management approval, if required.

Enclosed is a Chief Investigator Declaration. You should read it to familiarise yourself with the terms, sign the agreement on page four, completing the remaining details and return a copy to the TASC Research Governance Manager.

Following receipt of all relevant approvals, you should ensure that any subsequent amendments are notified to the Sponsor, REC and any relevant NHS R&D Office(s), and that an annual progress report is submitted to the Sponsor, REC and NHS R&D Tayside, as appropriate.

Please ensure yourself and your study staff are familiar with the University of Dundee Standard Operating Procedures and guidelines (available at http://www.tasc-research.org.uk/_page.php?id=157), and we strongly recommend that they have received Good Clinical Practice training, as appropriate, before the study commences.

Finally please contact Dr. Catrina Forde (c.forde@dundee.ac.uk) or Dr Keith Gillon (k.gillon@dundee.ac.uk) should you have any queries.

Signed for and on behalf of the University of Dundee

Catrina Forde.

Dr Keith Gillon
Senior R&D Manager, TASC

NHS Tayside

Dr Keith Gillon, TASC (Tayside Medical Science Centre),
Ninewells Hospital & Medical School, TASC Research & Development Office, Residency Block, Level 3, George Pirie Way, Dundee DD1 9SY.
k.gillon@dundee.ac.uk or PA , Leigh De Melo, l.demelo@dundee.ac.uk, Telephone 01382 740129

v. 2.3 260213

η Date 20th August 2012

Sponsor R&D No: 2013DE04*
Study Title: An in-vitro investigation of the effects of erosive beverage upon human hard tooth tissue and its restoration.
CI. Prof. RG Chadwick
Date Issued:190313

Insurance – Clinical Research Studies excluding Clinical Trials

A policy of Public Liability Insurance is held by the University of Dundee, last renewed with effect from 1st August 2012.

Insurers:	Zurich Municipal Insurance
Policy Number:	NHS-14CB07-0013
Cover application:	**Clinical Research** but excluding: 'Clinical Trials'/studies requiring Clinical Trials Authorisation from the Medical Healthcare Regulatory Agency (MHRA) and studies subject to the Medical Devices Regulations as amended or succeeded.
Limit of Indemnity:	**Legal Liability: £20,000,000** (Twenty million pounds sterling) In all for damages, costs and expenses arising out of all claims during any Period of Insurance for which the University is legally liable.
Territorial Limits:	Worldwide excluding the USA & Canada but excludes any services undertaken by the University outside the UK from any premises occupied on a local domiciled basis.

Other terms and conditions:

- Retroactive date 1 August 1994
- Maximum payable by insurers under the Legal Liability shall not exceed **£20,000,000** during any Period of Insurance
- Insured will provide particulars of any claim or circumstances which might give rise to a claim to Insurers without delay
- Human Materials – Tissue samples – subject to consent and regulatory approvals re collection, storage and disposal
- 'No fault' (Non-Negligent Harm) cover is not provided by this policy.

Signed on behalf of the University of Dundee

Gordon t. Campbell.

Gordon Campbell, Financial Manager

UoD Tel (Office hours): +44 (0)1382 384043 or 384045
UoD Tel (Out of hours): +44 (0)1382 388188
Email: insurance@dundee.ac.uk

TASC
TAyside medical Science Centre

Chief Investigator Declaration

The following Declaration and accompanying Insurance Checklist must be completed to enable the University of Dundee to adopt "Research Sponsor" status, for the following study: -

Title of Study	An in-vitro investigation of the effects of erosive beverage upon human hard tooth tissue and its restoration
Funding body	Internal
Chief Investigator	Prof. RG Chadwick
Dept./Div	Dental School
Sponsor R&D No	2013DE04*

Please provide confirmation that there is no reason why the University as Sponsor cannot adopt "Research Sponsor" status, as defined within the terms of the Research Governance Framework for Health & Community Care Scotland (RGF), for the above study. By signing below you are also confirming that you will comply with the responsibilities of a Chief Investigator (CI) as defined within the RGF **(available at http://www.cso.scot.nhs.uk/Publications/ResGovFramework/RGFEdTwo.pdf).**

Section 1 - Your responsibilities as CI with respect to the study referenced in this Declaration are to ensure

1. if appropriate, the study will receive research ethics committee approval;

   Information on the East of Scotland Research Ethics Service (EoSRES) is available at http://www.tasc-research.org.uk/_page.php?id=406

   Information on the University of Dundee Research Ethics Committee (UREC) is available at http://www.dundee.ac.uk/mainresearch/ethics/

2. if the study requires tissue from Tayside Tissue Bank, necessary approvals have been or will be obtained;
3. if an external collaborator is providing samples/tissues/data, necessary ethical and regulatory approval is or will be held on file;
4. the study has been subjected to expert independent review and judged to be of a high scientific standard and quality; it has also been judged to represent good value for money;
5. the study complies with all legal (informed consent, consent to use tissue, data protection, health and safety), and that all procedures in the study protocol will be adhered to;

   University guidance on research governance is available at http://www.dundee.ac.uk/rgp/policyroadmap/

6. any proposed changes or amendments to or deviations from the protocol are submitted for approval to the Sponsor, ethics committee, and any other appropriate body such as NHS R&D Office;
7. the dignity, rights, safety and well-being of participants in the study are given priority at all times by the study team;
8. steps have been taken to reduce the risks associated with the study and mechanisms are in place to manage and report any adverse events or incidents that may arise;
9. arrangements are in place for the Sponsor and other stakeholder organisations to be alerted if significant developments occur as the study progresses, whether in relation to the safety of individuals or to scientific direction;
10. arrangements are in place to ensure the integrity and confidentiality of clinical and other records used or generated during the study;
11. any care organisation, including NHS Boards or Trusts, involved in the study is informed of the details of the study, and that written approval from that organisation has been obtained for the study to commence;
12. when the study involves participants under the care of a doctor, nurse or social worker for the condition to which the study relates, those care professionals are informed that their patients are being invited to participate and agree to retain overall responsibility for their care;

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13. when the study involves a service user or carer or a child, looked after or receiving services under the auspices of a local authority, that the agency director agrees to the person (and/or their carer) being invited to participate and is fully aware of the arrangements for dealing with any disclosures or other relevant information;
14. unless participants or the relevant research ethics committee request otherwise, participants' care professionals are given information specifically relevant to their care that arises during the study;
15. a written agreement with the funder(s) of the study is in place and with any collaborators external to the Sponsor;
16. allocation of responsibilities during the study have been agreed and documented with any collaborators external to the Sponsor;
17. each member of the study team is qualified by education, training and experience to discharge his/her academic and/or managerial role; the recruitment practices for individual researchers employed on the study are transparent and open to scrutiny;
18. students and new researchers working on the study will have adequate supervision, support and training;
19. if staff working on the study are required to work to Good Clinical Practice, suitable training has been provided or experience confirmed:
20. procedures are in place to ensure collection of high quality, accurate data and to ensure the integrity and confidentiality of data during processing and storage;
21. reports on the progress and outcomes of the study required by the Sponsor, funder(s), or others with a legitimate interest are produced on time and to an acceptable standard;
22. complaints from patients or staff involved in the study and cases or a suspected case of misconduct are reported; **The University's policy on misconduct in research is available at http://www.somis.dundee.ac.uk/court/policy/miscinresearch.pdf**
23. arrangements are in place for the management of financial and other resources provided for the study, including the management of any intellectual property arising from the study;
24. all data and documentation associated with the study will be available for scientific or financial audit at the request of the appropriate auditing authority;
25. a plan is established for publication and dissemination of research findings from the study, including to the participants, subject to any commercial confidentiality constraints;
26. the findings of the study will be open to critical review through the accepted scientific and professional channels, subject to any commercial confidentiality constraints.
27. you have read and understand the requirements of the **University of Dundee Research Governance Policy Handbook available at http://www.dundee.ac.uk/rgp/policyroadmap/**

I confirm that having read the RGF compliance demands cited at 1 to 27 above, I know of no reason why the Sponsor cannot provide confirmation of my compliance with these demands as they apply to the study identified in this form.

The Sponsor may be required to undertake sample auditing of returns made in response to the issue of this form.

Signed R Graham Chadwick **Date** 3/4/13

Print Name R GRAHAM CHADWICK

Please now return a copy of this form to the Research Governance Manager, TASC.

EoSRES

NHS SCOTLAND

East of Scotland Research Ethics Service (EoSRES)
Tayside Medical Sciences Centre (TASC)
Residency Block C, Level 3
Ninewells Hospital & Medical School
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Dr R G Chadwick
Clinical Senior Lecturer and Hon Consultant in Restorative Dentistry
University of Dundee
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DD1 4HN

Date: 19 October 2012
Your Ref:
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Extension: Ninewells extension: 83839
Direct Line: 01382 383839
Email: caroline.ackland@nhs.net

Dear Dr Chadwick

Re: Determination of normal behaviour in the consumption of carbonated beverages in order to inform the development of an in vitro testing environment

You have sought advice from the Research Ethics Office on the above project. I have considered this and can advise that this does not require ethical review under the terms of the Governance Arrangement for Research Ethics Committees (GAfREC) in the UK. The advice is based on the following documentation provided:

Document	Version	Date
Cover letter	N/A	26 September 2012
Protocol	Not Specified	Not Specified
Participant Information Sheet	1.0	10 September 2012
Consent Form	1.0	10 September 2012
Questionnaire	Not Specified	Not Specified
Participant Information Sheet	2.0	16 October 2012

- You are undertaking an observational study with dental students
- You are undertaking an anonymous questionnaire survey
- You are conducting a video recorded lunch
- You may still require Research and Development approval

Please note that this advice is issued on behalf of the Research Ethics Service Office and does not constitute an opinion of a Research Ethics Committee (REC). It is intended to satisfy journal editors and conference organisers, who may require evidence of consideration of the need for ethical review prior to publication or presentation of your results.

You should keep a copy of this letter within your project file.

Yours sincerely,

cyAckland.

Caroline Ackland
Scientific Officer, East of Scotland Research Ethics Service

Cc: Dr Catrina Forde, Research Governance Manager, TASC

8.2 Appendix 2 – Questionnaire I

8.2.1 The Human Tissue Act cover letter

25 June, 2012

Dear Colleague,

You will no doubt remember your days as an undergraduate dental student. You may well have practiced clinical techniques at that time upon extracted human teeth and also learned the evidence for these obtained from research conducted upon them.

I am a postgraduate research student undertaking a research PhD in dental erosion under the supervision of Dr. RG Chadwick. We are interested in the use of extracted teeth in both teaching and research. I should be grateful if you could spend approximately five minutes of your time completing the enclosed survey.

You can do this either by completing the enclosed paper copy returning it in the pre-paid envelope or by using the web link http://www.survey.dundee.ac.uk/teeth.

We wish to guarantee anonymity so either approach bears no hidden identifiers. As a result you will not be contacted again should you prefer not to respond. Only respond by one method (post/web).

Your name was selected at random from the dentists register search engine at the General Dental Council.

Thank you for your assistance.

Yours sincerely,

Abubaker S. Qutieshat

8.2.2 The Human Tissue Act questionnaire

Extracted Teeth Survey

Welcome

Welcome to the Extracted Teeth Survey. This survey aims to gain a better understanding of how dentists in the United Kingdom deal with extracted teeth.

The survey is completed anonymously and takes around 5-10 minutes to complete.

Remember, you can alternatively use the web link **http://www.survey.dundee.ac.uk/teeth** to complete the survey. If you are able to complete this questionnaire please do so by one method only.

Extracted Teeth Survey

Basic Information

1. How long have you been a qualified dentist? *"to the nearest whole number of years"*

- O 3 years or less
- O 4-6 years
- O 7-9 years
- O 10-12 years
- O 13 years or more

2. Did you qualify in the UK?

- O Yes
- O No

If the answer is "yes" skip to question 3

a. State the country where you qualified

b. When did you first register with the General Dental Council to practice dentistry in the UK?

Extracted Teeth

3. Indicate if you collect extracted teeth in your practice

- O Yes
- O No

The reason for collecting extracted teeth. *(select all that apply)*

- ☐ For dental education on behalf of students or dental institutions
- ☐ For research on behalf of dental institutions
- ☐ Own collection "difficult extractions, rare cases, anomalies etc..."
- ☐ Other *(please specify):*

4. Before September, 2006; did you collect extracted teeth?

- O Never
- O Sometimes
- O Most of the time
- O Always

5. After September, 2006; did you collect extracted teeth?

- O Never
- O Sometimes
- O Most of the time
- O Always

6. Do you currently have extracted teeth stored in your practice?

- O Yes
- O No

If the answer is "No", skip to question 7

a. Approximately how many?

- O 1-20
- O 21-40
- O 41-60
- O More then 60

b. In what medium do you store your extracted teeth?

7. If approached by an institution to collect teeth for which purpose if any would you agree to do this?

	Agree	Refuse
a. Dental education	O	O
b. Research	O	O

8. Indicate the type of consent you think is required for tooth collection for the following purposes:

	None	Verbal consent	Record in notes	Written consent at time of extraction	Written consent at least 24 hours before extraction
a. Dental education	O	O	O	O	O
b. Research	O	O	O	O	O

9. When you were training or developing your clinical skills, how did you find practicing this upon extracted human teeth?

O Never did this

O Not helpful at all

O Somewhat helpful

O Very helpful

10. Did you attempt to collect extracted teeth during your dental undergraduate study?

- O Yes
- O No

a. When did you graduate?

b. On seeking to collect the teeth, how did you find the people you approached to do this?

- O Did not deliver
- O Obstructive but delivered
- O Reluctant but delivered
- O Helpful

11. In your opinion, who owns the freshly extracted teeth immediately after their extraction?

- O The dentist and/or the dental clinic or institution
- O The patient
- O No one
- O Other *(please specify)*:

12. Regarding the collection of teeth for the purposes of dental research

(select all that apply)

- ☐ A human Tissue Authority license must be held
- ☐ Consent for this use must be obtained
- ☐ The tooth must be traceable back to the donor/patient from whom the tooth was extracted
- ☐ The tooth has to be non-traceable and totally anonymous
- ☐ Consent for use of the tooth can be withdrawn
- ☐ The use that the tooth is put to can be altered without consent
- ☐ The donor may be charged for storing the tooth
- ☐ The donor must not be charged for storing his tooth

13. Regarding the collection of teeth for the purposes of dental undergraduate education

(select all that apply)

- ☐ A human Tissue Authority license must be held
- ☐ Consent for this use must be obtained
- ☐ The tooth must be traceable back to the donor/patient from whom the tooth was extracted
- ☐ The tooth has to be non-traceable and totally anonymous
- ☐ Consent for use of the tooth can be withdrawn
- ☐ The use that the tooth is put to can be altered without consent
- ☐ The donor may be charged for storing the tooth
- ☐ The donor must not be charged for storing his tooth

Extracted Teeth Survey

Final Page

Thank you for taking part in this survey.

For more information and other inquiries, please contact

a.s.qutieshat@dundee.ac.uk

8.3 Appendix 3 – Questionnaire II

Pre-Experimental Questionnaire

PIZZA AND SOFT DRINK PARTY PROJECT

Greetings from the Restorative Dentistry Research Team,

An experimental pizza and soft drink party is designed to more fully understand the aspects of human behaviour while drinking in a social environment and to apply this behaviour to an artificial mouth model.

Please fill out this quick survey and help us recruit participants for the party. An invitation to attend will reach you via your university email and you can either accept or decline.

Survey link: **www.survey.dundee.ac.uk/party**

Thank you

PIZZA AND SOFT DRINK PARTY PROJECT

Welcome

Welcome to the PIZZA AND SOFT DRINK PARTY survey.

An experimental pizza and soft drink party is designed to more fully understand the aspects of human behaviour while drinking in a social environment and to apply this behaviour to an artificial mouth model. Depending on this survey a decision will be made based on your answers whether to invite you to the aforementioned party or not. An invitation will reach you via your university email and you can either accept or decline.

The personal information you provide (including your email address) will be used only to invite you to the experiment and provide you with participation instructions. Any personal information you provide will be held in accordance with the provisions of the Data Protection Act 1998 and used only for the reasons specified above. Cookies, personal data stored by your Web browser, are not used in this survey.

Completing the survey will take less than 5 minutes.

Selection Survey

Intro

11 Have you ever had any allergic reaction after ingestion of any food or drink?

Yes.

No.

Part I

Personal Details

Date of birth

Dates need to be in the format 'DD/MM/YYYY', for example 27/03/1980.

Please make sure the date is between 01/01/1974 and 31/12/1996.

(dd/mm/yyyy)

Sex:

- Male
- Female

UOD Email:

Part II

Generally,which of the following soft drinks would be your **first** choice?

How often do you consume this drink?

- once a week or less
- 2-3 times a week
- 4-6 times a week
- once a day
- 2 times a day
- 3 times a day or more

How much do you consume each time?

- 1 can or equivalent
- 2 cans or equivalent
- More than 2 cans or equivalent

Which of the following soft drinks would be your **second** choice?

How often do you consume this drink?

- once a week or less
- 2-3 times a week
- 4-6 times a week
- once a day
- 2 times a day
- 3 times a day or more

How much do you consume each time?

- 1 can or equivalent
- 2 cans or equivalent
- More than 2 cans or equivalent

Which of the following soft drinks would be your **third** choice?

How often do you consume this drink?

- once a week or less
- 2-3 times a week
- 4-6 times a week
- once a day
- 2 times a day
- 3 times a day or more

How much do you consume each time?

- 1 can or equivalent
- 2 cans or equivalent
- More than 2 cans or equivalent

When do you usually consume soft drinks?

- With food
- After meals
- While travelling
- After sports or certain physical activities
- In parties
- To quench the thirst
- With hard drinks
- While smoking
- During meetings or gatherings
- In cinemas
- Watching TV

- [ ] Studying
- [ ] Other

If you selected Other, please specify:

How often would you use a straw while drinking?

- ○ Always
- ○ Only if available
- ○ Sometimes, depends on the mood
- ○ Rarely
- ○ Never, even when available

How many times do you refill your drink in a restaurant? *(consider that refills are free)*

- ○ Usually I don't!
- ○ 1 time
- ○ 2 times
- ○ More than 2 times

Part III

"While enjoying your pizza.."

What type of the aforementioned soft drinks would you most likely choose?

What would be the ideal serving temperature for your chosen drink?

- ○ Chilled
- ○ Ambient "room temperature"
- ○ Doesn't matter

What would be the ideal serving options for your chosen drink?

- [ ] Regular
- [ ] Sugar-free "No added sugar"
- [ ] Diet "Low calorie"

How would you like your drink to be served in?

- Can
- Bottle
- Glass cup
- Plastic cup

Would you prefer your beverage to be presented according to your answers above? Required

- Presentation doesn't matter
- Yes
- No (please specify)

Part IV

Oral Hygiene

Regarding the frequency of your oral hygiene habits:

	Frequency					
	Never	Occasionally but not on daily basis	Once daily	Twice daily	Thrice daily	More than 3 times daily
Brushing	○	◉	○	○	○	○
Rinsing	○	○	○	○	○	○

Regarding the timing of your oral hygiene habits: *(select all that apply)*

	Timing				If you selected Other, please specify:
	After a meal	First thing in the morning	Before I go to bed	Other	
Brushing	☐	☐	☐	☐	
Rinsing	☐	☐	☐	☐	

Part V

Parties will be held on the following dates, choose the date that suits you:

- [ ] XX-Mar-2013 from 12:00
- [ ] YY-Mar-2013 from 12:00

If a friend of yours is willing to come along and join this event; provide us with his/her UOD email: *You can invite more than one friend*

Thank you!

Thank you for taking part in the survey. You will be contacted soon.

All the information and data obtained in the study will be stored securely in the Dundee Dental Hospital and School and be destroyed once the study is complete. Only the researchers in charge of the project will have access to the data.

For more information contact Dr. A S Qutieshat a.s.qutieshat@dundee.ac.uk

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Key for selection options

Coca Cola, Sprite, Fanta, Irn Bru, Barr Cola, Tango, Pepsi, 7-Up, Dr. Pepper, Tizer, Mountain Dew, Schweppes Lemon, Shloer, Grapetiser, Appletiser

---

Post-experimental Questionnaire

PIZZA AND SOFT DRINK PARTY: A POST-EXPERIMENTAL QUESTIONNAIRE

- On a scale of 1 to 10, how did you feel during the experiment?
  "10 = Relaxed and acting normal"

1	2	3	4	5	6	7	8	9	10

Comments *(if any)*.....

- On a scale of 1 to 10, how would you describe your performance during the experiment?
  "10 = Consuming exactly the same amount of drink you would usually consume in a similar real-life scenario"

1	2	3	4	5	6	7	8	9	10

Comments *(if any)*.....

THANK YOU FOR PARTICIPATING IN OUR EXPERIMENT!

8.4 Appendix 4 – List of materials and equipment

A list of all materials and equipment used in the fabrication of Saltus together with the software packages used in its design:

Basic material set:

- Perspex® Clear Cast 3 mm sheets (Stockline Plastics Ltd., Dundee, UK)
- Perspex® Colours Cast 3 mm sheets (Perspex® Distribution Ltd., Blackburn, UK)
- Plastic liquid dispensers
- Syringe needle tips 18G
- Eppendorf tubes
- M12 Nylon plastic 80mm long hexagonal head bolts, full nuts and washers

Adjunctive material set:

- Tensol 70 cement (Bostik limited, Leicester, UK)
- Balsa wood 3mm thick sheets.
- Balsa wood square rods.
- Balsa cement (Humbrol, Hornby Hobbies Ltd., Kent, UK).
- Pendant drill/saw Milbro (Milbro Pendant Drill Flexi-Tool, Milnes Bros., Croydon, Surrey, UK)
- Vertical drill/saw stand Milbro (Milbro, Milnes Bros., Croydon, Surrey, UK)
- C-Clamps
- Level gauge
- Square ruler
- Hi Grade Perkins Cellulose thinner (J Perkins Distribution Ltd., Lenham, Kent, UK)
- Fume extractor (Prosthodontics Dental Laboratory, Dundee Dental School, Dundee, UK)

Softwares

- Blender™ 2.72 (Stichting Blender Foundation, Amsterdam, the Netherlands)
- AutoCAD 2012 (Autodesk Inc., San Rafael, California, US)
- SketchUp 2013 (Trimble Navigation Limited, Sunnyvale, CA, US)

8.5 Appendix 5 – Operation Manual

SALTUS Artificial Mouth System Operation Manual

Calibration

Prior to running any major set of diets; it is recommended to make sure that the systems' associated pump settings and connections are accurately calibrated.

For the large, medium and small tubing running at **13.3**, **5.0** and **0.3** ml/min respectively:

- On the main pump, set drive speed value at 2.33 using the arrows on the control panel

- Wrap an elastic rubber band (1" elastic bands, The Works Stores Centre, Sutton Coldfield, UK) around each tubing group (1 inch short of tubing terminals).
- Insert the large tubing group into a 200 ml graduated cylinder and the medium tubing group into a 50 ml cylinder.
- Set up a loop cycle for the small tubing group. See under the heading "setting a loop" later in this document on how to do this.
- Run pump for 60 seconds.
- Ideally, the large and medium tubing groups should yield 106.4 and 40 ml respectively.
- If one or both values vary by more than 2%, refer to the troubleshooting section.
- Repeat 2 times (3 runs in total)

For the small tubing group running at **0.3** ml/min:

- Set drive speed value at 2.33

- Wrap an elastic rubber band around tubes (1 inch short of tubing terminals).
- Insert the small tubing group into a 100 ml graduated cylinder.
- Set up a loop cycle for the large and medium tubing groups.
- Run pump for 30 minutes.
- Ideally, the small tubing group should yield 72 ml.
- If the values vary by more than 2%, refer to the troubleshooting section.
- Repeat 2 times (3 runs in total)

For the small tubing group running at **0.1** ml/min:

- Set drive speed value at 0.666

- Wrap an elastic rubber band around tubes (1 inch short of tubing terminals).
- Insert the small tubing group into a 50 ml graduated cylinder.
- Set up a loop cycle for the large and medium tubing groups.
- Run pump for 30 minutes.
- Ideally, the small tubing group should yield 24 ml.
- If the values vary by more than 2%, refer to the troubleshooting section.
- Repeat 2 times (3 runs in total)

For the medium tubing group running at **0.3** ml/min: *(Alternative)*

- Set drive speed value at 0.131

- Wrap an elastic rubber band around tubes (1 inch short of tubing terminals).
- Insert the medium tubing group into a 100 ml graduated cylinder.
- Set up a loop cycle for the large and small tubing groups.
- Run pump for 30 minutes.
- Ideally, the medium tubing group should yield 72 ml.
- If the values vary by more than 2%, refer to the troubleshooting section.
- Repeat 2 times (3 runs in total)

For the medium tubing group running at **0.1** ml/min: (Alternative)

- Set drive speed value at lowest limit (65.5µ)

- Wrap an elastic rubber band around tubes (1 inch short of tubing terminals).
- Insert the medium tubing group into a 50 ml graduated cylinder.
- Set up a loop cycle for the large and small tubing groups.
- Run pump for 30 minutes.
- Ideally, the medium tubing group should yield 24 ml.
- If the values vary by more than 2%, refer to the troubleshooting section.
- Repeat 2 times (3 runs in total)

Setting a loop

- Fill a 1000 ml Erlenmeyer flask with 800 ml distilled water. One flask for each loop.
- If a tubing group is intended to be set in a loop; put both ends, inlet and outlet ends, into the flask.
- Make sure that inlet ends are dipped in distilled water by at least 1 inch.

The full sequence

Timer controlled and temperature regulated container

- For the “two can diet”, pre-set the timer to operate twice; once at the beginning of the test cycle and again on approaching the middle time-point of the cycle. While for the “one can” diet, pre-set the switch to operate only once; at the beginning of the cycle.
- Fill the temperature-regulated container with the test beverage to be used (5.3 L for the “two can diet” and 2.7 L for the “1 can diet”).
- Make sure the temperature indicator shows 14°C on its screen.
- Hang outlet tubing directly above the main test beverage container using a vertical stand and a clamp.

Setting the timer

- Press the key “TIMER” to access the options for the **switch-on** settings
- Press the “HOUR” and “MINUTE” keys to set the **switch-on** time (HH:MM)
- Press the key “WEEK” to set the **switch-on** working days.
- Press the key “TIMER” again to set the **switch-off** settings
- Press the “HOUR” and “MINUTE” keys to set the **switch-off** time (HH:MM)
- Press the key “WEEK” to set the **switch-off** working days.
- Repeat step 1-6 to set more than one **switch-on**/**switch-off** setting.
- Make sure the displayed time is accurate before setting any programs. To adjust time press “HOUR”,”MINUTE” and “WEEK” while holding the “CLOCK” key down.
- Make sure the auto switch sign is on by pressing the “AUTO/MANU” key.

Source Reservoirs

- Allocate 5000 ml glass beaker as a reservoir to be filled with the test beverage to be used.
- Connect the test beverage reservoir with the main pump using the large tubing group
- Allocate two 2000 ml glass bottles for artificial saliva. The modified caps are to be used here for both bottles.
- Connect artificial saliva reservoirs with the main pump using the small and medium tubing groups.

Saliva Preparation

- Prepare saliva as described in the methodology chapter.
- Fill 2.3L of stimulated saliva into one glass bottle and 2.3L of unstimulated saliva into the other.
- Seal bottles with the modified caps.

Figure A5-1. (A) Tubing setting during the day and night cycles. Unstimulated saliva tubing (Dark Blue) is active while stimulated (Light blue) and test beverage (Red) tubing are set to loop (B) Tubing setting for the test cycle. Stimulated saliva (Light blue) and test beverage (red) tubing are active while unstimulated saliva (Dark blue) tubing is set to loop.

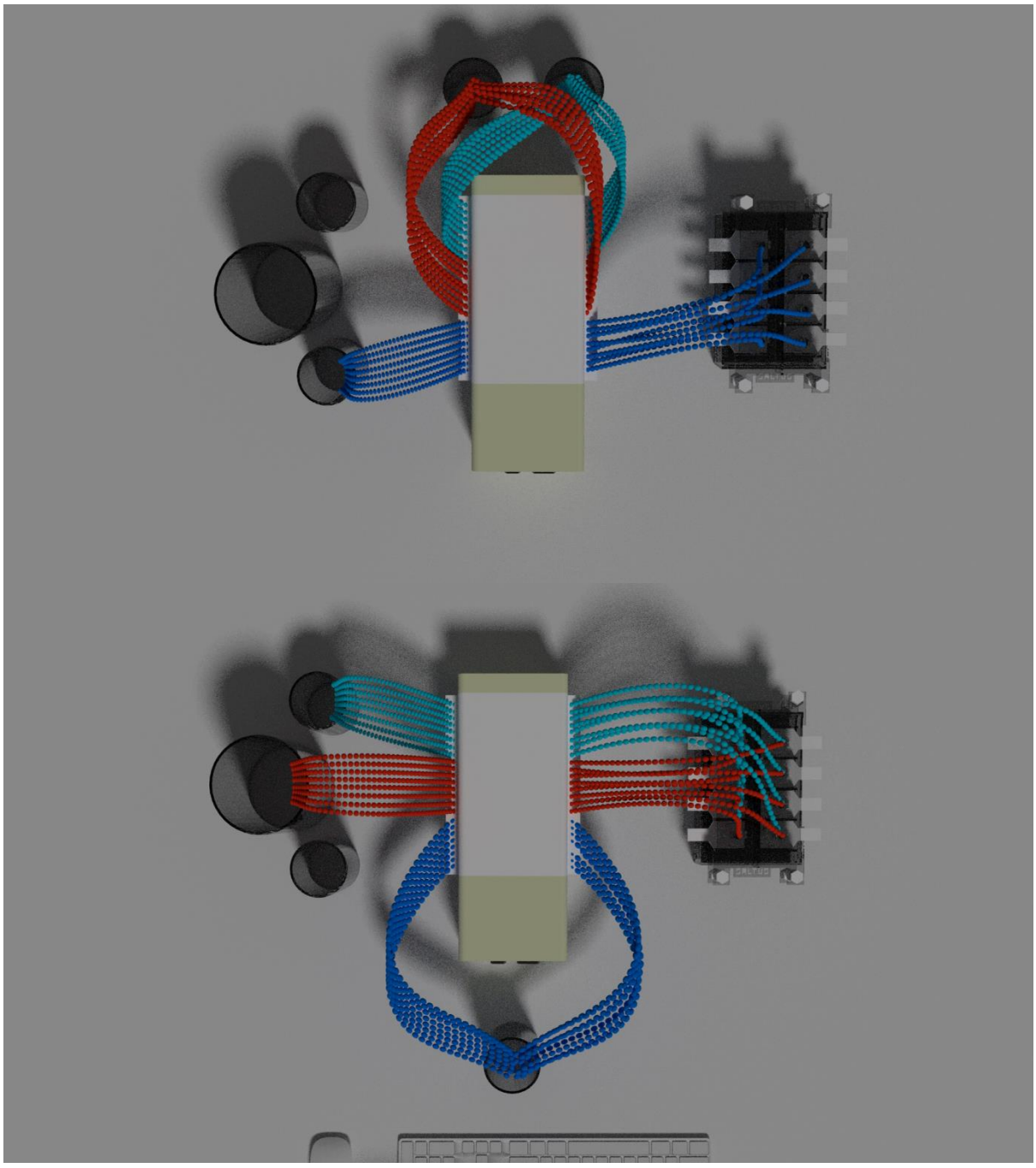
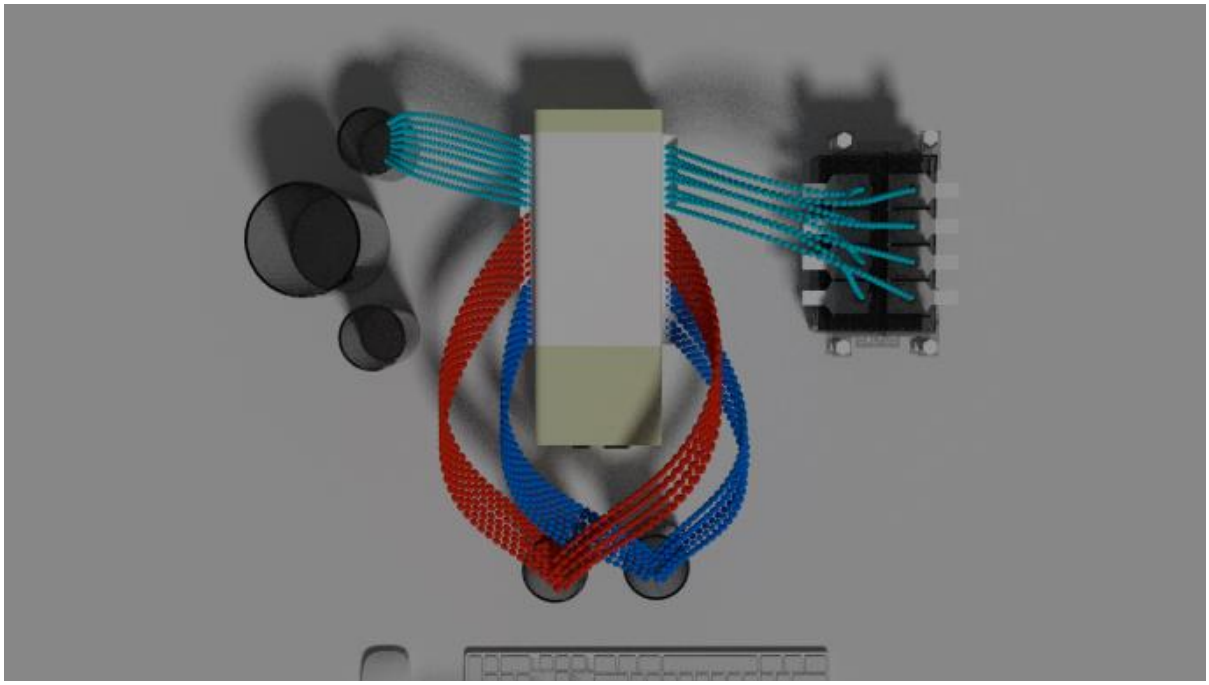


Figure A5-2. Alternative setting: Medium tubing to circulate unstimulated saliva**.**



Collector and transfer tubes

- Allocate a 10L tap-equipped Polyethylene aspirator along with five 20ml universal sample tubes.
- Connect the aspirator with Saltus via its outlet tubing.
- Secure the outlet tubing on top of the aspirator using a clamp.
- After each “complete” 24-hour cycle, using the tap, dispense the resultant solution into 3 universal sample tubes.
- Empty the aspirator and connect it back to the full sequence.

Self-cleansing cycle

Ideally, this cycle last for 48 hours.

- Fill the temperature-regulated container and the clearance container with distilled water then add Milton sterilising tablets (Milton BabyCare, Newmarket, UK). Add 1 tablet to the 5 L temperature-regulated container and 2 tablets to the 10 L clearance container. Keep for overnight.
- Fill all source containers with a diluted Lipsol detergent (Lipsol, SciLabware, Stoke-on-Trent, UK) and connect the outlet tubing with the sink.
- Run the system until the containers are empty.
- Fill all source containers again with a diluted Lipsol detergent (Lipsol, SciLabware, Stoke-on-Trent, UK) and connect the outlet tubing with the sink.
- Run the system until the containers are empty.
- Spot wipe and clean all visible stains or precipitations
- Repeat the procedure above using distilled water only to rinse the detergent out of the system.
- Dismantle Saltus and its components and soak overnight in a diluted detergent solution along with its specimen disk holders. Once complete, rinse all parts with distilled water before assembly.

Troubleshooting

Problem	Possible causes	Solution
Flow rate is lower than needed. Flow rate is higher than needed. No fluid out of tubing (1 or more)	Wrong pump speed value. Precipitations inside tubing. Tubing blocked.	Check the pump speed value on control panel. Clean the lumen of the tubing using the custom made mini-plumbing wire. Use the modified air syringe to clear the blockage.
Main pump fails to switch on. Main pump prematurely switches off.	Electric timer wrong settings	Check the timer's clock and settings
Clearance pump fails to switch on. Clearance pump prematurely switches off.	Electric timer wrong settings	Check the timer's clock and settings
Fountain pump fails to switch on. Fountain pump prematurely switches off.	Timer switch wrong settings	Check the timer's clock and settings. Check wiring
Tubing leaks	Connecter failure	Check for tubing-tubing bond failure. Check for blockage. Use the custom made mini-plumbing wire to check the connector's lumen. Once fully checked, reapply bond.
Dark precipitations in clearance tubing	Fungal growth	Stop the experiment. Run a self-cleansing cycle immediately.

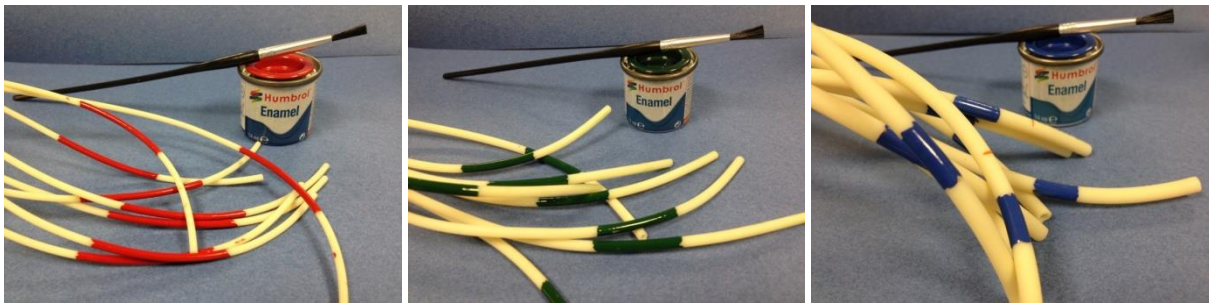
8.6 Appendix 6 – Flow rate algorithm

The standard procedure in calculating flow rates;

The work utilised three diameters of tubing as shown in figure A6-1.

Flow Rate Algorithm

Figure A6-1. (a) Small tubing has an internal diameter of 0.38 mm and was colour coded red (b) Medium tubing has an internal diameter of 1.65 mm and was colour coded green (c) Large tubing has an internal diameter of 2.79 mm and was colour coded blue.



1. Stimulated saliva flow rate and test beverage flow rate

- The following flow rate values were adopted:
    - Stimulated artificial-salivary flow rate of 5.0 ml/min during the acidic attack.
    - Test beverage flow rate of 13.3 ml/min.

- The following settings were chosen upon piloting
    - Day cycle (waking hours) pump drive speed: 2.33
    - Test beverage cycle: 2.33
    - Night cycle (sleeping hours) pump drive speed: 0.666
    - Unstimulated saliva tubing: **Small** tubing group
    - Stimulated saliva tubing: **Medium** tubing group
    - test beverage tubing: **Large** tubing group

- The following settings were also chosen (as an alternative) upon piloting

    - Day cycle (waking hours) pump drive speed: 0.131
    - Test cycle: 2.33
    - Night cycle (sleeping hours) pump drive speed: 65.5µ
    - Unstimulated saliva tubing: **Medium** tubing group
    - Stimulated saliva tubing: **Medium** tubing group
    - Test beverage tubing: **Large** tubing group

    Justification procedure:

        - Drive speed values to be tested started from 2.00 and in increments/reductions of 0.1 thereafter until the target value was reached.
        - An elastic rubber band was wrapped around each tubing group (8 tubes each) to facilitate their insertion into a 50 ml and 200 ml graduated cylinder for medium and large tubing respectively.
        - Small tubing was set to loop.
        - The pump was set to operate for 60 seconds.
        - Once the target value has been reached the procedure was repeated twice to yield a set of three trial values.
        - The expected volume to be produced by the pump for the duration of 1 minute would be:

            - Volume = flow rate X number of tubes X duration
            - Volume (large tubing) = 13.3 X 8 X 1 = 106.4 ml
            - Volume (medium tubing) = 5.0 X 8 X 1 = 40 ml

- Results:

Run	Volume @ 2.00 drive speed	
	Medium tubing	**Large tubing**
1	34.5	91.0

Rejected, and an increment of 0.1 was added.

Run	Volume @ 2.10 drive speed	
	Medium tubing	**Large tubing**
1	36.25	96.5

Rejected, and an increment of 0.1 was added.

Run	Volume @ 2.20 drive speed	
	Medium tubing	**Large tubing**
1	38.0	102.0

Rejected, and an increment of 0.1 was added.

Run	Volume @ 2.30 drive speed	
	Medium tubing	**Large tubing**
1	39.75	106.0

Rejected, and an increment of 0.03 was added.

Run	Volume @ 2.33 drive speed	
	Medium tubing	**Large tubing**
1	40.0	106.4
2	40.0	106.4
3	40.0	106.4
Mean±SD	**40.0 ± 0.0**	**106.4 ± 0.0**

Accepted, pump drive speed of **2.33** was confirmed.

2. Unstimulated saliva flow rate

Unstimulated artificial-salivary flow rate of 0.3 ml/min during the day (awake) and 0.1 ml/min during the night (asleep) were adopted.

- Day cycle: 0.3 ml/min (Group function)

  - Drive speed value was set to the predetermined value of 2.33.
  - An elastic rubber band was wrapped around the tubes of the small tubing group (8 tubes) to facilitate their insertion into a 100 ml graduated cylinder.
  - Medium and large tubing were set to loop.
  - The pump was set to operate for 30 minutes.
  - Once the target value has been reached, procedure was repeated twice to yield a set of three trials.
  - The expected volume to be produced by the pump for the duration of 30 minutes would be:
    - Volume = flow rate X number of tubes X duration
    - Volume = 0.3 X 8 X 30 = 72 ml

  - Results:

Run	Volume @ 2.33 drive speed
	Small tubing
1	72
2	72
3	72
Mean±SD	**72.0 ± 0.0**

Accepted, pump drive speed of **2.33** was confirmed.

Alternatively:

- Drive speed values to be tested started from 0.143 and in increments/reductions of 0.007 thereafter until the target value was reached.
- An elastic rubber band was wrapped around the medium tubing group (8 tubes) to facilitate their insertion into a 100 ml graduated cylinder.
- Large and small tubing were set to loop.
- The pump was set to operate for 30 minutes.
- Once the target value has been reached, procedure was repeated twice to yield a set of three trials.
- The expected volume to be produced by the pump for the duration of 30 minutes would be:
    - Volume = flow rate X number of tubes X duration
    - Volume = 0.3 X 8 X 30 = 72 ml

- Results:

Run	Volume @ 0.142 drive speed
	Medium tubing
1	78.5

Rejected, and of 0.007 was detucted.

Run	Volume @ 0.135 drive speed
	Medium tubing
1	76

Rejected, and 0.007 was deducted.

Run	Volume @ 0.128 drive speed
	Medium tubing
1	71

Rejected, and an increment of 0.001 was added.

Run	Volume @ 0.129 drive speed
	Medium tubing
1	71.5

Rejected, and an increment of 0.002 was added.

Run	Volume @ 0.131 drive speed
	Medium tubing
1	72
2	72
3	72
Mean±SD	**72.0 ± 0.0**

Accepted, pump drive speed of **0.131** was confirmed.

- Night cycle: 0.1 ml/min
    - Drive speed value was set to the predetermined value of 0.666.
    - An elastic rubber band was wrapped around the tubes of the small tubing group (8 tubes) to facilitate their insertion into a 50 ml graduated cylinder.
    - Medium and large tubing were set to loop.
    - The pump was set to operate for 30 minutes.
    - Once the target value has been reached, procedure was repeated twice to yield a set of three trials.
    - The expected volume to be produced by the pump for the duration of 30 minutes would be:
        - Volume = flow rate X number of tubes X duration
        - Volume = 0.1 X 8 X 30 = 24 ml

- Results:

Run	Volume @ 0.640 drive speed
	Small tubing
1	17

Rejected, and an increment of 0.01 was added.

Run	Volume @ 0.650 drive speed
	Small tubing
1	21

Rejected, and an increment of 0.01 was added.

Run	Volume @ 0.660 drive speed
	Small tubing
1	27

Rejected, and an increment of 0.01 was added.

Run	Volume @ 0.672 drive speed
	Small tubing
1	26

Rejected, and 0.012 was deducted.

Run	Volume @ 0.666 drive speed
	Small tubing
1	24
2	24
3	24
Mean±SD	**24.0 ± 0.0**

Accepted, pump drive speed of **0.666** was confirmed.

Alternatively:

- Drive speed value was set to the lowest value possible (0.0565).
- An elastic rubber band was wrapped around the tubes of the medium tubing group (8 tubes) to facilitate their insertion into a 50 ml graduated cylinder.
- Small and large tubing were set to loop.
- The pump was set to operate for 30 minutes.
- Once the target value has been reached, procedure was repeated twice to yield a set of three trials.
- The expected volume to be produced by the pump for the duration of 30 minutes would be:
    - Volume = flow rate X number of tubes X duration
    - Volume = 0.1 X 8 X 30 = 24 ml
- Results:

	Volume @ 0.0565 drive speed
	Medium tubing
1	24
2	24
3	24
Mean±SD	**24.0 ± 0.0**

Accepted, pump drive speed of 0.0565 was confirmed.

8.7 Appendix 7 – Raw data

Tables A7-1 – A7-46 present raw surface hardness and profile data sets for all human enamel and Ostrich eggshell specimens. Raw reference and post-diet calcium and phosphate ion concentration data sets are also presented. These table were referred to in Chapter 4.

Table A7-1. Pre- and post- diet raw surface hardness values (HV) of human enamel. (a) Pre-diet (b) Post-diet

Readings **Diet** 1	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	268	261	296	292	270	260	303	243	244	267	270.4	20.7
Sample 2	267	256	296	242	247	261	310	296	264	265	270.4	22.6
Sample 3	220	279	291	254	311	300	263	295	270	239	272.2	28.8
Sample 4	285	258	281	211	311	322	253	284	292	332	282.9	35.8
Sample 5	251	258	253	293	305	244	247	276	258	285	267.0	21.3
Sample 6	303	245	233	275	311	232	270	278	219	296	266.2	32.4
Sample 7	286	297	252	273	276	278	278	316	280	299	283.5	17.4
Sample 8	289	288	218	288	281	287	244	279	293	320	278.7	28.2

Readings **Diet** 1	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	252	278	309	270	255	303	288	327	284	310	287.6	24.7
Sample 2	280	291	261	261	250	244	262	267	257	264	263.6	13.6
Sample 3	317	316	257	217	275	289	289	310	289	318	287.7	31.9
Sample 4	313	304	311	269	242	329	264	269	316	297	291.4	28.4
Sample 5	309	248	263	236	292	298	247	289	248	241	267.2	27.2
Sample 6	248	272	290	294	225	243	248	306	284	253	266.3	26.6
Sample 7	259	257	274	270	290	289	282	254	265	298	273.8	15.3
Sample 8	265	297	322	270	232	250	265	261	282	292	273.6	25.6

Table A7-1 II. Pre- and post- diet raw surface hardness values (HV) of human enamel. (a) Pre-diet (b) Post-diet

Readings **Diet** 1R	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	302	302	263	301	271	289	239	279	282	281	280.9	19.8
Sample 2	251	262	268	259	268	271	234	252	216	273	255.4	18.2
Sample 3	276	272	292	232	228	299	227	280	227	291	262.4	30.2
Sample 4	274	291	260	262	302	285	264	268	279	260	274.5	14.5
Sample 5	312	247	286	281	285	271	282	295	251	253	276.3	20.9
Sample 6	253	277	296	279	285	234	314	304	235	259	273.6	27.7
Sample 7	254	231	285	230	262	261	243	247	258	276	254.7	17.8
Sample 8	228	261	238	299	263	292	300	247	253	317	269.8	30.1

Readings **Diet** 1R	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	296	324	277	333	278	273	340	304	302	266	299.3	26.2
Sample 2	198	232	196	279	267	288	306	325	277	283	265.1	43.3
Sample 3	260	235	239	297	285	312	283	300	323	286	282.0	29.2
Sample 4	231	248	234	236	244	283	296	253	288	280	259.3	24.8
Sample 5	241	249	314	277	270	280	280	282	242	283	271.8	22.4
Sample 6	279	287	296	269	259	281	236	294	252	268	272.1	19.2
Sample 7	270	240	274	269	225	235	289	243	285	301	263.1	25.7
Sample 8	292	219	313	280	297	253	251	242	228	225	260.0	33.3

Table A7-2. Pre- and post- diet raw surface hardness values (HV) of human enamel. (a) Pre-diet (b) Post-diet

Readings **Diet** 2	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	264	299	257	262	247	289	212	301	271	257	265.9	26.5
Sample 2	223	205	212	211	252	288	283	217	247	253	239.1	30.1
Sample 3	285	303	251	318	302	296	296	286	340	324	300.1	24.4
Sample 4	252	206	234	257	233	210	264	225	277	218	237.6	24.0
Sample 5	294	281	300	299	288	290	363	257	332	353	305.7	33.3
Sample 6	308	317	277	250	328	230	282	256	291	271	281.0	31.0
Sample 7	219	213	278	223	265	232	207	258	212	269	237.6	27.0
Sample 8	264	299	257	262	247	289	212	301	271	257	265.9	26.5

Readings **Diet** 2	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	231	265	259	233	238	256	254	235	254	231	245.6	13.2
Sample 2	223	248	259	230	275	275	277	231	240	246	250.4	20.2
Sample 3	288	253	263	288	255	249	265	245	261	283	265	16.0
Sample 4	251	253	259	243	240	214	230	256	239	249	243.4	13.6
Sample 5	294	306	278	328	287	267	304	330	314	272	298	22.2
Sample 6	261	279	311	272	264	247	285	284	242	320	276.5	25.2
Sample 7	243	247	271	267	247	247	219	253	291	255	254	19.2
Sample 8	256	263	291	249	232	222	266	294	306	269	264.8	26.9

Table A7-2 II. Pre- and post- diet raw surface hardness values (HV) of human enamel. (a) Pre-diet (b) Post-diet

Readings **Diet** 2R	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	289	212	301	271	257	264	299	257	262	247	265.9	26.5
Sample 2	288	283	217	247	253	223	205	212	211	252	239.1	30.1
Sample 3	296	296	286	340	324	285	303	251	318	302	300.1	24.4
Sample 4	210	264	225	277	218	252	206	234	257	233	237.6	24.0
Sample 5	290	363	257	332	353	294	281	300	299	288	305.7	33.3
Sample 6	230	282	256	291	271	308	317	277	250	328	281.0	31.0
Sample 7	232	207	258	212	269	219	213	278	223	265	237.6	27.0
Sample 8	289	212	301	271	257	264	299	257	262	247	265.9	26.5

Readings **Diet** 2R	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	288	252	275	218	225	254	243	205	244	266	247	25.8
Sample 2	217	233	256	233	267	266	251	258	277	240	249.8	18.7
Sample 3	243	259	284	246	283	300	292	290	235	253	268.5	23.7
Sample 4	281	281	274	274	241	279	270	253	273	256	268.2	13.6
Sample 5	277	299	321	303	276	322	225	269	267	277	283.6	29.0
Sample 6	262	300	297	289	263	256	290	202	281	332	277.2	34.6
Sample 7	282	270	228	261	282	245	222	277	253	261	258.1	21.3
Sample 8	199	197	242	236	252	231	233	228	267	255	234	22.5

Table A7-3. Pre- and post- diet raw surface hardness values (HV) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 3	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	331	310	328	332	312	341	284	372	322	340	327.2	23.1
Sample 2	344	387	332	342	328	298	338	324	349	323	336.5	22.9
Sample 3	311	290	304	346	304	300	273	319	359	316	312.2	25.2
Sample 4	299	319	300	302	324	354	284	273	312	305	307.2	22.3
Sample 5	299	319	300	302	324	354	284	273	312	305	307.2	22.3
Sample 6	296	321	329	303	339	275	245	292	354	294	304.8	32.1
Sample 7	292	277	269	280	286	285	300	309	226	302	282.6	23.4
Sample 8	225	295	280	300	306	260	282	270	288	344	285.0	31.2

Readings Diet 3	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	257	297	246	234	340	263	248	285	256	303	272.9	32.7
Sample 2	287	280	307	266	278	266	253	280	329	224	276.9	28.6
Sample 3	270	327	286	321	305	290	291	300	292	272	295.4	18.6
Sample 4	282	288	318	271	291	286	309	309	269	235	285.8	24.1
Sample 5	354	313	339	312	283	322	296	312	319	324	317.4	20.0
Sample 6	271	299	274	319	323	324	293	306	299	293	300.2	18.5
Sample 7	254	256	256	261	278	269	264	274	271	260	264.3	8.2
Sample 8	253	285	268	274	266	280	261	266	296	277	272.6	12.5

Table A7-3 II. Pre- and post- diet raw surface hardness values (HV) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 3R	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	282	281	263	301	239	279	302	302	271	289	280.9	19.8
Sample 2	216	273	268	259	234	252	251	262	268	271	255.4	18.2
Sample 3	227	291	292	232	227	280	276	272	228	299	262.4	30.2
Sample 4	279	260	260	262	264	268	274	291	302	285	274.5	14.5
Sample 5	251	253	286	281	282	295	312	247	285	271	276.3	20.9
Sample 6	235	259	296	279	314	304	253	277	285	234	273.6	27.7
Sample 7	258	276	285	230	243	247	254	231	262	261	254.7	17.8
Sample 8	253	317	238	299	300	247	228	261	263	292	269.8	30.1

Readings Diet 3R	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	295	258	253	268	260	261	223	231	274	292	261.5	23.0
Sample 2	206	191	235	257	290	265	223	268	268	206	240.9	33.3
Sample 3	268	225	222	181	203	315	243	278	183	292	241.0	46.2
Sample 4	249	222	246	267	258	205	200	200	273	232	235.2	27.6
Sample 5	331	260	296	299	322	311	229	230	266	227	277.0	40.0
Sample 6	223	277	235	261	282	242	277	270	234	208	250.9	25.9
Sample 7	201	282	247	205	279	189	273	277	251	235	243.9	35.2
Sample 8	220	274	253	305	253	296	220	272	277	259	262.9	28.2

Table A7-4. Pre- and post- diet raw surface hardness values (HV) of human enamel. (a) Pre-diet (b) Post-diet

Readings **Diet** 4	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	282	316	306	261	281	323	319	277	329	245	293.9	28.7
Sample 2	263	268	255	296	264	243	289	260	318	276	273.2	22.2
Sample 3	291	286	315	293	334	288	282	314	282	344	302.9	22.5
Sample 4	322	346	310	333	348	359	323	312	272	319	324.4	24.6
Sample 5	272	283	275	304	306	475	327	281	287	287	309.7	60.4
Sample 6	302	254	294	277	239	285	314	287	239	280	277.1	25.5
Sample 7	285	262	267	311	266	263	332	314	265	295	286.0	25.6
Sample 8	318	306	269	278	299	304	261	317	296	285	293.3	19.5

Readings **Diet** 4	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	290	257	288	262	324	311	313	263	303	307	291.8	23.9
Sample 2	295	269	205	305	284	327	274	187	289	264	269.9	43.2
Sample 3	292	256	282	299	308	317	295	260	311	236	285.6	26.8
Sample 4	270	243	291	231	269	311	281	211	287	283	267.7	30.5
Sample 5	273	263	335	305	260	218	339	291	203	325	281.2	46.9
Sample 6	211	208	252	327	253	200	253	246	239	310	249.9	41.5
Sample 7	253	278	216	281	293	306	277	306	334	310	285.4	33.1
Sample 8	235	185	330	277	209	261	246	285	272	223	252.3	41.9

Table A7-4 II. Pre- and post- diet raw surface hardness values (HV) of human enamel. (a) Pre-diet (b) Post-diet

Readings **Diet** 4R	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	304	304	237	311	290	332	296	291	328	314	300.7	26.5
Sample 2	222	327	279	283	234	213	287	268	337	280	273.0	41.0
Sample 3	332	296	291	328	314	304	304	237	311	290	300.7	26.5
Sample 4	292	253	268	349	244	294	297	303	227	282	280.9	34.8
Sample 5	237	311	290	332	296	291	328	314	304	304	300.7	26.5
Sample 6	294	297	303	227	282	292	253	268	349	244	280.9	34.8
Sample 7	227	282	268	349	244	294	297	303	292	253	280.9	34.8
Sample 8	244	294	297	303	227	282	292	253	268	349	280.9	34.8

Readings **Diet** 4R	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	286	272	302	243	264	248	271	270	283	287	272.6	18.0
Sample 2	274	238	266	238	265	238	230	222	259	261	249.1	17.8
Sample 3	302	301	306	291	295	286	274	268	261	262	284.6	17.1
Sample 4	266	261	275	241	274	251	243	270	210	285	257.6	22.0
Sample 5	328	297	290	313	301	327	309	287	281	309	304.2	16.0
Sample 6	241	241	273	261	289	240	207	268	262	273	255.5	23.5
Sample 7	232	256	261	212	256	246	264	230	223	261	244.1	18.5
Sample 8	241	223	220	239	235	247	238	255	250	240	238.8	11.0

Table A7-5. . Pre- and post- diet raw surface profile values (µm) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 1	1	2	3	4	5	Mean	SD
Sample 1	-4.7	-3.46	-3.33	-3.76		-3.81	0.62
Sample 2	1.28	1.1	0.98	0.69		1.01	0.25
Sample 3	-0.79	-0.6	-0.03	-0.2		-0.41	0.35
Sample 4	-24.74	-24.21	-24.47			-24.47	0.27
Sample 5	-4.32	-4.71	-4.5			-4.51	0.20
Sample 6	-2.26	-2.17	-2.2			-2.21	0.05
Sample 7	2.23	2.8	2.5			2.51	0.29
Sample 8	36.4	42.5	41.1			40.00	3.20

Readings Diet 1	1	2	3	4	5	Mean	SD
Sample 1	-20.70	-20.10	-20.55	-20.20	-20.83	-20.48	0.32
Sample 2	-6.79	-6.49	-6.50	-6.76	-6.19	-6.54	0.24
Sample 3	-14.03	-11.63	-14.37	-13.01	-12.58	-13.12	1.11
Sample 4	-37.05	-35.86	-36.01	-36.50	-36.65	-36.42	0.48
Sample 5	-16.48	-19.47	-16.74	-18.02	-17.59	-17.66	1.19
Sample 6	-11.98	-10.57	-11.00	-11.22	-11.44	-11.24	0.52
Sample 7	-11.03	-11.40	-11.10	-10.60	-11.15	-11.06	0.29
Sample 8	29.16	28.65	29.00	29.08	28.89	28.96	0.20

Table A7-5 II. Pre- and post- diet raw surface profile values (µm) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 1R	1	2	3	4	5	Mean	SD
Sample 1	-5.29	-5.01	-5.58	-5.56	-5.18	-5.32	0.24
Sample 2	22.87	23.23	23.26	22.93	22.38	22.94	0.36
Sample 3	-15.47	-16.34	-14.98	-15.34	-14.95	-15.42	0.56
Sample 4	22.50	22.98	22.84	21.81	22.55	22.53	0.45
Sample 5	8.67	7.75	8.75	8.92	7.77	8.37	0.56
Sample 6	14.11	13.90	14.07	12.28	13.02	13.48	0.80
Sample 7	-7.02	-7.37	-7.81	-7.63	-6.86	-7.34	0.40
Sample 8	8.02	7.69	9.10	10.14	8.27	8.65	0.98

Readings Diet 1R	1	2	3	4	5	Mean	SD
Sample 1	-14.83	-18.07	-15.63	-16.39	-15.98	-16.18	1.20
Sample 2	14.94	14.94	15.99	15.65	14.95	15.29	0.50
Sample 3	-28.69	-28.12	-28.03	-28.61	-27.93	-28.27	0.35
Sample 4	11.87	11.60	12.31	11.75	12.11	11.93	0.28
Sample 5	-1.92	-1.92	-2.00	-1.93	-1.96	-1.94	0.03
Sample 6	6.73	6.70	6.67	6.78	6.62	6.70	0.06
Sample 7	-17.27	-17.05	-16.47	-16.91	-16.94	-16.92	0.29
Sample 8	-3.98	-4.51	-3.98	-4.36	-3.97	-4.16	0.26

Table A7-6. . Pre- and post- diet raw surface profile values (µm) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 2	1	2	3	4	5	Mean	SD
Sample 1	6.09	6.60	6.99			6.56	0.45
Sample 2	43.16	45.76	42.13			43.68	1.87
Sample 3	32.08	30.29	32.70			31.69	1.25
Sample 4	0.04	0.80	-0.48			0.12	0.64
Sample 5	40.94	37.33	41.23			39.83	2.17
Sample 6	16.24	15.79	15.96			16.00	0.23
Sample 7	16.59	16.18	14.11			15.63	1.33
Sample 8	22.04	20.10	21.40			21.18	0.99

Readings Diet 2	1	2	3	4	5	Mean	SD
Sample 1	-13.98	-14.97	-14.31	-14.57	-14.29	-14.42	0.37
Sample 2	28.00	28.04	27.84	27.98	27.93	27.96	0.08
Sample 3	14.47	14.82	13.44	14.58	13.87	14.24	0.57
Sample 4	-15.99	-15.61	-15.49	-15.87	-15.56	-15.70	0.21
Sample 5	21.45	20.01	18.60	19.71	19.93	19.94	1.02
Sample 6	6.10	6.02	6.21	6.01	6.21	6.11	0.10
Sample 7	-8.26	-4.81	-7.89	-7.36	-6.65	-7.00	1.36
Sample 8	3.67	3.65	3.66	3.58	3.74	3.66	0.06

Table A7-6 II. Pre- and post- diet raw surface profile values (µm) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 2R	1	2	3	4	5	Mean	SD
Sample 1	-8.59	-9.52	-10.10	-9.11	-9.01	-9.27	0.57
Sample 2	-11.97	-12.69	-11.38	-12.06	-11.69	-11.96	0.49
Sample 3	10.58	10.17	10.56	9.98	10.33	10.32	0.25
Sample 4	-9.14	-8.70	-8.97	-9.41	-8.98	-9.04	0.26
Sample 5	13.95	13.87	13.93	13.27	13.54	13.71	0.30
Sample 6	19.22	20.24	19.02	19.84	17.96	19.25	0.87
Sample 7	0.41	0.21	-0.26	0.20	0.01	0.11	0.25
Sample 8	7.29	7.37	7.85	7.37	7.20	7.42	0.25

Readings Diet 2R	1	2	3	4	5	Mean	SD
Sample 1	-16.77	-16.65	-16.98	-16.67	-16.92	-16.79	0.15
Sample 2	-29.97	-30.05	-30.52	-30.16	-30.19	-30.17	0.21
Sample 3	-3.03	-4.54	-3.76	-3.78	-3.78	-3.77	0.53
Sample 4	-23.17	-23.27	-23.15	-23.13	-23.26	-23.20	0.06
Sample 5	-3.87	-2.55	-3.25	-3.51	-2.91	-3.21	0.51
Sample 6	-1.08	-1.64	-0.20	-0.87	-1.07	-0.97	0.52
Sample 7	-9.76	-9.03	-8.10	-8.90	-9.01	-8.96	0.59
Sample 8	-9.23	-9.06	-8.96	-9.22	-8.94	-9.09	0.14

Table A7-7. . Pre- and post- diet raw surface profile values (µm) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 3	1	2	3	4	5	Mean	SD
Sample 1	-1.68	-1.89	-1.29	-1.44	-1.37	-1.53	0.25
Sample 2	8.42	8.68	8.22	7.36	8.69	8.27	0.55
Sample 3	4.25	4.37	4.85	4.74	4.38	4.52	0.26
Sample 4	6.97	6.72	6.68	6.65	5.71	6.55	0.48
Sample 5	-2.42	-2.83	-2.40	-2.48	-2.52	-2.53	0.17
Sample 6	19.21	18.21	18.16	17.94	16.95	18.09	0.81
Sample 7	-1.09	-0.97	-1.14	-2.86	-2.49	-1.71	0.89
Sample 8	2.64	2.59	2.80	2.67	2.77	2.69	0.09

Readings Diet 3	1	2	3	4	5	Mean	SD
Sample 1	-19.33	-20.91	-22.15	-21.28	-20.56	-20.85	1.03
Sample 2	-12.71	-12.42	-12.73	-12.73	-12.21	-12.56	0.24
Sample 3	-21.52	-21.71	-20.69	-20.88	-22.01	-21.36	0.56
Sample 4	-16.30	-16.46	-13.89	-15.30	-14.76	-15.35	1.07
Sample 5	-20.78	-22.73	-23.28	-23.11	-23.11	-22.60	1.04
Sample 6	4.83	4.72	4.48	4.52	4.59	4.63	0.15
Sample 7	-20.44	-21.56	-19.96	-21.13	-21.57	-20.93	0.71
Sample 8	-22.78	-22.32	-22.54	-23.62	-22.56	-22.76	0.51

Table A7-7 II. . Pre- and post- diet raw surface profile values (µm) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 3R	1	2	3	4	5	Mean	SD
Sample 1	6.43	5.60	6.89	6.63	6.88	6.49	0.53
Sample 2	3.16	3.06	2.71	2.58	2.70	2.84	0.25
Sample 3	4.94	4.91	4.00	5.22	4.98	4.81	0.47
Sample 4	-3.87	-3.95	-3.98	-3.89	-4.29	-3.99	0.17
Sample 5	16.08	15.86	14.90	17.09	16.12	16.01	0.78
Sample 6	-2.65	-4.31	-3.96	-2.60	-2.48	-3.20	0.87
Sample 7	1.18	1.05	1.15	1.02	0.97	1.07	0.09
Sample 8	6.10	5.31	6.13	5.88	6.06	5.90	0.34

Readings Diet 3R	1	2	3	4	5	Mean	SD
Sample 1	-15.89	-16.58	-17.98	-17.03	-15.14	-16.53	1.08
Sample 2	-28.68	-29.00	-27.78	-27.64	-28.64	-28.35	0.60
Sample 3	-21.04	-21.41	-19.54	-22.35	-22.43	-21.35	1.18
Sample 4	-32.97	-32.06	-32.97	-32.31	-32.95	-32.66	0.44
Sample 5	-8.70	-8.07	-8.07	-8.33	-8.76	-8.38	0.33
Sample 6	-19.22	-20.02	-19.53	-19.60	-18.39	-19.35	0.61
Sample 7	-22.41	-22.06	-20.02	-22.21	-21.67	-21.67	0.96
Sample 8	-24.13	-25.85	-24.92	-24.20	-24.85	-24.79	0.69

Table A7-8. . Pre- and post- diet raw surface profile values (µm) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 4	1	2	3	4	5	Mean	SD
Sample 1	70.65	73.84	73.88	74.06	73.91	73.27	1.47
Sample 2	2.66	2.86	2.08	2.10	2.06	2.35	0.38
Sample 3	7.91	7.64	8.05	7.86	6.86	7.66	0.47
Sample 4	6.17	7.21	7.20	8.85	7.01	7.29	0.97
Sample 5	25.96	25.41	26.13	25.72	25.02	25.65	0.44
Sample 6	30.22	29.56	30.41	30.24	29.47	29.98	0.43
Sample 7	13.88	15.08	14.31	15.21	15.16	14.73	0.60
Sample 8	6.27	6.13	6.90	6.22	6.33	6.37	0.31

Readings Diet 4	1	2	3	4	5	Mean	SD
Sample 1	44.58	44.83	43.89	43.20	42.71	43.84	0.90
Sample 2	-38.66	-38.78	-39.49	-38.95	-39.00	-38.97	0.32
Sample 3	-21.36	-23.63	-19.40	-21.47	-21.48	-21.46	1.50
Sample 4	-24.48	-23.05	-22.88	-23.61	-23.35	-23.48	0.63
Sample 5	-9.72	-7.74	-7.32	-8.43	-8.12	-8.27	0.91
Sample 6	-9.51	-10.35	-8.19	-9.02	-9.67	-9.35	0.80
Sample 7	-9.08	-7.98	-6.58	-5.85	-7.36	-7.37	1.25
Sample 8	-27.60	-27.35	-27.20	-27.44	-27.32	-27.38	0.15

Table A7-8 II. . Pre- and post- diet raw surface profile values (µm) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 4R	1	2	3	4	5	Mean	SD
Sample 1	6.13	6.01	5.84	5.91	6.19	6.02	0.15
Sample 2	11.70	11.57	12.10	12.48	11.79	11.93	0.36
Sample 3	18.96	18.33	19.43	18.37	18.65	18.75	0.46
Sample 4	18.23	19.16	18.88	18.60	18.59	18.69	0.35
Sample 5	11.28	11.23	10.64	11.16	10.63	10.99	0.32
Sample 6	11.39	11.52	11.23	11.10	11.54	11.36	0.19
Sample 7	-25.28	-25.13	-25.31	-25.54	-25.02	-25.25	0.19
Sample 8	-3.67	-3.70	-4.26	-3.73	-3.64	-3.80	0.26

Readings Diet 4R	1	2	3	4	5	Mean	SD
Sample 1	-24.43	-23.69	-23.14	-23.70	-22.46	-23.49	0.73
Sample 2	-18.28	-18.42	-18.46	-18.34	-18.29	-18.36	0.08
Sample 3	-10.26	-10.34	-10.71	-10.82	-8.70	-10.17	0.85
Sample 4	-10.83	-11.26	-11.19	-11.51	-11.53	-11.26	0.29
Sample 5	-14.03	-14.87	-13.85	-14.58	-14.84	-14.44	0.47
Sample 6	-19.47	-17.90	-18.26	-17.99	-18.19	-18.37	0.64
Sample 7	-47.66	-47.38	-47.34	-47.24	-48.23	-47.57	0.40
Sample 8	-43.22	-43.22	-44.03	-44.58	-43.73	-43.76	0.58

Table A7-9. Reference and post-diet raw ion concentration values (mmol). (a) Reference Saliva/Beverage mixture solution (b) Post-diet calcium ion concentration values excluding reference ion content values. (c) Post-diet phosphate ion concentration values excluding reference ion content values.

(a)

Ref. Sal/Bev Mix. Diet 1	1	2	3	4	5	6	Mean	SD
Calcium	0.81	0.8	0.79	0.8	0.8	0.8	**0.80**	0.006
Phosphate	4.95	4.96	4.95	4.92	4.94	4.96	**4.95**	0.015

*Based on 1:0.528 saliva to test-beverage ratio

(b)

Diet 1	Test cycle 1	Test cycle 2	Test cycle 3
Calcium			
1	0.06	0.07	0.07
2	0.04	0.06	0.08
3	0.07	0.05	0.08
Mean	**0.06**	**0.06**	**0.08**
SD	0.02	0.01	0.01

(c)

Diet 1	Test cycle 1	Test cycle 2	Test cycle 3
Phosphate			
1	0.12	0.12	0.12
2	0.12	0.15	0.12
3	0.12	0.07	0.10
Mean	**0.12**	**0.11**	**0.11**
SD	0.00	0.04	0.01

Table A7-10. Reference and post-diet raw ion concentration values (mmol). (a) Reference Saliva/Beverage mixture solution (b) Post-diet calcium ion concentration values excluding reference ion content values. (c) Post-diet phosphate ion concentration values excluding reference ion content values.

(a)

Ref. Sal/Bev Mix. Diet 2	1	2	3	4	5	6	Mean	SD
Calcium	0.79	0.8	0.8	0.79	0.79	0.8	**0.80**	0.005
Phosphate	4.93	4.97	4.97	4.95	4.95	4.91	**4.95**	0.023

*Based on 1:0.528 saliva to beverage ratio.

(b)

Diet 2	Test cycle 1	Test cycle 2	Test cycle 3	Test cycle 4	Test cycle 5
Calcium					
1	0.10	0.05	0.06	0.07	0.05
2	0.09	0.03	0.12	0.07	0.08
3	0.09	0.08	0.10	0.08	0.11
Mean	**0.09**	**0.05**	**0.09**	**0.07**	**0.08**
SD	0.01	0.03	0.03	0.01	0.03

(c)

Diet 2	Test cycle 1	Test cycle 2	Test cycle 3	Test cycle 4	Test cycle 5
Phosphate					
1	0.13	0.16	0.06	0.08	0.07
2	0.12	0.12	0.05	0.06	0.07
3	0.13	0.10	0.09	0.06	0.08
Mean	**0.13**	**0.13**	**0.07**	**0.07**	**0.07**
SD	0.01	0.03	0.02	0.01	0.01

Table A7-11. Reference and post-diet raw ion concentration values (mmol). (a) Reference Saliva/Beverage mixture solution (b) Post-diet calcium ion concentration values excluding reference ion content values. (c) Post-diet phosphate ion concentration values excluding reference ion content values.

(a)

Ref. Sal/Bev Mix. Diet 3	1	2	3	4	5	6	Mean	SD
Calcium	0.79	0.79	0.8	0.78	0.79	0.8	**0.79**	0.008
Phosphate	4.8	4.8	4.84	4.79	4.82	4.81	**4.81**	0.018

*Based on 1:1.056 saliva to test-beverage ratio.

(b)

Diet 3	Test cycle 1	Test cycle 2	Test cycle 3
Calcium			
1	0.12	0.11	0.11
2	0.12	0.12	0.11
3	0.12	0.13	0.11
Mean	**0.12**	**0.12**	**0.11**
SD	0.00	0.01	0.00

(c)

Diet 3	Test cycle 1	Test cycle 2	Test cycle 3
Phosphate			
1	0.13	0.12	0.14
2	0.16	0.15	0.16
3	0.15	0.18	0.15
Mean	**0.15**	**0.15**	**0.15**
SD	0.02	0.03	0.01

Table A7-12. Reference and post-diet raw ion concentration values (mmol). (a) Reference Saliva/Beverage mixture solution (b) Post-diet calcium ion concentration values excluding reference ion content values. (c) Post-diet phosphate ion concentration values excluding reference ion content values.

(a)

Ref. Sal/Bev Mix. Diet 4	1	2	3	4	5	6	Mean	SD
Calcium	0.81	0.8	0.79	0.78	0.8	0.79	**0.80**	0.010
Phosphate	4.93	4.86	4.94	4.93	4.94	4.92	**4.92**	0.030

*Based on 1:1.056 saliva to test-beverage ratio.

(b)

Diet 4	Test cycle 1	Test cycle 2	Test cycle 3	Test cycle 4	Test cycle 5
Calcium					
1	0.14	0.14	0.10	0.11	0.11
2	0.14	0.14	0.10	0.12	0.11
3	0.15	0.14	0.10	0.11	0.12
Mean	**0.14**	**0.14**	**0.10**	**0.11**	**0.11**
SD	0.01	0.00	0.00	0.01	0.01

(c)

Diet 4	Test cycle 1	Test cycle 2	Test cycle 3	Test cycle 4	Test cycle 5
Phosphate					
1	0.15	0.17	0.16	0.16	0.16
2	0.13	0.14	0.18	0.14	0.17
3	0.16	0.18	0.17	0.17	0.16
Mean	**0.15**	**0.16**	**0.17**	**0.16**	**0.16**
SD	0.02	0.02	0.01	0.02	0.01

Table A7-13. Pre- and post- diet raw surface hardness values (HV) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 1R	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	118	140	132	142	123	130	116	118	132	124	127.5	9.2
Sample 2	151	137	113	117	118	122	131	138	137	113	127.7	12.9
Sample 3	133	150	130	142	146	127	125	131	141	117	134.2	10.3
Sample 4	127	132	130	134	147	102	112	113	105	146	124.8	16.1
Sample 5	137	126	118	141	104	119	110	108	153	128	124.4	15.7
Sample 6	141	133	125	138	130	133	138	119	141	140	133.8	7.4
Sample 7	136	133	128	134	146	110	137	119	132	129	130.4	10.0
Sample 8	129	100	128	140	132	126	104	121	132	139	125.1	13.4

Readings Diet 1R	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	85	78	105	104	85	105	74	81	83	88	88.8	11.6
Sample 2	93	89	82	91	94	98	99	98	89	103	93.6	6.1
Sample 3	103	103	82	90	74	82	87	79	74	85	85.9	10.4
Sample 4	79	89	94	81	85	78	99	89	84	78	85.6	7.1
Sample 5	75	93	76	85	102	85	93	81	92	77	85.9	8.9
Sample 6	75	96	95	77	84	87	82	85	79	87	84.8	7.0
Sample 7	78	82	90	88	78	80	78	85	90	98	84.7	6.8
Sample 8	81	109	102	85	87	97	99	90	87	100	93.7	9.0

Table A7-13 II. Pre- and post- diet raw surface hardness values (HV) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 1R	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	107	122	144	128	137	126	155	147	138	141	134.5	14.0
Sample 2	146	150	147	103	142	132	113	144	121	138	133.6	16.1
Sample 3	131	151	145	136	141	146	133	147	115	142	138.7	10.5
Sample 4	131	123	134	128	128	122	107	127	128	122	125.0	7.4
Sample 5	153	101	120	141	117	130	134	150	133	132	131.1	15.6
Sample 6	125	130	130	107	126	136	132	111	92	120	120.9	13.7
Sample 7	125	131	127	146	137	140	121	144	132	145	134.8	8.9
Sample 8	109	146	129	150	150	112	143	124	134	125	132.2	15.0

Readings Diet 1R	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	92	92	91	102	107	95	104	103	103	105	99.4	6.2
Sample 2	86	96	89	79	90	81	83	93	87	83	86.7	5.4
Sample 3	82	97	98	81	80	82	97	88	91	87	88.3	7.1
Sample 4	89	86	89	90	86	88	92	93	89	89	89.1	2.3
Sample 5	84	92	88	98	92	85	85	85	89	87	88.5	4.4
Sample 6	90	92	94	86	75	91	94	85	98	87	89.2	6.4
Sample 7	89	97	105	108	101	92	94	101	98	93	97.8	6.0
Sample 8	87	97	93	90	93	96	91	88	91	87	91.3	3.5

Table A7-14. Pre- and post- diet raw surface hardness values (HV) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 2	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	113	146	133	135	121	128	131	125	149	126	130.7	10.9
Sample 2	126	132	123	104	115	125	111	111	120	145	121.2	11.9
Sample 3	106	110	103	102	100	113	111	122	103	120	109.0	7.6
Sample 4	133	120	149	147	147	127	149	137	138	153	140.0	10.9
Sample 5	147	133	153	144	138	119	148	141	143	131	139.7	9.9
Sample 6	119	90	112	130	85	99	137	120	123	118	113.3	16.9
Sample 7	113	146	131	144	128	149	121	130	146	143	135.1	12.3
Sample 8	105	118	103	125	141	102	100	126	103	117	114.0	13.7

Readings Diet 2	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	78	79	85	81	71	80	70	84	83	84	79.5	5.3
Sample 2	68	67	73	78	76	72	84	80	77	79	75.4	5.4
Sample 3	68	77	73	76	82	75	75	73	71	77	74.8	3.8
Sample 4	81	82	94	94	87	77	85	88	86	87	86.1	5.3
Sample 5	91	91	75	87	91	79	83	88	92	83	86.0	5.8
Sample 6	90	80	83	84	87	86	77	85	81	84	83.6	3.7
Sample 7	78	77	79	80	75	74	79	77	73	72	76.4	2.8
Sample 8	77	75	77	85	75	81	74	81	75	76	77.6	3.6

Table A7-14 II. Pre- and post- diet raw surface hardness values (HV) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 2R	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	107	122	144	128	137	126	155	147	138	141	134.5	14.0
Sample 2	146	150	147	103	142	132	113	144	121	138	133.6	16.1
Sample 3	131	151	145	136	141	146	133	147	115	142	138.7	10.5
Sample 4	131	123	134	128	128	122	107	127	128	122	125.0	7.4
Sample 5	153	101	120	141	117	130	134	150	133	132	131.1	15.6
Sample 6	125	130	130	107	126	136	132	111	92	120	120.9	13.7
Sample 7	125	131	127	146	137	140	121	144	132	145	134.8	8.9
Sample 8	109	146	129	150	150	112	143	124	134	125	132.2	15.0

Readings Diet 2R	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	85	58	71	88	86	78	77	86	101	65	79.5	12.5
Sample 2	70	80	92	64	101	93	67	83	82	72	80.5	12.4
Sample 3	80	95	104	64	111	84	83	62	69	68	82.0	17.0
Sample 4	72	84	86	72	79	77	78	77	83	74	77.7	4.9
Sample 5	63	99	90	83	75	62	74	82	70	86	78.5	11.9
Sample 6	75	74	83	79	71	72	80	77	74	72	75.7	3.9
Sample 7	95	86	83	81	93	85	94	80	83	83	86.2	5.6
Sample 8	82	94	96	82	89	87	88	87	93	84	87.7	4.9

Table A7-15. Pre- and post- diet raw surface hardness values (HV) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 3	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	104	132	120	114	108	97	116	111	137	131	117.0	13.0
Sample 2	122	135	154	111	119	145	127	138	127	137	131.5	12.8
Sample 3	114	113	112	113	131	95	114	109	122	121	114.4	9.4
Sample 4	127	129	97	126	137	148	122	128	141	111	126.6	14.6
Sample 5	104	107	132	131	137	119	141	130	140	127	126.8	12.9
Sample 6	109	140	132	134	97	157	141	127	122	103	126.2	18.7
Sample 7	104	107	132	131	137	119	141	130	140	127	126.8	12.9
Sample 8	116	92	140	109	105	141	129	130	131	159	125.2	19.8
Mean	118	115	100	127	97	107	106	98	116	110	109.4	9.7

Readings Diet 3	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	70	75	70	75	70	82	82	65	79	70	73.8	5.8
Sample 2	70	64	86	77	84	90	80	67	96	88	80.2	10.6
Sample 3	72	56	63	47	58	90	76	66	48	57	63.6	13.3
Sample 4	58	55	63	74	73	59	67	74	55	63	64.4	7.5
Sample 5	56	57	72	73	56	63	80	67	59	57	64.0	8.6
Sample 6	55	73	62	66	79	80	75	68	70	73	70.1	7.7
Sample 7	73	70	60	82	68	87	66	60	70	63	69.9	8.9
Sample 8	69	59	50	67	75	67	62	66	77	66	65.6	7.7

Table A7-15 II. Pre- and post- diet raw surface hardness values (HV) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 3R	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	130	116	118	132	124	118	140	132	142	123	127.5	9.2
Sample 2	122	131	138	137	113	151	137	113	117	118	127.7	12.9
Sample 3	127	125	131	141	117	133	150	130	142	146	134.2	10.3
Sample 4	102	112	113	105	146	127	132	130	134	147	124.8	16.1
Sample 5	119	110	108	153	128	137	126	118	141	104	124.4	15.7
Sample 6	133	138	119	141	140	141	133	125	138	130	133.8	7.4
Sample 7	110	137	119	132	129	136	133	128	134	146	130.4	10.0
Sample 8	126	104	121	132	139	129	100	128	140	132	125.1	13.4

Readings Diet 3R	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	79	71	70	83	87	68	65	58	94	78	75.3	10.9
Sample 2	69	69	69	78	63	60	67	78	70	67	69.0	5.7
Sample 3	84	65	60	51	61	57	71	64	80	70	66.3	10.2
Sample 4	67	80	76	67	82	85	61	73	75	74	74.2	7.3
Sample 5	60	59	76	86	58	59	72	78	64	79	68.7	10.3
Sample 6	62	65	69	71	63	69	76	72	65	73	68.5	4.6
Sample 7	73	64	72	73	78	64	60	67	74	69	69.4	5.6
Sample 8	72	62	68	56	67	76	71	69	64	68	67.6	5.6

Table A7-16. Pre- and post- diet raw surface hardness values (HV) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 4	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	101	115	115	88	92	124	109	122	109	106	108.1	11.8
Sample 2	131	113	142	116	118	121	131	112	133	121	123.8	9.9
Sample 3	80	103	93	97	82	90	118	113	118	103	99.7	13.8
Sample 4	87	119	109	129	105	113	111	122	118	92	110.5	13.1
Sample 5	103	117	123	133	98	124	135	125	130	105	119.3	13.1
Sample 6	94	82	83	119	112	77	118	137	81	128	103.1	22.2
Sample 7	110	127	127	116	124	133	103	132	116	110	119.8	10.3
Sample 8	119	96	113	83	88	123	115	120	105	131	109.3	15.8

Readings Diet 4	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	69	55	58	53	53	55	56	51	59	53	56.2	5.1
Sample 2	48	50	45	47	60	54	62	45	59	60	53	6.8
Sample 3	59	53	50	59	50	48	49	52	47	51	51.8	4.2
Sample 4	60	58	56	58	57	54	57	57	56	57	57	1.6
Sample 5	55	55	56	43	63	62	44	49	76	45	54.8	10.3
Sample 6	54	59	52	65	60	64	59	73	73	64	62.3	7.0
Sample 7	47	57	54	70	62	55	50	55	71	48	56.9	8.4
Sample 8	62	68	72	55	74	64	76	56	62	64	65.3	7.1

Table A7-16 II. Pre- and post- diet raw surface hardness values (HV) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 4R	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	109	122	115	88	109	106	92	124	101	115	108.1	11.8
Sample 2	131	112	142	116	133	121	118	121	131	113	123.8	9.9
Sample 3	118	113	93	97	118	103	82	90	80	103	99.7	13.8
Sample 4	111	122	109	129	118	92	105	113	87	119	110.5	13.1
Sample 5	135	125	123	133	130	105	98	124	103	117	119.3	13.1
Sample 6	118	137	83	119	81	128	112	77	94	82	103.1	22.2
Sample 7	103	132	127	116	116	110	124	133	110	127	119.8	10.3
Sample 8	115	120	113	83	105	131	88	123	119	96	109.3	15.8

Readings Diet 4R	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	57	43	46	60	63	48	54	50	62	49	53.2	7.0
Sample 2	64	70	68	75	67	68	77	76	69	68	70.2	4.3
Sample 3	44	43	38	34	65	55	37	53	48	50	46.7	9.5
Sample 4	52	49	59	54	56	43	53	51	53	49	51.9	4.4
Sample 5	41	63	61	60	64	48	94	70	38	78	61.7	16.9
Sample 6	41	64	63	48	60	67	54	71	77	69	61.4	11.0
Sample 7	56	58	73	89	50	70	64	55	56	51	62.2	12.1
Sample 8	59	58	65	45	55	55	65	53	50	59	56.4	6.2

Table A7-17. Pre- and post- diet raw surface profile values (µm) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 1	1	2	3	Mean	SD
Sample 1	9.56	6.67	9.97	8.73	1.80
Sample 2	2.61	1.34	2.36	2.10	0.67
Sample 3	-5.39	-4.91	-7.11	-5.80	1.16
Sample 4	-10.56	-9.84	-10.00	-10.13	0.38
Sample 5	10.70	11.45	11.00	11.05	0.38
Sample 6	3.58	5.30	4.44	4.44	0.86
Sample 7	2.75	3.35	2.98	3.03	0.30
Sample 8	4.03	7.16	3.59	4.93	1.95

Readings Diet 1	1	2	3	4	5	Mean	SD
Sample 1	1.15	0.06	-0.32	0.46	0.09	0.29	0.56
Sample 2	-3.66	-3.61	-3.64	-3.67	-3.63	-3.64	0.02
Sample 3	-13.73	-15.42	-14.01	-14.45	-14.30	-14.39	0.64
Sample 4	-14.86	-13.72	-14.02	-14.33	-14.08	-14.21	0.43
Sample 5	5.47	5.71	6.61	6.23	5.64	5.93	0.47
Sample 6	-7.77	-4.19	-6.50	-5.51	-6.79	-6.15	1.36
Sample 7	2.19	2.83	2.07	2.39	2.37	2.37	0.29
Sample 8	0.50	-3.08	-2.24	-1.99	-1.19	-1.60	1.35

Table A7-17 II. Pre- and post- diet raw surface profile values (µm) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 1R	1	2	3	4	5	Mean	SD
Sample 1	27.64	25.94	28.93	26.07	26.80	27.08	1.24
Sample 2	25.68	28.19	27.43	26.66	26.65	26.92	0.94
Sample 3	6.89	6.74	5.18	6.55	5.13	6.10	0.87
Sample 4	7.20	7.52	6.78	6.41	7.59	7.10	0.50
Sample 5	-4.77	-5.19	-5.41	-6.10	-4.90	-5.27	0.53
Sample 6	-0.59	-0.87	-0.52	-0.30	-0.47	-0.55	0.21
Sample 7	-1.57	-1.73	-1.80	-2.31	-2.12	-1.91	0.30
Sample 8	20.58	19.31	22.21	18.81	20.83	20.35	1.34

Readings Diet 1R	1	2	3	4	5	Mean	SD
Sample 1	19.37	18.98	19.09	19.06	19.23	19.14	0.15
Sample 2	22.23	22.44	22.41	22.42	22.29	22.36	0.09
Sample 3	-2.26	-4.39	-3.40	-3.65	-3.05	-3.35	0.78
Sample 4	1.25	1.67	1.44	1.39	1.52	1.45	0.16
Sample 5	-9.24	-9.22	-9.25	-9.34	-9.16	-9.24	0.06
Sample 6	-7.94	-8.56	-8.67	-8.47	-8.30	-8.39	0.28
Sample 7	-7.58	-7.08	-7.38	-7.04	-7.65	-7.34	0.28
Sample 8	12.50	11.59	10.85	12.36	10.96	11.65	0.77

Table A7-18. Pre- and post- diet raw surface profile values (µm) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 2	1	2	3	4	5	Mean	SD
Sample 1	20.98	23.06	22.80	21.49	24.48	22.56	1.38
Sample 2	3.62	3.19	4.69	3.98	4.57	4.01	0.63
Sample 3	26.85	30.16	30.98	27.65	29.02	28.93	1.71
Sample 4	6.16	6.26	6.05	6.41	5.99	6.17	0.17
Sample 5	24.96	26.35	24.82	25.68	25.74	25.51	0.63
Sample 6	-6.65	-6.82	-5.62	-4.36	-6.02	-5.89	0.98
Sample 7	2.01	0.75	1.29	2.58	1.67	1.66	0.70
Sample 8	14.63	15.67	14.51	14.50	14.73	14.81	0.49

Readings Diet 2	1	2	3	4	5	Mean	SD
Sample 1	12.61	12.02	11.76	12.54	11.75	12.14	0.42
Sample 2	-11.66	-12.68	-10.90	-11.08	-12.43	-11.75	0.79
Sample 3	22.34	23.69	21.05	22.81	21.92	22.36	0.99
Sample 4	-17.28	-17.73	-15.67	-16.37	-17.44	-16.89	0.85
Sample 5	18.12	18.77	17.34	18.52	17.64	18.08	0.59
Sample 6	-21.04	-21.09	-21.12	-21.30	-20.88	-21.09	0.15
Sample 7	-13.44	-13.69	-12.57	-12.87	-13.57	-13.23	0.48
Sample 8	3.83	4.06	4.18	4.04	4.02	4.02	0.13

Table A7-18 II. Pre- and post- diet raw surface profile values (µm) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 2R	1	2	3	4	5	Mean	SD
Sample 1	3.01	2.32	2.89	1.97	1.55	2.35	0.61
Sample 2	28.51	25.28	26.61	24.50	27.72	26.52	1.66
Sample 3	4.33	4.68	4.27	4.44	4.53	4.45	0.16
Sample 4	22.54	23.37	23.43	22.67	24.02	23.20	0.61
Sample 5	-6.99	-5.77	-7.38	-7.99	-8.16	-7.26	0.95
Sample 6	-0.29	0.96	0.08	0.41	-0.81	0.07	0.67
Sample 7	12.53	12.53	12.75	12.65	13.66	12.82	0.48
Sample 8	-2.79	-2.94	-2.87	-3.17	-3.37	-3.03	0.24

Readings Diet 2R	1	2	3	4	5	Mean	SD
Sample 1	-5.14	-4.54	-5.41	-5.02	-5.29	-5.08	0.34
Sample 2	17.22	16.86	17.11	17.15	17.04	17.08	0.14
Sample 3	-9.05	-8.41	-9.99	-9.90	-10.22	-9.52	0.76
Sample 4	3.78	3.56	3.77	3.75	3.47	3.67	0.14
Sample 5	-16.20	-16.67	-16.86	-16.34	-16.10	-16.43	0.32
Sample 6	-12.95	-14.60	-14.57	-13.66	-14.78	-14.11	0.78
Sample 7	-5.99	-6.09	-3.05	-3.31	-3.55	-4.39	1.51
Sample 8	-13.99	-13.49	-13.88	-13.93	-14.17	-13.89	0.25

Table A7-19. Pre- and post- diet raw surface profile values (µm) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 3	1	2	3	Mean	SD
Sample 1	1.79	1.60	2.01	1.80	0.21
Sample 2	13.09	14.46	11.37	12.97	1.55
Sample 3	1.80	1.32	1.13	1.42	0.35
Sample 4	32.45	36.89	31.20	33.51	2.99
Sample 5	0.19	-0.80	-1.68	-0.76	0.94
Sample 6	9.19	9.01	9.10	9.10	0.09
Sample 7	-2.42	-1.33	-2.00	-1.92	0.55
Sample 8	40.93	39.73	40.00	40.22	0.63

Readings Diet 3	1	2	3	4	5	Mean	SD
Sample 1	-14.08	-14.43	-14.38	-14.25	-14.36	-14.30	0.14
Sample 2	1.47	0.21	0.25	0.98	0.30	0.65	0.56
Sample 3	-8.03	-6.91	-5.35	-7.56	-5.95	-6.76	1.11
Sample 4	13.01	11.60	11.11	11.57	12.24	11.91	0.74
Sample 5	-24.34	-17.19	-20.77	-19.99	-21.54	-20.77	2.59
Sample 6	-13.76	-12.75	-15.70	-14.45	-13.70	-14.07	1.09
Sample 7	-16.21	-20.21	-22.82	-19.91	-19.61	-19.75	2.36
Sample 8	21.14	20.28	21.00	20.34	21.27	20.81	0.46

Table A7-19 II. Pre- and post- diet raw surface profile values (µm) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 3R	1	2	3	4	5	Mean	SD
Sample 1	0.48	0.50	0.46	1.04	1.23	0.74	0.37
Sample 2	6.27	6.08	5.11	6.13	5.87	5.89	0.46
Sample 3	5.44	7.04	5.26	4.44	5.45	5.53	0.94
Sample 4	23.81	23.41	22.73	23.64	23.11	23.34	0.43
Sample 5	27.96	27.79	27.05	27.77	27.13	27.54	0.42
Sample 6	12.34	13.21	13.17	11.92	13.09	12.75	0.58
Sample 7	5.15	4.49	4.60	4.54	4.41	4.64	0.30
Sample 8	11.94	11.59	11.97	12.03	11.60	11.83	0.21

Readings Diet 3R	1	2	3	4	5	Mean	SD
Sample 1	-15.84	-16.93	-17.31	-16.25	-17.15	-16.69	0.63
Sample 2	-8.87	-8.82	-8.85	-9.07	-8.58	-8.83	0.17
Sample 3	-11.40	-13.09	-11.68	-12.50	-11.57	-12.05	0.72
Sample 4	10.68	10.75	10.45	10.66	10.62	10.63	0.11
Sample 5	12.96	13.20	13.09	13.19	12.96	13.08	0.12
Sample 6	-8.46	-7.58	-7.19	-8.13	-7.35	-7.75	0.54
Sample 7	-5.20	-5.26	-5.32	-5.38	-5.17	-5.27	0.09
Sample 8	-4.71	-5.18	-4.34	-5.16	-4.33	-4.75	0.42

Table A7-20. Pre- and post- diet raw surface profile values (µm) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 4	1	2	3	4	5	Mean	SD
Sample 1	13.99	13.55	13.90	13.54	13.93	13.78	0.19
Sample 2	15.27	15.55	15.97	15.89	15.95	15.73	0.27
Sample 3	7.68	7.97	7.91	7.78	7.61	7.79	0.14
Sample 4	14.45	13.74	13.65	13.52	14.06	13.89	0.34
Sample 5	20.53	20.81	21.13	20.48	21.62	20.92	0.42
Sample 6	20.76	20.75	20.38	21.34	21.05	20.86	0.32
Sample 7	13.09	12.55	13.22	13.16	12.56	12.91	0.30
Sample 8	13.03	13.48	13.33	13.46	13.17	13.30	0.17
Mean							
SD							

Readings Diet 4	1	2	3	4	5	Mean	SD
Sample 1	-5.90	-5.94	-5.56	-5.78	-5.30	-5.69	0.27
Sample 2	-13.39	-15.39	-11.19	-12.59	-13.39	-13.19	1.52
Sample 3	-17.77	-19.17	-20.57	-13.77	-14.77	-17.21	2.88
Sample 4	-19.60	-21.80	-17.60	-19.60	-20.20	-19.76	1.51
Sample 5	2.08	-1.33	1.88	0.88	-0.93	0.52	1.57
Sample 6	-0.76	1.54	0.22	0.78	-0.42	0.27	0.92
Sample 7	-0.74	-1.74	-1.71	-1.40	-1.34	-1.38	0.40
Sample 8	-16.39	-17.38	-16.78	-20.63	-14.36	-17.11	2.27

Table A7-20 II. Pre- and post- diet raw surface profile values (µm) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 4R	1	2	3	4	5	Mean	SD
Sample 1	8.13	6.81	7.54	7.73	4.93	7.03	1.27
Sample 2	-8.44	-7.90	-8.17	-6.77	-6.30	-7.52	0.93
Sample 3	-11.24	-11.92	-10.71	-11.78	-11.08	-11.35	0.50
Sample 4	2.77	1.71	3.07	1.93	3.60	2.62	0.79
Sample 5	4.37	4.86	5.24	2.37	5.41	4.45	1.23
Sample 6	30.18	27.60	28.03	29.58	27.84	28.64	1.16
Sample 7	-2.01	-1.31	-2.47	-1.50	-0.76	-1.61	0.66
Sample 8	12.15	11.16	12.49	11.64	12.21	11.93	0.53

Readings Diet 4R	1	2	3	4	5	Mean	SD
Sample 1	-14.29	-14.35	-16.86	-11.81	-11.80	-13.82	2.11
Sample 2	-24.42	-24.30	-24.08	-24.02	-24.49	-24.26	0.21
Sample 3	-27.07	-30.13	-27.97	-27.57	-29.59	-28.46	1.33
Sample 4	-8.18	-9.80	-9.23	-8.96	-9.26	-9.09	0.59
Sample 5	-24.48	-24.98	-21.97	-23.98	-24.03	-23.89	1.15
Sample 6	4.48	2.34	6.30	4.95	3.84	4.39	1.46
Sample 7	-19.50	-18.45	-18.66	-18.01	-19.30	-18.78	0.61
Sample 8	-13.04	-13.99	-14.01	-15.58	-10.57	-13.44	1.84

Table A7-21. . Reference and post-diet raw ion concentration values (mmol). (a) Reference Saliva/Beverage mixture solution (b) Post-diet calcium ion concentration values excluding reference ion content values. (c) Post-diet phosphate ion concentration values excluding reference ion content values.

(a)

Ref. Sal/Bev Mix. Diet 1	1	2	3	4	5	6	Mean	SD
Calcium	0.81	0.8	0.79	0.8	0.8	0.8	**0.80**	0.006
Phosphate	4.95	4.96	4.95	4.92	4.94	4.96	**4.95**	0.015

*Based on 1:0.528 saliva to test-beverage ratio

(b)

Diet 1	Test cycle 1	Test cycle 2	Test cycle 3
Calcium			
1	0.09	0.06	0.06
2	0.08	0.07	0.07
3	0.08	0.07	0.08
Mean	**0.08**	**0.07**	**0.07**
SD	0.01	0.01	0.01

(c)

Diet 1	Test cycle 1	Test cycle 2	Test cycle 3
Phosphate			
1	0.11	0.15	0.07
2	0.11	0.10	0.12
3	0.12	0.07	0.13
Mean	**0.11**	**0.11**	**0.11**
SD	0.01	0.04	0.03

Table A7-22. . Reference and post-diet raw ion concentration values (mmol). (a) Reference Saliva/Beverage mixture solution (b) Post-diet calcium ion concentration values excluding reference ion content values. (c) Post-diet phosphate ion concentration values excluding reference ion content values.

(a)

Ref. Sal/Bev Mix. Diet 2	1	2	3	4	5	6	Mean	SD
Calcium	0.79	0.8	0.8	0.79	0.79	0.8	**0.80**	0.005
Phosphate	4.93	4.97	4.97	4.95	4.95	4.91	**4.95**	0.023

*Based on 1:0.528 saliva to test-beverage ratio.

(b)

Diet 2	Test cycle 1	Test cycle 2	Test cycle 3	Test cycle 4	Test cycle 5
Calcium					
1	0.09	0.09	0.02	0.12	0.15
2	0.10	0.13	0.10	0.13	0.11
3	0.09	0.09	0.14	0.12	0.11
Mean	**0.09**	**0.10**	**0.09**	**0.12**	**0.12**
SD	0.01	0.02	0.06	0.01	0.02

(c)

Diet 2	Test cycle 1	Test cycle 2	Test cycle 3	Test cycle 4	Test cycle 5
Phosphate					
1	0.09	0.08	0.17	0.13	0.14
2	0.10	0.17	0.11	0.14	0.17
3	0.09	0.14	0.11	0.11	0.16
Mean	**0.09**	**0.13**	**0.13**	**0.13**	**0.16**
SD	0.01	0.05	0.03	0.02	0.02

Table A7-23. . Reference and post-diet raw ion concentration values (mmol). (a) Reference Saliva/Beverage mixture solution (b) Post-diet calcium ion concentration values excluding reference ion content values. (c) Post-diet phosphate ion concentration values excluding reference ion content values.

(a)

Ref. Sal/Bev Mix. Diet 3	1	2	3	4	5	6	Mean	SD
Calcium	0.79	0.79	0.8	0.78	0.79	0.8	**0.79**	0.008
Phosphate	4.8	4.8	4.84	4.79	4.82	4.81	**4.81**	0.018

*Based on 1:1.056 saliva to test-beverage ratio

(b)

Diet 3	Test cycle 1	Test cycle 2	Test cycle 3
Calcium			
1	0.14	0.13	0.15
2	0.13	0.16	0.15
3	0.16	0.14	0.16
Mean	**0.14**	**0.14**	**0.13**
SD	0.02	0.02	0.01

(c)

Diet 3	Test cycle 1	Test cycle 2	Test cycle 3
Phosphate			
1	0.14	0.22	0.12
2	0.15	0.15	0.12
3	0.14	0.18	0.21
Mean	**0.14**	**0.18**	**0.15**
SD	0.01	0.04	0.05

Table A7-24. . Reference and post-diet raw ion concentration values (mmol). (a) Reference Saliva/Beverage mixture solution (b) Post-diet calcium ion concentration values excluding reference ion content values. (c) Post-diet phosphate ion concentration values excluding reference ion content values.

(a)

Ref. Sal/Bev Mix. Diet 4	1	2	3	4	5	6	Mean	SD
Calcium	0.81	0.8	0.79	0.78	0.8	0.79	**0.80**	0.010
Phosphate	4.93	4.86	4.94	4.93	4.94	4.92	**4.92**	0.030

*Based on 1:1.056 saliva to test-beverage ratio.

(b)

Diet 4	Test cycle 1	Test cycle 2	Test cycle 3	Test cycle 4	Test cycle 5
Calcium					
1	0.17	0.17	0.18	0.21	0.18
2	0.17	0.17	0.19	0.20	0.19
3	0.17	0.15	0.20	0.22	0.19
Mean	**0.17**	**0.16**	**0.19**	**0.21**	**0.19**
SD	0.00	0.01	0.01	0.01	0.01

(c)

Diet 4	Test cycle 1	Test cycle 2	Test cycle 3	Test cycle 4	Test cycle 5
Phosphate					
1	0.20	0.17	0.21	0.18	0.20
2	0.14	0.18	0.19	0.21	0.20
3	0.18	0.17	0.17	0.18	0.18
Mean	**0.17**	**0.17**	**0.19**	**0.19**	**0.19**
SD	0.03	0.01	0.02	0.02	0.01

Table A7-25. Pre- and post- diet raw surface hardness values (HV) of human enamel. (a) Pre-diet. (b) Post-period 1. (c) Post-period 2. (d) Post period 3.

Readings Diet 5	1	2	3	4	5	6	7	8	Mean	SD
Sample 1	235	307	343	291	205	312	246	316	281.9	47.7
Sample 2	309	290	302	325	267	333	318	363	313.4	28.9
Sample 3	283	324	252	259	324	310	279	248	284.9	31.3
Sample 4	303	222	257	277	320	336	279	306	287.5	36.6
Sample 5	269	220	246	278	252	291	218	200	246.8	32.0
Sample 6	303	274	272	276	318	216	237	282	272.3	32.9
Sample 7	244	237	253	286	242	230	240	258	248.8	17.4
Sample 8	243	299	262	306	280	226	280	309	275.6	30.1

Readings Diet 5	1	2	3	4	5	6	7	8	9	Mean	SD
Sample 1	218	208	211	232	220	260	233	176	221	219.9	22.6
Sample 2	244	274	275	209	261	247	274	259	252	255.0	20.9
Sample 3	207	256	268	230	286	207	201	257	281	243.7	33.2
Sample 4	224	176	262	287	273	200	261	254	292	247.7	39.6
Sample 5	262	274	245	249	260	181	264	224	194	239.2	32.7
Sample 6	275	268	231	243	269	300	265	220	267	259.8	24.4
Sample 7	216	271	250	280	296	290	277	248	226	261.6	28.1
Sample 8	225	274	213	257	224	236	263	213	296	244.6	29.3

Readings Diet 5	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	293	255	218	268	232	230	262	252	281	207	249.8	27.7
Sample 2	226	239	240	255	262	257	269	255	286		254.3	17.8
Sample 3	243	217	243	229	296	175	250	277	244		241.6	34.4
Sample 4	195	215	242	252	255	224	239	188	224		226.0	23.7
Sample 5	243	259	291	286	279	207	231	292	252		260.0	29.6
Sample 6	296	180	219	302	252	293	258	242	317	256	261.5	42.0
Sample 7	235	253	271	258	222	229	265	277	242		250.2	19.3
Sample 8	278	250	229	221	255	243	272	243	239		247.8	18.5

Readings Diet 5	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	268	278	329	293	282	278	260	253	264	290	279.5	21.6
Sample 2	292	260	284	266	287	310	295	272	283	310	285.9	16.8
Sample 3	264	243	274	208	259	278	275	284	319	254	265.8	28.9
Sample 4	316	236	283	300	265	254	312	322	291	282	286.1	28.1
Sample 5	227	289	253	247	309	249	200	242	256	252	252.4	30.0
Sample 6	286	252	275	265	283	310	226	226	275	267	266.5	26.2
Sample 7	319	210	208	225	264	291	257	202	247	286	250.9	39.8
Sample 8	269	295	317	321	300	305	241	318	245	243	285.4	32.8

Table A7-26. Pre- and post- diet raw surface hardness values (HV) of human enamel. (a) Pre-diet. (b) Post-period 1. (c) Post-period 2. (d) Post period 3.

Readings Diet 5	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	126	79	141	153	141	132	155	117	89	127	126.0	25.2
Sample 2	135	140	141	127	139	149	147	125	141	130	137.4	8.1
Sample 3	108	138	143	147	149	134	128	144	129	124	134.4	12.6
Sample 4	122	108	141	132	116	127	145	141	134	145	131.1	12.7
Sample 5	131	121	127	129	137	118	139	116	135	107	126.0	10.3
Sample 6	145	134	140	137	150	127	162	123	117	142	137.7	13.3
Sample 7	132	110	134	131	144	112	143	127	146	149	132.8	13.6
Sample 8	113	149	145	148	149	118	143	131	134	159	138.9	14.7

Readings Diet 5	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	84	79	69	80	77	61	76	81	76		75.9	7.0
Sample 2	68	70	68	74	71	68	68	70	68	74	69.9	2.4
Sample 3	67	70	71	70	79	66	67	70	71	70	70.1	3.6
Sample 4	80	60	70	74	72	80	60	70	74	72	71.2	6.9
Sample 5	63	70	77	69	67	67	75				69.7	4.9
Sample 6	76	72	65	65	67	64	76	72	65	65	68.7	4.8
Sample 7	61	64	64	65	65	61	64	64	65	65	63.8	1.5
Sample 8	82	75	70	77	72	82	75	70	77	72	75.2	4.4

Readings Diet 5	1	2	3	4	5	6	7	8			Mean	SD
Sample 1	58	61	63	68	68	66	66	63			64.1	3.5
Sample 2	73	78	69	82	78	74	65	85			75.5	6.6
Sample 3	58	61	62	61	68	81	66	70			65.9	7.3
Sample 4	55	55	59	64	65	67	71	63			62.4	5.7
Sample 5	65	62	62	61	62	76	58	66			64.0	5.4
Sample 6	63	66	67	67	70	67	67	62			66.1	2.5
Sample 7	65	66	63	64	71	59	69	67			65.5	3.7
Sample 8	65	68	62	68	61	58	66	65			64.1	3.5

Readings Diet 5	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	84	90	92	83	100	88	76	85	96	85	87.9	6.9
Sample 2	80	86	79	83	89	92	76	77	81	82	82.5	5.1
Sample 3	92	90	94	97	99	91	87	92	90	97	92.9	3.8
Sample 4	83	81	76	85	87	71	87	74	72	79	79.5	6.0
Sample 5	78	81	88	88	80	90	86	80	84	75	83.0	4.9
Sample 6	110	85	96	97	85	82	83	80	86	97	90.1	9.5
Sample 7	85	81	68	79	68	82	68	66	76	64	73.7	7.7
Sample 8	74	82	77	75	76	72	79	72	86	77	77.0	4.4

Table A7-27. Pre- and post- diet raw surface profile values (µm) of human enamel. (a) Pre-diet (b) Post-period 1. (c) Post-period 2. (d) Post-period 3.

Readings Diet 5	1	2	3	Mean	SD
Sample 1	-0.60	-0.57	-0.63	-0.60	0.03
Sample 2	-8.51	-7.24	-7.65	-7.80	0.65
Sample 3	1.30	1.30	1.30	1.30	0.00
Sample 4	7.19	8.03	7.60	7.61	0.42
Sample 5	1.72	0.58	0.30	0.87	0.75
Sample 6	0.44	0.23	0.33	0.33	0.11
Sample 7	-5.68	-5.32	-5.50	-5.50	0.18
Sample 8	0.63	1.11	1.11	0.95	0.28

Readings Diet 5	1	2	3	Mean	SD
Sample 1	-3.33	-3.73	-3.52	-3.53	0.20
Sample 2	-15.49	-14.64	-15.15	-15.09	0.43
Sample 3	0.72	0.57	0.65	0.65	0.08
Sample 4	6.30	6.66	6.45	6.47	0.18
Sample 5	-1.71	-1.18	-1.45	-1.45	0.27
Sample 6	-0.90	-0.93	-0.85	-0.89	0.04
Sample 7	-17.76	-14.14	-14.01	-15.30	2.13
Sample 8	0.43	0.47	0.44	0.45	0.02

Readings Diet 5	1	2	3	Mean	SD
Sample 1	-9.85	-9.53	-10.22	-9.87	0.35
Sample 2	-19.30	-17.18	-20.89	-19.12	1.86
Sample 3	-1.68	-2.10	-1.90	-1.89	0.21
Sample 4	3.71	5.37	5.45	4.84	0.98
Sample 5	-7.80	-9.40	-10.21	-9.14	1.23
Sample 6	-3.64	-5.00	-4.15	-4.26	0.69
Sample 7	-18.87	-21.33	-19.50	-19.90	1.28
Sample 8	-0.87	-0.80	-0.75	-0.81	0.06

Readings Diet 5	1	2	3	Mean	SD
Sample 1	-10.98	-11.53	-11.25	-11.25	0.28
Sample 2	-23.07	-23.45	-23.22	-23.25	0.19
Sample 3	-6.44	-5.49	-6.00	-5.98	0.48
Sample 4	-1.71	-1.77	-1.74	-1.74	0.03
Sample 5	-13.44	-12.85	-13.10	-13.13	0.30
Sample 6	-8.16	-8.14	-8.18	-8.16	0.02
Sample 7	-26.28	-25.34	-26.01	-25.88	0.48
Sample 8	-7.03	-6.94	-6.99	-6.99	0.05

Table A7-28. Pre- and post- diet raw surface profile values (µm) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 5	1	2	3	Mean	SD
Sample 1	-6.87	-5.44	-5.03	-5.78	0.97
Sample 2	13.95	13.13	13.66	13.58	0.42
Sample 3	3.53	2.34	2.80	2.89	0.60
Sample 4	16.43	14.41	13.52	14.79	1.49
Sample 5	10.72	10.58	10.30	10.53	0.21
Sample 6	11.32	11.33	11.33	11.33	0.01
Sample 7	0.36	0.00	0.12	0.16	0.18
Sample 8	-5.33	-6.71	-6.26	-6.10	0.70

Readings Diet 5	1	2	3	Mean	SD
Sample 1	-9.47	-8.83	-9.09	-9.13	0.32
Sample 2	10.33	10.52	10.40	10.42	0.10
Sample 3	1.11	0.73	0.99	0.94	0.19
Sample 4	8.90	7.74	5.16	7.27	1.91
Sample 5	7.24	7.74	6.97	7.32	0.39
Sample 6	11.15	11.29	9.08	10.51	1.24
Sample 7	-5.95	-6.64	-3.34	-5.31	1.74
Sample 8	-12.64	-10.10	-14.47	-12.40	2.19

Readings Diet 5	1	2	3	Mean	SD
Sample 1	-11.07	-14.76	-12.01	-12.61	1.92
Sample 2	6.12	7.03	4.95	6.03	1.04
Sample 3	-1.45	-2.74	-1.90	-2.03	0.65
Sample 4	3.71	5.37	5.45	4.84	0.98
Sample 5	2.39	2.39	2.38	2.39	0.01
Sample 6	9.25	9.57	8.24	9.02	0.69
Sample 7	-13.33	-12.90	-14.45	-13.56	0.80
Sample 8	-17.31	-17.32	-17.32	-17.32	0.01

Readings Diet 5	1	2	3	Mean	SD
Sample 1	-16.12	-15.89	-15.99	-16.00	0.12
Sample 2	1.55	2.63	2.02	2.07	0.54
Sample 3	-4.47	-3.81	-3.39	-3.89	0.54
Sample 4	3.00	3.01	3.52	3.18	0.30
Sample 5	-0.97	-0.95	-0.78	-0.90	0.10
Sample 6	4.51	4.01	6.05	4.86	1.06
Sample 7	-12.23	-15.49	-14.97	-14.23	1.75
Sample 8	-22.68	-19.16	-16.43	-19.42	3.13

Table A7-29. Pre- and post- diet raw surface hardness values (HV) of human enamel. (a) Pre-diet (b) Post-diet

Readings **Diet** 6	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	256	266	240	292	274	317	261	260	283	250	269.9	22.5
Sample 2	287	266	262	196	204	284	231	188	314	318	255.0	47.9
Sample 3	284	259	286	353	292	352	285	228	279	309	292.7	38.2
Sample 4	262	310	256	246	263	272	217	214	198	258	249.6	32.7
Sample 5	281	287	298	329	302	254	296	310	255	245	285.7	27.1
Sample 6	224	279	218	207	275	267	214	316	215	197	241.2	39.7
Sample 7	288	235	228	239	236	242	263	228	240	237	243.6	18.4
Sample 8	268	240	228	207	287	265	234	253	251	307	254.0	29.3

Readings **Diet** 6	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	255	278	251	262	274	253	242	248	252	299	261.4	17.4
Sample 2	253	243	243	254	252	293	279	304	216	288	262.5	27.4
Sample 3	263	272	246	225	256	218	263	215	293	253	250.4	25.0
Sample 4	259	286	247	264	253	223	261	223	277	301	259.4	25.0
Sample 5	245	269	285	258	327	279	309	254	279	272	277.7	24.9
Sample 6	231	280	297	253	214	247	295	246	310	243	261.6	31.8
Sample 7	262	262	295	281	241	275	277	277	302	295	276.7	18.4
Sample 8	296	220	303	306	270	261	281	286	286	302	281.1	26.0

Table A7-30. Pre- and post- diet raw surface hardness values (HV) of human enamel. (a) Pre-diet (b) Post-diet

Readings **Diet** 7	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	327	322	338	319	345	313	242	314	310	329	315.9	28.3
Sample 2	296	267	275	243	230	252	278	241	267	301	265.0	23.5
Sample 3	262	327	306	263	340	292	324	273	300	260	294.7	29.6
Sample 4	306	274	269	257	288	239	258	295	292	259	273.7	21.1
Sample 5	327	297	314	279	313	300	325	301	276	313	304.5	17.4
Sample 6	250	309	259	352	285	365	215	238	290	369	293.2	54.7
Sample 7	248	272	274	250	274	218	259	278	278	292	264.3	21.2
Sample 8	223	232	261	297	273	291	258	211	319	243	260.8	34.7

Readings **Diet** 7	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	301	317	299	303	280	300	247	261	295	283	288.6	21.2
Sample 2	309	276	271	309	268	268	309	271	276	309	286.6	19.5
Sample 3	334	349	362	341	349	340	277	388	348	362	345.0	28.4
Sample 4	259	282	287	276	253	247	238	294	265	285	268.6	19.0
Sample 5	302	348	304	363	277	337	277	302	304	348	316.2	30.6
Sample 6	260	276	255	240	265	260	276	255	240	265	259.2	12.5
Sample 7	268	279	307	293	260	251	268	279	307	293	280.5	19.2
Sample 8	271	255	232	240	215	215	240	232	255	271	242.6	20.2

Table A7-31. . Pre- and post- diet raw surface profile values (µm) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 6	1	2	3	Mean	SD
Sample 1	3.29	5.26	4.44	4.33	0.99
Sample 2	-0.92	-1.57	-0.77	-1.09	0.43
Sample 3	-0.80	-0.83	-0.82	-0.82	0.02
Sample 4	-1.11	-0.86	-0.51	-0.83	0.30
Sample 5	6.15	6.44	6.31	6.30	0.15
Sample 6	12.79	12.31	12.55	12.55	0.24
Sample 7	-2.88	-2.89	-2.55	-2.77	0.19
Sample 8	-7.66	-8.08	-7.90	-7.88	0.21

Readings Diet 6	1	2	3	Mean	SD
Sample 1	-4.68	-5.08	-3.97	-4.58	0.56
Sample 2	-11.22	-11.24	-11.23	-11.23	0.01
Sample 3	-9.37	-8.96	-9.13	-9.15	0.21
Sample 4	-13.00	-12.66	-12.93	-12.86	0.18
Sample 5	1.44	1.01	1.21	1.22	0.22
Sample 6	2.43	2.85	2.70	2.66	0.21
Sample 7	-9.81	-9.37	-9.66	-9.61	0.22
Sample 8	-14.65	-15.82	-15.01	-15.16	0.60

Table A7-32. . Pre- and post- diet raw surface profile values (µm) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 7	1	2	3	Mean	SD
Sample 1	0.75	0.29	0.45	0.50	0.23
Sample 2	2.28	1.28	1.58	1.71	0.51
Sample 3	0.61	1.07	0.88	0.85	0.23
Sample 4	-0.31	-0.16	-0.21	-0.23	0.08
Sample 5	-4.05	-4.99	-4.49	-4.51	0.47
Sample 6	-5.34	-6.45	-5.77	-5.85	0.56
Sample 7	-0.45	-0.10	-0.25	-0.27	0.18
Sample 8	1.48	2.78	2.05	2.10	0.65

Readings Diet 7	1	2	3	Mean	SD
Sample 1	-1.83	-1.93	-1.79	-1.85	0.07
Sample 2	-0.26	-0.21	0.74	0.09	0.56
Sample 3	-0.83	-1.85	-1.01	-1.23	0.54
Sample 4	-1.59	-1.78	-1.64	-1.67	0.10
Sample 5	-5.59	-5.95	-5.77	-5.77	0.18
Sample 6	-7.41	-7.75	-7.61	-7.59	0.17
Sample 7	-2.79	-2.71	-2.55	-2.68	0.12
Sample 8	-1.60	-1.03	-0.97	-1.20	0.35

Table A7-33. Pre- and post- diet raw surface hardness values (HV) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 6	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	113	139	108	129	126	132	108	143	124	133	125.5	12.3
Sample 2	139	140	137	105	117	143	124	119	130	134	128.8	12.3
Sample 3	147	135	144	113	107	137	136	119	143	117	129.8	14.4
Sample 4	128	140	154	150	147	165	124	132	142	102	138.4	17.8
Sample 5	117	130	131	124	123	130	131	132	152	115	128.5	10.3
Sample 6	127	135	135	116	126	115	125	131	139	145	129.4	9.6
Sample 7	135	123	112	123	148	138	118	129	126	124	127.6	10.4
Sample 8	122	148	151	126	133	138	109	133	162	156	137.8	16.5

Readings Diet 6	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	81	81	80	78	79	80	80	88	85	86	81.8	3.3
Sample 2	83	84	81	89	79	90	79	83	82	80	83.0	3.8
Sample 3	85	82	82	87	83	95	94	97	107	101	91.3	8.8
Sample 4	84	92	86	85	89	79	85	77	89	89	85.5	4.7
Sample 5	90	76	88	97	94	78	77	85	84	104	87.3	9.2
Sample 6	88	89	81	79	90	85	90	79	86	85	85.2	4.3
Sample 7	88	87	87	75	74	87	78	85	81	86	82.8	5.4
Sample 8	93	95	97	83	87	97	93	85	86	99	91.5	5.8

Table A7-34. Pre- and post- diet raw surface hardness values (HV) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 7	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	141	118	131	139	145	108	123	104	129	113	125.1	14.3
Sample 2	131	134	130	100	100	108	123	150	134	121	123.1	16.2
Sample 3	99	118	107	110	135	143	125	115	120	141	121.3	14.7
Sample 4	122	154	124	138	131	149	110	140	110	135	131.3	14.9
Sample 5	133	116	134	153	140	134	149	128	111	155	135.3	14.7
Sample 6	105	128	119	148	101	140	107	158	115	122	124.3	19.1
Sample 7	128	128	120	131	106	103	140	129	85	128	119.8	16.7
Sample 8	90	112	118	141	123	109	141	134	110	109	118.7	16.3

Readings Diet 7	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	108	115	120	114	105	120	121	130	121	131	118.5	8.4
Sample 2	119	143	123	111	125	114	109	108	121	123	119.6	10.3
Sample 3	139	111	132	113	111	131	108	113	137	111	120.6	12.5
Sample 4	100	114	102	112	100	109	113	108	114	119	109.1	6.6
Sample 5	130	125	115	126	107	135	146	143	115	131	127.3	12.5
Sample 6	130	116	103	109	143	113	119	118	107	105	116.3	12.3
Sample 7	97	101	107	98	94	91	108	90	91	97	97.4	6.4
Sample 8	93	117	115	123	126	88	88	104	100	107	106.1	13.9

Table A7-35. Pre- and post- diet raw surface profile values (µm) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 6	1	2	3	Mean	SD
Sample 1	-7.93	-7.87	-7.9	-7.90	0.03
Sample 2	-3.64	-2.92	-3.11	-3.22	0.37
Sample 3	-4.73	-5.34	-5	-5.02	0.31
Sample 4	-2.63	-2.93	-2.8	-2.79	0.15
Sample 5	19.18	18.85	19.01	19.01	0.17
Sample 6	-5.97	-5.53	-5.75	-5.75	0.22
Sample 7	-4.89	-5.1	-4.99	-4.99	0.11
Sample 8	3.69	3.88	3.8	3.79	0.10

Readings Diet 6	1	2	3	Mean	SD
Sample 1	-13.88	-13.47	-11.51	-12.95	1.27
Sample 2	-8.19	-8.95	-8.54	-8.56	0.38
Sample 3	-11.01	-10.74	-11.44	-11.06	0.35
Sample 4	-5.88	-5.4	-5.66	-5.65	0.24
Sample 5	10.69	11.55	11.03	11.09	0.43
Sample 6	-13.3	-13.93	-13.6	-13.61	0.32
Sample 7	-12.26	-11.79	-12.01	-12.02	0.24
Sample 8	-0.31	0.61	0.93	0.41	0.64

Table A7-36. Pre- and post- diet raw surface profile values (µm) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 7	1	2	3	Mean	SD
Sample 1	10.41	10.37	10.38	10.39	0.02
Sample 2	9.94	10.55	10.20	10.23	0.31
Sample 3	4.66	4.57	4.60	4.61	0.05
Sample 4	5.99	6.80	6.44	6.41	0.41
Sample 5	9.06	8.76	9.00	8.94	0.16
Sample 6	4.97	4.73	4.88	4.86	0.12
Sample 7	-2.83	-2.28	-2.55	-2.55	0.28
Sample 8	-7.86	-7.59	-7.61	-7.69	0.15

Readings Diet 7	1	2	3	Mean	SD
Sample 1	9.26	9.13	8.93	9.11	0.17
Sample 2	9.70	9.44	9.61	9.58	0.13
Sample 3	4.02	3.45	3.78	3.75	0.29
Sample 4	4.98	5.78	5.29	5.35	0.40
Sample 5	6.46	6.84	7.90	7.07	0.75
Sample 6	3.60	3.24	3.23	3.36	0.21
Sample 7	-4.20	-4.07	-3.92	-4.06	0.14
Sample 8	-9.00	-9.31	-8.27	-8.86	0.53

Table A7-37. Pre- and post- diet raw surface hardness values (HV) of human enamel. (a) Pre-diet (b) Post-diet

Readings **Diet** 8	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	280	293	268	330	313	267	255	222	279	297	280.4	30.5
Sample 2	281	289	341	350	344	344	258	197	223	191	281.8	62.7
Sample 3	306	341	277	252	329	262	230	296	249	290	283.2	35.9
Sample 4	273	231	227	209	242	277	285	280	214	212	245.0	30.7
Sample 5	280	293	268	330	313	267	255	222	279	297	280.4	30.5
Sample 6	281	289	341	350	344	344	258	197	223	191	281.8	62.7
Sample 7	306	341	277	252	329	262	230	296	249	290	283.2	35.9
Sample 8	273	231	227	209	242	277	285	280	214	212	245.0	30.7

Readings **Diet** 8	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	289	228	258	233	252	263	263	243	260	214	250.3	21.5
Sample 2	244	191	267	228	224	262	254	269	227	206	237.2	26.5
Sample 3	298	213	219	248	245	261	247	245	296	257	252.9	27.7
Sample 4	249	244	229	261	275	243	255	247	282	248	253.3	15.7
Sample 5	251	188	219	232	238	272	218	213	197	247	227.5	25.6
Sample 6	233	280	266	310	254	262	279	261	260	319	272.4	25.8
Sample 7	269	303	319	269	274	267	252	266	245	215	267.9	28.8
Sample 8	216	291	275	268	246	306	251	204	268	270	259.5	31.4

Table A7-38. Pre- and post- diet raw surface profile values (µm) of human enamel. (a) Pre-diet (b) Post-diet

Readings **Diet 8**	1	2	3	Mean	SD
Sample 1	4.03	4.33	4.19	4.18	0.15
Sample 2	16.18	16.23	16.21	16.21	0.03
Sample 3	12.62	12.48	12.50	12.53	0.08
Sample 4	22.06	22.12	22.10	22.09	0.03
Sample 5	10.03	10.33	10.19	10.18	0.15
Sample 6	17.78	17.38	17.11	17.42	0.34
Sample 7	12.62	12.48	12.50	12.53	0.08
Sample 8	22.06	22.12	22.10	22.09	0.03

Readings **Diet 8**	1	2	3	Mean	SD
Sample 1	-10.40	-9.87	-10.12	-10.13	0.27
Sample 2	6.22	6.58	6.56	6.45	0.20
Sample 3	-2.64	-2.23	-2.49	-2.45	0.21
Sample 4	8.42	9.54	7.45	8.47	1.05
Sample 5	-6.55	-6.37	-6.43	-6.45	0.09
Sample 6	7.65	7.85	7.94	7.81	0.15
Sample 7	-2.66	-2.91	-2.81	-2.79	0.13
Sample 8	9.50	8.49	9.00	9.00	0.51

Table A7-39. Pre- and post- diet raw surface hardness values (HV) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 8	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	153	117	132	132	117	151	107	168	142	138	135.7	18.8
Sample 2	119	124	115	127	131	125	133	128	140	115	125.7	8.0
Sample 3	136	125	128	135	145	133	124	145	131	109	131.1	10.6
Sample 4	140	120	105	128	136	122	123	110	124	121	122.9	10.5
Sample 5	153	117	132	132	117	151	107	168	142	138	135.7	18.8
Sample 6	119	124	115	127	131	125	133	128	140	115	125.7	8.0
Sample 7	136	125	128	135	145	133	124	145	131	109	131.1	10.6
Sample 8	140	120	105	128	136	122	123	110	124	121	122.9	10.5

Readings Diet 8	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	83	96	97	87	72	95	100	95	97	97	91.9	8.7
Sample 2	85	105	74	68	73	62	87	86	81	85	80.6	12.1
Sample 3	80	78	80	96	84	99	90	109	82	115	91.3	13.0
Sample 4	77	74	73	73	73	70	76	64	70	85	73.5	5.4
Sample 5	98	86	92	91	97	83	81	64	100	79	87.1	10.9
Sample 6	79	97	84	81	82	88	76	93	85	83	84.8	6.4
Sample 7	81	88	73	82	71	95	90	90	93	85	84.8	8.1
Sample 8	78	70	74	71	71	79	71	85	72	80	75.1	5.1

Table A7-40. Pre- and post- diet raw surface profile values (µm) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet 8	1	2	3	Mean	SD
Sample 1	-1.27	-1.86	-1.55	-1.56	0.30
Sample 2	8.94	8.10	8.44	8.49	0.42
Sample 3	1.20	1.07	1.13	1.13	0.07
Sample 4	6.89	5.88	6.32	6.36	0.51
Sample 5	-1.72	-1.68	-1.55	-1.65	0.09
Sample 6	8.49	8.01	8.44	8.31	0.26
Sample 7	1.02	1.70	1.31	1.34	0.34
Sample 8	3.89	3.23	3.88	3.67	0.38

Readings Diet 8	1	2	3	Mean	SD
Sample 1	-12.05	-13.27	-12.98	-12.77	0.64
Sample 2	-5.15	-5.39	-5.39	-5.31	0.14
Sample 3	-10.06	-10.10	-10.09	-10.08	0.02
Sample 4	-7.63	-7.84	-7.70	-7.72	0.11
Sample 5	-16.54	-15.00	-16.81	-16.12	0.98
Sample 6	-4.53	-6.94	-5.51	-5.66	1.21
Sample 7	-11.07	-11.06	-11.10	-11.08	0.02
Sample 8	-9.85	-10.60	-10.08	-10.18	0.38

Table A7-41. Pre- and post- diet raw surface hardness values (HV) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet Ø	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	212	301	257	262	271	257	247	289	264	299	265.9	26.5
Sample 2	283	217	212	211	247	253	252	288	223	205	239.1	30.1
Sample 3	296	286	251	318	340	324	302	296	285	303	300.1	24.4
Sample 4	264	225	234	257	277	218	233	210	252	206	237.6	24.0
Sample 5	363	257	300	299	332	353	288	290	294	281	305.7	33.3
Sample 6	282	256	277	250	291	271	328	230	308	317	281.0	31.0
Sample 7	207	258	278	223	212	269	265	232	219	213	237.6	27.0
Sample 8	212	301	257	262	271	257	247	289	264	299	265.9	26.5

Readings Diet Ø	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	183	211	203	179	192	172	172	187	198	203	190	13.6
Sample 2	221	184	218	189	206	229	175	206	187	208	202.3	17.8
Sample 3	182	171	204	171	186	177	184	188	184	179	182.6	9.5
Sample 4	169	182	199	208	160	176	191	149	166	169	176.9	18.2
Sample 5	200	188	185	234	197	145	223	260	172	185	198.9	32.8
Sample 6	172	183	216	191	169	193	174	160	221	172	185.1	20.3
Sample 7	206	190	189	168	204	194	195	183	186	206	192.1	11.8
Sample 8	170	152	160	183	151	169	211	197	206	236	183.5	28.3

Table A7-42. Pre- and post- diet raw surface hardness values (HV) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet Ø	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	140	132	118	132	142	123	124	118	130	116	127.5	9.2
Sample 2	137	113	138	137	117	118	113	151	122	131	127.7	12.9
Sample 3	150	130	131	141	142	146	117	133	127	125	134.2	10.3
Sample 4	132	130	113	105	134	147	146	127	102	112	124.8	16.1
Sample 5	126	118	108	153	141	104	128	137	119	110	124.4	15.7
Sample 6	133	125	119	141	138	130	140	141	133	138	133.8	7.4
Sample 7	133	128	119	132	134	146	129	136	110	137	130.4	10.0
Sample 8	100	128	121	132	140	132	139	129	126	104	125.1	13.4

Readings Diet Ø	1	2	3	4	5	6	7	8	9	10	Mean	SD
Sample 1	65	55	63	56	50	60	64	67	54	75	60.9	7.4
Sample 2	57	68	47	43	69	58	67	47	45	72	57.3	11.2
Sample 3	49	56	51	46	56	45	43	52	49	43	49.0	4.7
Sample 4	47	51	34	47	47	52	37	62	55	51	48.3	8.2
Sample 5	49	55	60	53	52	58	46	48	54	51	53.0	4.3
Sample 6	44	54	61	53	52	56	58	64	48	60	55.0	6.1
Sample 7	42	53	45	40	52	41	41	51	44	37	44.6	5.6
Sample 8	53	44	62	51	68	45	60	62	79	61	58.5	10.7

Table A7-43. . Pre- and post- diet raw surface profile values (µm) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet Ø	1	2	3	4	5	Mean	SD
Sample 1	-7.8	-7.7	-7.27	-8.23	-8.82	-7.96	0.59
Sample 2	-10.85	-10.46	-10.75	-11.49	-10.14	-10.74	0.50
Sample 3	11.88	12.24	12.49	12.07	12.47	12.23	0.26
Sample 4	-8.11	-7.67	-7.84	-7.38	-7.66	-7.73	0.27
Sample 5	-4.14	-3.75	-3.87	-3.58	-4.16	-3.90	0.25
Sample 6	25.23	24.66	25.16	25.54	25.57	25.23	0.37
Sample 7	-14.23	-13.82	-14.36	-15.26	-13.86	-14.31	0.58
Sample 8	24.07	24.83	24.78	25.28	25.13	24.82	0.47

Readings Diet Ø	1	2	3	4	5	Mean	SD
Sample 1	-68.86	-67.39	-66.28	-67.40	-64.93	-66.98	1.46
Sample 2	-71.16	-71.44	-71.52	-71.27	-71.17	-71.31	0.16
Sample 3	-45.78	-45.95	-46.68	-46.90	-42.67	-45.60	1.70
Sample 4	-66.78	-67.64	-67.50	-68.14	-68.18	-67.65	0.57
Sample 5	-53.94	-55.61	-53.58	-55.04	-55.55	-54.74	0.93
Sample 6	-36.428	-33.278	-34.008	-33.468	-33.858	-34.21	1.28
Sample 7	-79.126	-78.556	-78.476	-78.276	-80.266	-78.94	0.81
Sample 8	-34.022	-34.032	-35.642	-36.752	-35.042	-35.10	1.15

Table A7-44. Pre- and post- diet raw surface profile values (µm) of human enamel. (a) Pre-diet (b) Post-diet

Readings Diet Ø	1	2	3	4	5	Mean	SD
Sample 1	10.78	9.6	10.53	9.58	10.61	10.22	0.58
Sample 2	14.25	15.01	16.13	15.92	16.09	15.48	0.82
Sample 3	-6.28	-5.48	-5.65	-6.01	-6.46	-5.98	0.41
Sample 4	12.04	10.11	9.86	9.52	10.97	10.50	1.01
Sample 5	28.46	29.22	30.08	28.33	31.41	29.50	1.28
Sample 6	29.07	29.06	28.06	30.65	29.87	29.34	0.97
Sample 7	8.34	6.88	8.69	8.54	6.93	7.88	0.90
Sample 8	8.2	9.41	9.01	9.34	8.58	8.91	0.51

Readings Diet Ø	1	2	3	4	5	Mean	SD
Sample 1	-21.54	-24.06	-23.55	-23.33	-23.53	-23.20	0.97
Sample 2	-34.46	-35.30	-35.70	-40.17	-30.13	-35.15	3.58
Sample 3	-36.39	-42.73	-39.21	-39.96	-42.14	-40.08	2.53
Sample 4	-25.27	-21.72	-23.60	-23.18	-24.88	-23.73	1.42
Sample 5	-3.28	-3.12	-3.39	-3.26	-3.18	-3.25	0.10
Sample 6	-20.06	-23.26	-15.34	-17.43	-20.26	-19.27	3.01
Sample 7	-34.15	-35.69	-39.78	-28.60	-30.00	-33.65	4.49
Sample 8	-37.25	-39.62	-33.30	-37.30	-38.26	-37.15	2.36

Table A7-45. Reference and post-diet raw ion concentration values (mmol). (a) Reference Saliva/Beverage mixture solution (b) Post-diet calcium ion concentration values excluding reference ion content values. (c) Post-diet phosphate ion concentration values excluding reference ion content values.

(a)

Ref. Sal/Bev Mix. Diet Ø	1	2	3	4	5	6	Mean	SD
Calcium	0.07	0.06	0.04	0.04	0.08	0.05	**0.06**	0.016
Phosphate	4.81	4.81	4.82	4.81	4.82	4.8	**4.81**	0.008

*Based on 1:0.528 saliva to test-beverage ratio.

(b)

Diet Ø	Test cycle 1	Test cycle 2	Test cycle 3
Calcium			
1	0.31	0.30	0.30
2	0.30	0.31	0.29
3	0.30	0.30	0.30
Mean	**0.30**	**0.30**	**0.30**
SD	0.01	0.01	0.01

(c)

Diet Ø	Test cycle 1	Test cycle 2	Test cycle 3
Phosphate			
1	0.35	0.35	0.36
2	0.35	0.35	0.30
3	0.34	0.33	0.35
Mean	**0.35**	**0.34**	**0.34**
SD	0.01	0.01	0.03

Table A7-46. Reference and post-diet raw ion concentration values (mmol). (a) Reference Saliva/Beverage mixture solution (b) Post-diet calcium ion concentration values excluding reference ion content values. (c) Post-diet phosphate ion concentration values excluding reference ion content values.

(a)

Ref. Sal/Bev Mix. Diet Ø	1	2	3	4	5	6	Mean	SD
Calcium	0.07	0.06	0.04	0.04	0.08	0.05	**0.06**	0.016
Phosphate	4.81	4.81	4.82	4.81	4.82	4.8	**4.81**	0.008

*Based on 1:0.528 saliva to test-beverage ratio

(b)

Diet Ø	Test cycle 1	Test cycle 2	Test cycle 3
Calcium			
1	0.34	0.34	0.33
2	0.33	0.33	0.33
3	0.33	0.33	0.33
Mean	**0.33**	**0.33**	**0.33**
SD	0.01	0.01	0.00

(c)

Diet Ø	Test cycle 1	Test cycle 2	Test cycle 3
Phosphate			
1	0.22	0.21	0.22
2	0.29	0.22	0.23
3	0.21	0.28	0.20
Mean	**0.24**	**0.24**	**0.22**
SD	0.04	0.04	0.02

8.8 Appendix 8 – Published paper

Journal of Dentistry 43 (2015) 1352–1357

Contents lists available at ScienceDirect

Journal of Dentistry

journal homepage: www.intl.elsevierhealth.com/journals/jden

Informing a realistic laboratory erosion-testing regime - observations.

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ARTICLE INFO

Article history:
Received 12 May 2015
Received in revised form 18 August 2015
Accepted 18 August 2015

Keywords:
Erosion
Observation
Behaviour
Carbonated beverages

ABSTRACT

Objectives: To measure aspects of fizzy drink consumption in a social environment to inform the development of a laboratory testing regime.
Methods: This was an observational study in which participants were invited to attend one of four pizza and soft drink parties. All such foods and drinks were served in an air conditioned room at a temperature of 24 °C. All drinks were at a temperature of 4 °C and each participant was asked to spit out, into graduated cups, their first and second sips. Both the temperature and volume of these were measured. Upon completion of the party the volume of drinks consumed was determined. Video footage of the experiment was recorded for subsequent analysis to determine sip count and the elapsed time period between first and last sip. These values were compared to the analogous measured value of expectorated volume in order to assess the usefulness of video observation in the context of this work.
Results: The mean expectorated beverage temperature was 14.9 ± 2.0 °C. The mean time spent drinking was 44.2 ± 17.4 minutes with a mean consumption rate of 13.3 ± 6.0 ml/min. Only the sip volume and sip count per can values were significantly different between sexes ($P < 0.05$) with females displaying lower values for volume and a higher sip count. There was close agreement between the sip volume values observed and calculated using video observation derived parameters.
Conclusions: Several human drinking behaviour values were reported in this study and these will be of value in the development of more realistic laboratory erosion-testing regimes. It is concluded, within the limitations of this work, that (1) there are differences in the drinking behaviour of males and females with respect to sip volume and count, (2) the intraoral rise in temperature of a 4 °C beverage is lower than that used in previous laboratory simulations and (3) the values derived from video observation agree with those measured directly validating this technique for use in further studies.
Clinical significance: The work provides valuable quantitative data on which to base simulated laboratory erosion work. Perhaps the most significant finding is that sipped beverages attain a temperature of only 14.9 °C.

1. Introduction

Many papers are reported in the dental literature that seek to evaluate the erosiveness of foods and drinks by laboratory assessment as summarised well by Barbour and Lussi [1]. Commonly these use a range of accepted laboratory techniques to reach their conclusions. Often, in order to obtain measurable effects, the regimes adopted to expose the tooth tissues to the food/beverage under investigation are severe representing many cumulative exposures [2]. They thus do not represent what could be considered as normal eating/drinking behaviour but atypical behaviours of greater erosive risk. If more realistic testing regimes are to be developed it is important that an assessment of normal eating and drinking behaviour is made. Surprisingly the literature contains few reports of this and where it does it is concerned with the consumption of hard rather than soft drinks [3,4]. This work therefore sought to determine this, by means of an observational study conducted upon human volunteers, as a first stage in the development of an artificial mouth for erosion testing. From the literature factors thought to be of significance in the development of such a system, and measured in this work, are

- The temperature at which in vivo erosion occurs for this can significantly affect dental erosion. In laboratory studies of dental erosion Shellis et al. [5] suggested adopting body temperature (37 °C), oral cavity temperature (36 °C) or room temperature (25 °C). Similarly other attempts to design and run

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http://dx.doi.org/10.1016/j.jdent.2015.08.008
0300-5712/

demineralization/remineralization cycle regimes have kept the process running under a fixed temperature of 37 °C [6–8].
- The sip volume of the beverage [9–19].
- Beverage swallowing [14,20] and consumption rates [21–23].
- Exposure time of the tooth substance to the beverage [22–24].

A recent systematic review on the prevalence of tooth wear in children and adolescents [25] highlighted that gender could influence the degree of wear. The null hypothesis of the present work was that there would be no differences between the drinking behaviour of subjects singly and according to gender.

2. Materials and methods

Prior to the commencement of the study a copy of the experimental protocol was sent to the East of Scotland Research Ethics Service (EoSRES) to determine the need or otherwise for ethical approval. The reply received stated that no ethical approval was required for this work.

Potential participants were sought from all University of Dundee students by the weekly email they receive advertising events in that institution. This contained a link to the project that gave information on what it entailed. The purpose stated was to gather data to develop an artificial mouth. Students enrolled on dental courses were excluded from the study as it was felt by the researchers their knowledge of erosion may affect their dietary behaviour.

Those who consented to participate in the study were asked to complete a pre-visit questionnaire that assessed their beliefs concerning their personal fizzy drink consumption and preferences (beverage choice, method of drinking, serving temperature, quantity and rate of drinking). It also served as a method of checking for any food or drink allergies. This had previously been piloted upon a convenience sample of potential respondents. Upon receipt of the completed questionnaire an invitation was issued to attend one of a series of four "pizza and soft drink parties". This title was chosen in an endeavour to foster a relaxed atmosphere in which to observe normal behaviour.

2.1. Pizza and soft drink party

On each occasion this was held in the same air conditioned room at a temperature of 24 °C. Each participant was allocated to one of four observers and was issued with two graduated 60 ml measuring cups (Nutriculture, Skelmersdale, UK). Prior to the serving of food they were invited to select a drink from those on display. The choice of beverages available represented the previously declared preferences of those attending. All drinks were at a temperature of 4 °C having been refrigerated for at least 24 h before the commencement of the experiment. All participants were asked to spit out, into the graduated cups, their first and second sips. The observers immediately measured the temperature of these, using a digital thermometer (Basetech BT-80, Conrad, Colchester, UK) allowing a period of 60 s for equilibrium to be reached before the reading was taken. A note was also made of the volumes of each sip.

Thereafter a standard selection of pizzas were served and supplies of drinks at 4 °C were made continuously available. Table 1 gives details of the available pizzas and beverages. Throughout the experiment a music video was played to foster a casual atmosphere and encourage social interaction amongst the participants. No time limit for the activity was imposed.

Upon completion of the party analysis of the retained opened beverage containers of the participants permitted the researchers to calculate the volume of drinks apparently consumed and adjust this downwards to the actual volume consumed by measuring any residual drink by means of a 250 ml measuring cylinder (MBL Volumetrics, SciLabware, Stoke-on-Trent, UK). The total consumed volume per subject (V_T) was calculated using the formula:

$$V_T = (C\ V_c) - (V_R + V_E)$$

V_T = Total consumed volume per subject; C = Number of containers opened for the subject; V_c = Container volume; V_R = Residual volume; V_E = Total expectorated volume

Throughout the experiment two video cams with fish-eye lenses (3 in 1 lens, Olloclip, Huntington Beach, USA) mounted on opposite corners of the room, recorded video footage of the

Table 1
The pizza and beverages served in this work.

Pizza type	
Ristorante mozzarella	Dr. Oetker Ristorante, Bielefeld, Germany
Ristorante pollo	Dr. Oetker Ristorante, Bielefeld, Germany
Ristorante funghi	Dr. Oetker Ristorante, Bielefeld, Germany
Ristorante vegetale	Dr. Oetker Ristorante, Bielefeld, Germany
Ristorante spinace	Dr. Oetker Ristorante, Bielefeld, Germany
Beverage type	
Coca-cola	Coca-Cola Great Britain, London, UK
Coca-cola diet	Coca-Cola Great Britain, London, UK
Coca-cola zero	Coca-Cola Great Britain, London, UK
Sprite	Coca-Cola Great Britain, London, UK
Sprite zero	Coca-Cola Great Britain, London, UK
Fanta	Coca-Cola Great Britain, London, UK
Fanta zero	Coca-Cola Great Britain, London, UK
Schweppes	Coca-Cola Great Britain, London, UK
Dr. Pepper	Coca-Cola Great Britain, London, UK
Dr. Pepper zero	Coca-Cola Great Britain, London, UK
Pepsi	Britvic Soft Drinks Limited, London, UK
Pepsi diet	Britvic Soft Drinks Limited, London, UK
Pepsi Max	Britvic Soft Drinks Limited, London, UK
7Up	Britvic Soft Drinks Limited, London, UK
Mountain dew	Britvic Soft Drinks Limited, London, UK
Irn Bru	A.G.Barr Public Limited Company, Cumbernauld, Scotland, UK
Irn Bru sugar free	A.G.Barr Public Limited Company, Cumbernauld, Scotland, UK
Grapetiser	Coca-Cola Enterprises Limited, Uxbridge, UK
Appletiser	Coca-Cola Enterprises Limited, Uxbridge, UK

experiment at two different angles. This was subsequently analysed to yield for each participant sip count and the elapsed time period between first and last sip. Based on these observations for each subject a calculated sip volume (V_{cal}) and consumption rate (R) were calculated:

$$V_{cal} = \frac{V_T}{S}$$

$$R = \frac{V_t}{t}$$

V_{cal} = Calculated sip volume per subject; S = Sip count; R = Consumption rate; t = Time period from first sip until last sip

The values derived in this way were compared to the analogous measured value of expectorated volume in order to assess the usefulness of video observation in the context of this work.

Analysis of variance of all values obtained in this study was undertaken, with *post hoc* students t comparison to identify significant differences between the sexes, using commercial statistical software (Prism, Version 6, GraphPad Software Incorporation., San Diego California, USA).

3. Results

A total of 303 students responded to the recruitment advert and of these 132 (43.6 %) were male and 171 (56.4%) female. On receipt of the timetable of experimental sessions eighty one potential participants, 48 (59.3%) males and 33 (40.7%) females aged 17–31 were able to attend the experiment and did so. The individual sessions were attended by 20, 21, 19 and 21 participants. The ratio of the invited to participating volunteers was 0.27.

3.1. Directly measured values

Table 2 gives the mean expectorated volumes for each of the two expectorated sips for the participants collectively and according to sex. The standard deviations of these observations are also given. Although in all cases the first expectorated sip volume was lower than the second expectorated one this difference was of no statistical significance ($P > 0.05$). The expectorated sip volume mean value for females (14.8 ± 6.9 ml) was considerably less than that for males (19.1 ± 8.2 ml) and this was statistically significantly ($P < 0.05$) different.

Table 2 also gives for each expectorated sip the beverage temperatures for the participants collectively and according to sex. There was no difference between the overall expectorated beverage temperature mean values for the first two sips ($P > 0.05$). According to gender however, the expectorated temperatures were higher for the sips of females compared to those of the males (15.3 ± 1.9 °C and 15.0 ± 1.9 °C versus 14.8 ± 2.1 °C and 14.6 ± 2.3 °C respectively) though this was of no statistical significance ($P > 0.05$). This table also contains the mean period of time over which the beverages were consumed for all participants collectively and according to sex. The maximum and minimum values of this quantity are also given. The subjects consumed their beverages over considerably different time periods ranging from 10.6 to 95.4 min with a mean of 44.2 ± 17.4 min. Comparable time period mean values were noticed for both sexes with no statistical difference ($P > 0.05$) between them. Observation of the video footage showed sip (single intake) duration to range from a fraction of a second to a maximum of 6.5 s.

3.2. Calculated values

Table 3 contains the standard deviations of all observations within it. It gives the mean volume of beverage consumption for the participants collectively and according to sex. The maximum and minimum values are also given. The mean consumption per person was 654.9 ± 348.8 ml. No statistically significant ($P > 0.05$) gender differences were found despite the lower consumption mean value for females in respect of this quantity. This table also contains the mean period of time over which the beverages were consumed for all participants collectively and according to sex. The maximum and minimum values of this quantity are also given. The subjects consumed their beverages over considerably different time periods ranging from 10.6 to 95.4 min with a mean of 44.2 ± 17.4 min. Comparable time period mean values were noticed for both sexes with no statistical difference ($P > 0.05$) between them. Observation of the video footage showed sip (single intake) duration to range from a fraction of a second to a maximum of 6.5 s.

Table 3 also gives the mean time and mean sip count for the participants to consume a 330 ml can collectively and according to sex. Although it is clear that females spent more time drinking a can than males this is of no statistical significance ($P > 0.05$). Females however took more sips per can compared to males (23.3 ± 12.1 sips and 18.4 ± 8.0 sips respectively); and this difference was statistically significant ($P < 0.05$). The mean sip volume and mean consumption rates as calculated using the parameters observed (sip count (S) and the time period from first to last sip (t)) in the video footage are also in Table 3. Both calculated sip volumes and consumption rates were lower for females compared to the values for males. Statistical analysis however showed that only sip volume was significantly different among the sexes ($P < 0.05$).

The measured overall expectorated volume (17.2 ± 7.9 ml) did not differ significantly ($P > 0.05$) from the calculated sip volume value (16.8 ± 5.9 ml). This too was the case when comparing the measured expectorated volume and calculated sip volume values for males (19.1 ± 8.2 ml versus 18.0 ± 5.9 ml) and females (14.8 ± 6.9 ml versus 15.1 ± 5.5 ml). This gives validity to the method of video observation used in this study.

4. Discussion

In the present study, food was served to relax people and stimulate the desire to drink in a comfortable atmosphere; perhaps mimicking a group lunch break or a social gathering atmosphere. Generally, fast food triggers thirstiness more than other food types

Table 2
Summary of the first, second and overall expectorated beverage volumes and temperatures for the participants collectively and according to gender (direct measurement).

	Male ($n = 48$)			Female ($n = 33$)			Overall ($n = 81$)		
	1st sip	2nd sip	Mean	1st sip	2nd sip	Mean	1st sip	2nd sip	Mean
Sip volume (ml)	18.3	19.9	19.1[a]	14.0	15.6	14.8[a]	16.4	18.0	17.2
SD	8.6	8.6	8.2	6.2	8.6	6.9	7.9	8.8	7.9
Sip temp. (°C)	14.8	14.6	14.7	15.3	15.0	15.1	15.0	14.8	14.9
SD	2.1	2.3	2.1	1.9	1.9	1.8	2.0	2.1	2.0

Values marked with same superscript letters within the table are significantly different at $p < 0.05$.

Table 3
Summary of human drinking behaviour mean values for the participants collectively and according to gender.

	Male ($n=48$)	Female ($n=33$)	Overall ($n=81$)
Consumed volume per subject (ml)	719.9	562.3	654.9
SD, max, min	393.8	249.9	348.8
	1625	1200	1625
	162	181	162
Time period of consumption (min)	43.1	45.8	44.2
SD, max, min	14.7	17.7	17.4
	95.4	85.7	95.4
	13.5	10.6	10.6
Time period per can (min)	21.4	24.4	22.6
SD	11.4	10.9	11.2
Sip count per can	18.4[a]	23.3[a]	20.4
SD	8.0	12.1	10.1
Sip volume "*calculated*" (ml)	18.0[b]	15.1[b]	16.8
SD, max, min	5.9	5.5	5.9
	30.0	28.5	30.0
	7.4	7.2	7.2
Consumption rate (ml/min)	14.4	11.8	13.3
SD, max, min	6.4	5.0	6.0
	30.2	31.2	31.2
	4.4	4.9	4.4

Values marked with same superscript letters within the table are significantly different at $p<0.05$.

and is preferred more by the targeted age group of the subjects of this study [26]. It was therefore why pizza was selected as the food to be served as well as a reflection of its ease of preparation. In order to conduct this work 108 pizzas were prepared and 115 cans of drinks were opened. In order to fully observe the subjects it was important therefore that food and beverages were easily prepared so the researchers were not distracted by preparation duties. Although as indicated in the materials and methods section the precise nature of parameters to be observed was unknown to the participants it is possible that their preconceived ideas concerning the exercise may have influenced behaviour.

Although soft drink type has a potential impact on drinking behaviour; researchers have rarely offered subjects a selection of soft drinks to choose from [27]. In this work however the subjects were able to select their preferred drink as the researchers wished to observe as normal a behaviour as possible. Drinking an unpreferred drink could result in atypical consumption values. Throughout the current experiment participants were served with their drink of choice with no imposed time or quantity limit.

In the present work participant observation data was obtained by monitoring subjects as they drank. Drinking might be held under either experimental or natural settings. Limited research has been undertaken exploring patterns of consumption in social environments; with their focus being on hard rather than soft drinks [3,4]. In the present study, normal drinking behaviour, we believe, was successfully simulated in a social atmosphere. To our knowledge, this is the first study to report drinking behaviour values for carbonated beverages in such an environment.

It is generally accepted that temperature can significantly affect dental erosion. In the present study the overall expectorated sip temperature was found to be 14.9 ± 2.0 °C. In light of previous recommendations concerning the temperature at which to conduct *in vitro* erosion studies (body temperature 37 °C/ oral cavity temperature 36 °C/ room temperature 25 °C) [5] this was surprising but such recommendations could of course be accounted for by the desire to accelerate the erosive process in the laboratory. In our view it is reasonable to suggest that a more physiological temperature at which to conduct such studies is around 14.9 °C based upon our observation that a carbonated beverage stored at 4 °C is found to have reached this temperature upon expectoration having been in the mouth only for a few seconds. On an anatomical basis the oral cavity, unlike the nasal cavity with its turbinate anatomical structure, is not designed to heat [28].

4.1. Sip volume

Several researchers report liquid sip volume values that can be of use as comparators to the results reported here. It is however important to note that some use liquids other than carbonated beverages. Some have shown that there is no significant difference in sip volumes between water and carbonated beverages [12], a more recent study showed that water sip size differs from carbonated beverages sip size [16]; this difference might be attributed to differences in both flavour and density of the imbibed liquid which appears to influence sip-sizing behaviours [12,16]. In the present study, mean calculated sip volume of carbonated beverages in a social environment was found to be 16.8 ± 5.9 ml overall among genders. In addition, the mean expectorated sip volume (non-social) was 17.2 ± 7.9 ml. Both values are in accord with a rather wide range of liquid sip volume values reported in the literature, ranging from 12 to 37.5 ml [9–15,17–19]. The close agreement between the measured and calculated values, that utilised video observation in their derivation, gives pedigree to the method of observation used in this study. Such technique could therefore be of value in other studies.

Several studies have demonstrated that the sip volume for males is significantly larger compared with females [12,15]. This difference is in agreement with the results obtained from the current study, where both calculated sip volume and expectorated sip volume values for males (18 ± 5.9 ml and 19.1 ± 8.2 ml respectively) were significantly larger than the value for females (15.1 ± 5.5 ml and 14.8 ± 6.9 respectively) ($P<0.05$). This difference is attributed, we believe, to anatomical differences among the sexes. In view of the observed differences between the genders in the work reported in this paper the null hypothesis that there

would be no differences between the drinking behaviour of subjects singly and according to gender is rejected.

Unlike the present study, the aforementioned attempts to report sip volumes were conducted during non-social atmospheres in which subjects had to follow certain instructions. Some workers [29] have reported sip volume in a more natural water drinking setting, in which subjects did not realise they were monitored while sipping, and compared it with values obtained under instructed conditions. Their reported sip volume mean value for the none instructed natural drinking task was 16 ml which is in agreement with the results reported in the present study (16.8 ± 5.9 ml) [29]. Thus, the experimental setting adopted in our study does not appear to have generated artificial behaviour.

4.2. Consumption rate

Most previous attempts to measure consumption rates (i.e.: water drinking) have been aimed at determining swallowing velocities (known also as swallowing capacity or swallowing rate) rather than consumption rate *per se*. Swallowing velocity has been reported to be greater in males compared to females [14,20].

Attempts to assess consumption rates by other workers have focused on hard rather than soft drinks [21–23]. In such work the consumption rate calculations depend on two factors, namely, quantity and time. In the present study, the mean consumption per person was found to be 719.9 ± 393.8 ml for males and 562.3 ± 249.9 ml for females. This difference in consumption between the genders is consistent with that reported in studies investigating the consumption of alcoholic drinks [22,23].

Generally, in the current study, the overall mean consumption per person was 654.9 ± 348.8 ml. However, it has been demonstrated that the mean consumption of beer and mixed hard drinks within 30 min of non-social laboratory drinking was 543 ± 240 ml and 519 ± 268 ml respectively [27]. Although these values look different at first, understanding the effects of time-limit and a non-social atmosphere on subject performance, presumably, render them comparable. There might be a possibility that the subjects did not intend to drink that much at the outset but greater fizzy drink consumption might actually be due to drinkers feeling tempted to drink more just because it was free.

In contrast to an experiment that measured the time a beverage was kept in the mouth before swallowing [30] the current experiment measured the total time that a beverage was being placed into the oral cavity. Both these factors place an erosive burden upon the teeth and so are important in assessing erosive risk. Subjects consumed their beverages over a period of 44.2 min on average which is alarming with respect to oral pH levels; for exposure to acids for periods longer than 10 min have potential to cause loss of tooth structure in depth [24]. Such a lengthy exposure to beverages results in a continuous source of acid in the oral cavity which could have a catastrophic effect on the integrity of tooth structure. This observation however needs to be treated with some caution for the work cited to support it, Hara and Zero [24], is a laboratory based study in which there is no salivary buffering. Contemporary preventive advice for patients to prevent erosion stresses limiting drink to tooth contact time [31] and reinforces our belief that both the duration of drinking and holding time of a sip in the mouth before swallowing are important factors to be considered when evaluating erosive risk.

In the present study, the overall mean consumption rate value was calculated to be 13.3 ± 6.0 ml/min, with males drinking at a higher consumption rate compared with females (14.4 ± 6.4 ml/min and 11.8 ± 5.0 ml/min respectively). This is in agreement with a study by Rosenbluth et al. [22] where males consumed beer at a higher consumption rate than did females. In another barroom observation study beer drinking consumption rates for males were reported to be significantly higher than for females (26.1 ml/min and 15.9 ml/min respectively) ($P < 0.01$) [23].

A laboratory study reported mean total consumed volume within 30 min of non-social laboratory drinking to be 543 ± 240 ml for beer and 519 ± 268 ml for mixed hard drinks [27]. Interpretation of the aforementioned data by simple mathematical calculations yields two consumption rate values, 18.1 ± 8 ml/min for beer and 17.3 ± 8.9 ml/min for mixed hard drinks. These values along with the ones provided by Geller et al. [23] when weighed up against the values from the current study (i.e. 13.3 ± 6.0 ml/min) presumably gives a sensible explanation of the slight rate difference, bearing in mind the differences in beverage type, social atmosphere and the presence of food. In other words, when comparing two social scenarios, the first a social gathering over lunch or dinner with food and soft drinks being served, and the second a social gathering in a barroom with only hard drinks; consumption rate of drinks will be less in the former scenario compared with the latter owing to the presence of food and the distinctive social atmosphere of each.

Previous attempts to design and run demineralisation/remineralisation cycle regimes utilised acid flow rates ranging from 0.15 to 5 ml/min and durations from 1 to 10 min that replicated a daily acid intake of 30 ml at most [6–8,32,33] which, we believe, does not reflect a realistic human drinking behaviour for the present study has shown that daily acid intake can exceed this.

More participant observation studies are needed in which to extend this research to look at factors that influence fizzy drinks consumption of individuals and to incorporate more age groups to include teenagers and older individuals. This would help to determine the generalizability of the reported drinking behaviour values and to reflect the observed behaviour on the atmosphere and experimental setting. It is acknowledged that the dental erosion state of the participants was not ascertained in this study. Though potentially being related to drinking habits it has been demonstrated previously that *in vivo* erosion is not correlated significantly to the quantity of beverage intake [34] being more a product of individual susceptibility factors when the teeth are exposed to such risk. A recent systematic review [35] concludes that consumption of soft drinks, acidic snacks/sweets and acidic fruit juices increases the odds of an individual developing dental erosion.

5. Conclusions

Several human drinking behaviour values were reported in this study and these will be of value in the development of more realistic laboratory erosion-testing regimes. It is concluded, within the limitations of this work, that (1) there are differences in the drinking behaviour of males and females with respect to sip volume and count, (2) the intraoral rise in temperature of a 4 °C beverage is lower than that used in previous laboratory simulations and (3) the values derived from video observation agree with those measured directly validating this technique for use in further studies.

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