The Influence of Salivary Factors on Dental Erosion

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"I can do everything through Him who gives me strength." Philippians, 4:13.

Declaration

This is the original work of the author.

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List of Publications

The following papers were published during the course of this study:

Scientific papers

Edwards M, Ashwood RA, Littlewood SJ, Brocklebank LM & Fung DE.

A videofluoroscopic comparison of straw and cup drinking: the potential influence on dental erosion.

British Dental Journal, 1998;**185:**244-249.

Edwards M, Creanor SL, Foye RH & Gilmour WH.

Buffering capacities of soft drinks: the potential influence on dental erosion

Journal of Oral Rehabilitation, 1999;**26:**923-927.

Professional publications

Edwards M & Creanor S.

Soft drinks and dental erosion.

Scottish Dentist; 1999; Issue 37:20-21.

Edwards M.

Videofluoroscopy, straws and dental erosion.

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27.

The following abstracts were presented at conferences and also published during the course of this study:

Ashwood RA, Edwards M, Littlewood S & Brocklebank L.

A videofluoroscopic comparison of straw and cup drinking.

Journal of Dental Research, 1996;**75:**1133.

Edwards M, Creanor SL & Foye RH.

Buffering capacities of soft drinks: the potential influence on dental erosion.

Journal of Dental Research, 1996;**75:**688.

Edwards M, Creanor SL & Foye RH.

Preliminary *in vivo* investigations into the erosive potential of a cola-type drink.

Caries Research, 1997;**31:**319-320.

Edwards M, Creanor SL & Foye RH.

Buffering capacity of saliva *in vitro.*

Journal of Dental Research, 1999;**78:**1075.

Edwards M, Creanor SL & Foye RH.

An investigation into salivary pH changes when exposed to various soft drinks.

Caries Research, 1999;**33:**327.

Creanor SL, Edwards M & Foye RH.

Salivary pH profiles during consumption of fruit drinks.

Caries Research, 1999;**33:**327.

The following paper has been submitted for publication during the course of this study:

Edwards M, Creanor SL, Foye RH & Gilmour WH.

The change in salivary pH when exposed repeatedly to an acidic soft drink: the potential influence in dental erosion.

List of Abbreviations

AC	all control subjects
AE	all erosion subjects
ANOVA	analysis of variance
APS	ammonium persulphate
BE	best of erosive group
BN	best of the normal group
C	*Coca-Cola*
CBB	Coomassie Brilliant Blue
CD	carbonated drinks
DC	*Diet Coke*
DL	*Diet Lilt*
DMFS	decayed, missing and filled surfaces
DMFT	decayed, missing and filled teeth
DTT	dithiothreitol
E	erosion, erosive
FBCD	fruit-based carbonated drinks
FC	female control subjects
FE	female erosion subjects
FJ	fruit juices
FW	flavoured waters
kD	kilodaltons
L	*Lilt*
M	molar
M&S	*Marks & Spencer*
MC	male control subject
ME	male erosion subjects
min	minute
mL	millilitres
mm	millimetres
MW	molecular weight

n	number
N	normal
NaOH	sodium hydroxide
NS	not significant
NUP	not under paraffin
OJ	orange juice
P	*Perrier*
PAGE	polyacrylamide gel electrophoresis
ppm	parts per million
PRPs	proline-rich proteins
PW	plain sparkling mineral water
rpm	revolutions per minute
SD	standard deviation
SDS	sodium dodecyl sulphate
sec	second
SW	still mineral water
TCA	trichloracetic acid
TEMED	tetramethylethylenediamine
TrisHCl	Trishydroxymethylmethylamine with pH adjusted by hydrochloric acid
UP	under paraffin
V	Volts
WE	worst of erosive group
WN	worst of normal group
µL	microlitres
°C	degrees of Centigrade

Summary

The Influence of Salivary Factors on Dental Erosion

Dental erosion appears to be an increasing problem in patients of all ages, with the rising incidence linked to the escalating consumption of soft drinks. The research discussed here has evaluated differences between various soft drinks. Variations between the salivary buffering capacity of normal individuals and subjects with erosion have also been identified.

Most soft drinks are acidic and have a low pH value. However, pH determines only the free acid ions, whereas titratable acidity gives an indication of the total acid present in the drink. The acids in a drink also contribute to the drink's buffering capacity. Drinks with a higher buffering capacity resist the rise in pH that saliva attempts to bring about and, therefore, have the potential to keep the pH of the oral cavity lower for longer.

Total acidity can be assessed by carrying out an acid-base titration. The slope of the graph, that is the total amount of alkali needed to bring about a rise in pH, gives an estimate of the buffering capacity of the drink. Several different groups of drinks have been tested. Carbonated drinks such as cola, which are not fruit-based, fruit-flavoured carbonated drinks such as *Lilt*, sparkling mineral waters, both plain and flavoured, still mineral water and pure fruit juices have all been included.

The initial pH values showed cola drinks to have the lowest pH and still mineral water the highest. Significant differences in buffering capacity were found between

each of the groups of drinks tested. The total acid found in fruit juices and fruit-based drinks was far greater than that found in cola drinks. It is clear that the addition of fruit flavouring, and hence more acid, to drinks increases their buffering capacity. This was despite the fact that the initial pH values of the fruit juices were higher than the cola drinks, indicating that pH values alone do not predict accurately the total amount of acid present in a drink. Caution must be exercised, however, when extrapolating these results to the oral cavity, but it is clear that in terms of acid content, fruit drinks and fruit juices have more potential demineralise tooth tissue.

In the mouth, saliva becomes a major modifying factor, as it neutralises and buffers acidic substances and acts in effect as a biological base. By carrying out titrations of saliva with soft drinks, it can be seen how effective saliva is at coping with the challenge of acidic drinks.

Drinks from each of the main groups were titrated with saliva from various volunteers. Again, clear differences were seen between the various groups of drinks, with pure fruit juices causing the greatest changes in salivary pH, followed by fruit-based carbonated drinks. Carbonated cola drinks and sparkling mineral waters caused smaller changes in salivary pH.

Saliva was also collected from volunteers with diagnosed dental erosion. It was interesting to note that the pH of saliva from those with erosion fell more quickly than that of normal individuals. Saliva from individuals with erosion may, therefore, be less able to cope with the acidic challenge of soft drinks.

The oral cavity is a dynamic physiological model. There are many complex factors involved in the interactions of saliva with acidic drinks, which *in vitro* experiments only go some way to explaining. The monitoring of salivary pH during drinking involved observing changes in pH while consuming an entire can of beverage. A measured sip of the chosen soft drink was taken every minute, with saliva being collected shortly after each sip for pH measurement. Three different drinks were tested, including a pure fruit juice and a diet drink.

For most of the volunteers, salivary pH fell during drinking. There were a few individuals for whom the pH values of saliva rose during drinking, highlighting the excellent ability of saliva as a buffering agent. However, the most common finding was a fall in salivary pH, which often extended beyond the drinking time, indicating that the effects of soft drinks on the saliva may continue for some time after drinking has stopped. It was felt important to include patients with diagnosed erosion in this trial, to see how their saliva reacted to an erosive insult. The fall in salivary pH was particularly marked for these volunteers with erosion. Once again, it emerged that there may be some deficiency in the saliva of those who are prone to erosion, in that the buffering capacity of the saliva appeared to be reduced. There were also differences between the various drinks tested. Consumption of the pure fruit juice caused the pH of saliva to fall to a lower value than when the cola drink was taken. These results underline the results from the earlier *in vitro* tests, indicating that pure fruit juices may have more potential to cause erosion because of their ability to lower the pH of the oral cavity for longer.

There is evidence that many factors contribute to the buffering ability of saliva. Salivary proteins are believed to play a role, especially at extremes of acidity. An investigation using gel electrophoresis to separate proteins in the saliva from a range of normal and erosion volunteers showed that some erosion individuals had more proteins of low molecular weight. Salivary proteins are also involved in the formation of pellicle, which is thought to give a degree of protection against acid erosion. Any differences in protein content may play a significant role in disease development for those prone to erosion.

In summary, these studies have allowed significant insights to be made into the aetiology of dental erosion. It has been identified that, not only do drinks vary in their ability to lower salivary pH, but that saliva also varies considerably in its ability to counteract erosive changes. However, further research in this area is ongoing and investigation into other factors involved in dental erosion continues.

1 INTRODUCTION AND REVIEW OF LITERATURE

1.1 Introduction

Dental erosion is clinically distinct from caries and is attracting considerable interest from both the dental profession and the popular press (Connor, 1996; Christie, 1998).

1.2 General

1.2.1 Definition and terminology

Pindborg (1970) defined erosion as the superficial loss of dental hard tissue by a chemical process which does not involve bacteria. Eccles (1982a) suggested that chemical etching by acid might be supplemented by chelation. Possible chelating action by citrates was first proposed by Zipkin & McClure (1949). The generic terms tooth surface loss (Eccles, 1982a) and tooth wear (Smith & Knight, 1984a) include other chronic destructive processes such as abrasion and attrition, which lead to irreversible loss of tooth structure. It is unusual to see any process acting alone and difficult to determine the part played by each (Eccles, 1982a) and, therefore, erosion can be seen as a multifactorial process, similar to caries.

Attrition describes the wear of teeth at sites of direct contact between the teeth (Mair, 1992). Attrition is often the result of bruxism and facets are usually seen on the occlusal and incisal surfaces (Robb, Cruwys & Smith, 1991). Wear can also occur interproximally during mastication (Eccles, 1982a).

Abrasion is mechanical wear caused by objects other then another tooth (Mair, 1992) and its occurrence was found to be infrequent compared to other types of tooth wear (Smith & Knight, 1984b). Abrasion is usually the result of over vigorous toothbrushing (Knight, 1969) but other agents, such as pipe stems and hair grips, have been thought to cause abrasion (Eccles, 1982a).

Other terms are now coming into use to describe certain forms of tooth wear. Demastication is a term used to describe lesions which are often a combination of abrasion and attrition when an abrasive diet causes pathological interproximal wear (Imfeld, 1996a). Abfraction, meaning "breaking away" is the result of interocclusal forces creating flexure and thus, physical microfractures (abfractions) at the cervical region (Grippo & Simring, 1995). The resulting fracture at the cementoenamel junction causes a classic wedge-shaped defect (Imfeld, 1996a). Non-carious tooth substance loss can also be the result of resorption, either physiological or pathological (Imfeld, 1996a). Perimylolysis is a special form of erosion that describes the defect which occurs when the patient suffers from chronic regurgitation and lesions are seen on the palatal surfaces of maxilliary teeth (Pindborg, 1970).

1.2.2 Classification

Eccles (1979) developed a classification based on clinical severity of erosion, graded from slight to severe, by expanding a previous proposal (Eccles & Jenkins, 1974). The classification is as follows:

- Class I denotes a superficial lesion involving enamel only
- Class II signifies a localised lesion involving dentine of less than one third of the tooth surface
- Class III indicates a generalised lesion, with more than one third of the surface having exposed dentine.

Smith & Knight (1984a) proposed a new index for tooth wear, to record damage by the triad of attrition, erosion and abrasion, either singly or in combination. The index involves scoring each tooth from 0 – 4, although varying criteria apply to different tooth surfaces. Broadly, the index is scored as below:

0: no change

1: loss of enamel surface characteristics

2: dentine exposed for less than one third of the surface

3: dentine exposed for more than one third of the surface

4: complete loss of enamel or pulp exposure

Figure 1.1 shows a labial view of a patient with erosion. The enamel surface is shiny, with loss of surface features. In addition, wear has progressed to the stage where there is shortening of the incisal edge. A palatal view of a patient with erosion is shown in Figure 1.2. There has been loss of tooth tissue into dentine, with a characteristic rim of enamel remaining around the edge of the tooth.

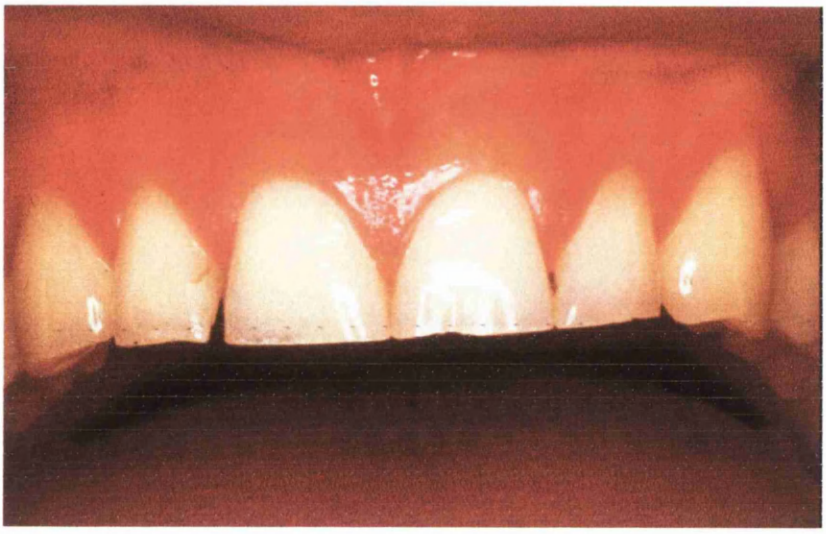

Figure 1.1
Labial view of a patient with erosion.

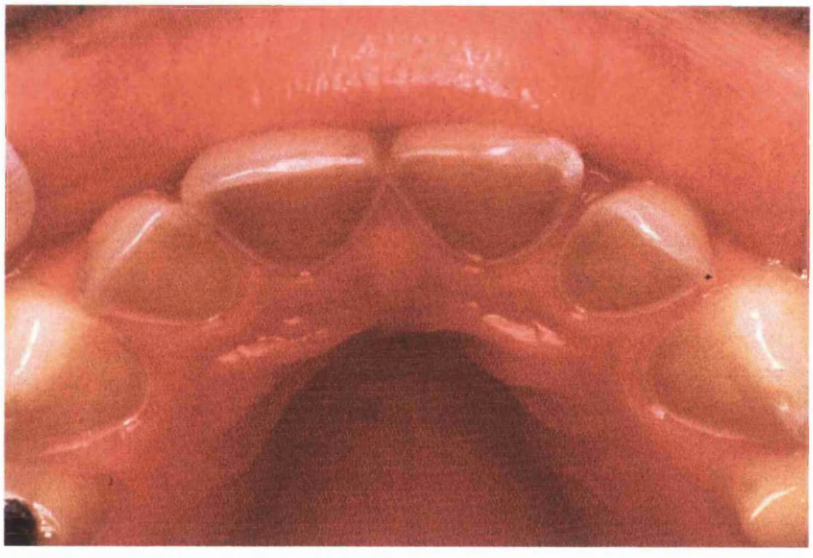

Figure 1.2
Palatal view of a patient with erosion.

1.2.3 Pathology: mechanisms of tooth wear and tissue loss

Jenkins (1981) summarised the acid theory of caries and pH changes at the tooth surface as follows:

- Apatite solubility at neutral pH is low, calcium and phosphate ions in saliva and plaque provide a supersaturated solution, preventing enamel dissolution;
- At lower pH values, around pH 5.5, apatite solubility increases, calcium and phosphate in the environment can no longer saturate the enamel and so mineral dissolves;
- As the pH rises, saturation is reached again and apatite is re-formed in enamel, completing the demineralisation/remineralisation cycle.

Caries, however, consists of a penetrating lesion, rather than the superficial spreading decalcification seen in erosion (Jenkins, 1966). Erosive agents dissolve the apatite crystals and, because the dissolved minerals are removed immediately, enamel is lost layer by layer from the surface (ten Cate & Imfeld, 1996).

Acid is the principal agent in enamel dissolution for both caries and erosion, although the source differs. Bacterial metabolism produces weak acids which cause caries, while the relatively stronger acids causing erosion come from dietary sources, the environment or endogenous sites such as the stomach (Larsen, 1973). Larsen (1973) proposed that the two types of enamel dissolution were chemically distinct. Erosion occurred when teeth were exposed to a buffered solution undersaturated with respect to both hydroxyapatite (HA) and fluorapatite (FA), similar to the degrees of

saturation found in fruit juices and soft drinks. The teeth showed surface etching but no sign of subsurface demineralisation. Saliva is undersaturated with respect to HA and FA at pH levels below 4. When teeth were placed in a buffer supersaturated with respect to FA but undersaturated with respect to HA, they exhibited subsurface, caries-like lesions, with the original enamel surface remaining intact. FA was depositing into the surface enamel while HA was dissolving from the surface enamel. The conditions at which saliva is supersaturated with respect to FA and undersaturated with respect to HA are found in the pH range 4.5 – 5.5.

This work was carried further by Larsen in 1974, to show that changing the saturation degrees of fluids could indeed dissolve the subsurface enamel while preserving the surface layers and yet at other times cause erosive injury. However, no account was taken of the role of saliva in remineralisation or the potential effects of overlying plaque and pellicle. The use of a diffusion barrier (Larsen, 1991a) to create a local supersaturation with respect to FA changed the formation of erosion lesions to those of caries. The relationship between the development of erosion and caries was investigated by Larsen (1991b) who showed that erosion could develop over caries by changing the saturation conditions with respect to FA.

The actual mechanism of enamel surface loss is still uncertain. Meurman & Frank (1991b) used scanning electron microscopy (SEM) to study the progression of erosion in human and bovine enamel immersed in various soft drinks. They described dissolution of the prism cores following a prolonged acidic challenge, with eventual involvement of the interprismatic areas. The study also observed irregular dissolution in aprismatic enamel, with areas of distinctly eroded enamel adjacent to less affected areas, suggesting that aprismatic enamel is not as liable to erosive

destruction as prismatic enamel. To simulate mechanical wear, some samples were diamond-polished and erosion progressed more readily in these specimens. This suggested that the outermost layer was probably more acid resistant than the deeper layers of enamel and was thought to represent the clinical situation where erosion is associated with other forms of tooth wear. Millward, Shaw & Smith (1995), in developing an *in vitro* technique for erosive lesion formation, observed that citric acid pumped across enamel caused loss of the aprismatic enamel surface. As exposure times increased, micropitting became more severe and the underlying prismatic enamel was exposed. Changes in enamel surface texture were examined by Whitehead *et al.* (1997) using SEM and profilometry. Eroded enamel appeared smoother with a significant increase in the depth of pits and pores.

Meurman, Drysdale & Frank (1991) also investigated erosion of dentine by similar methods to those used in their enamel studies. Peritubular dentine was the first tissue to be affected, with destruction and tubular hollowing. The lesions progressed to the intertubular areas, causing a rough and porous surface. The tubules became significantly enlarged, allowing easier access to the odontoblast processes and, therefore, explaining the painful sensitivity experienced by many patients.

The chemistry of dental erosion was summarised by McIntyre (1992). The ions from strong acids overwhelm the phosphate buffers in saliva before bicarbonate can be generated from saliva stimulation. The acid ions will then react with the phosphate groups of HA and even FA, preventing further buffering or remineralisation, resulting in enamel dissolution, which proceeds until bicarbonate buffering becomes effective. This situation is in contrast to the weaker, more prolonged acidic challenge of caries which allows sufficient time for effective buffering of acids and

remineralisation of at least the surface layer. Fluoride incorporation into enamel is particularly effective in the prevention of caries, but less so in the erosion process because at the low pH values present during erosion, both HA and FA are unstable. Fluoride in either the enamel or in the ambient solution will not affect the degree of undersaturation with respect to mineral (Meurman & ten Cate, 1996).

In summary, the main differences between erosion and caries are manifested by the fact that they rarely occur on the same site. Caries occurs on plaque-covered sites whereas erosion occurs in areas which are free from plaque. The concentrations of the acids involved in erosion are greater than the concentrations of the acids produced at specific sites by plaque microorganisms in caries (Meurman & ten Cate, 1996). Erosion involves rapid demineralisation (Millward, Shaw & Smith, 1995) with remineralisation occurring during prolonged periods between acid exposure. In contrast, caries tends to be a slower process, with a dynamic equilibrium between de- and remineralisation (Nunn, Shaw & Smith, 1996).

1.2.4 Prevalence and epidemiology

Very few population-based surveys have been published and this may be due, in part, to the lack of well-defined criteria for assessing erosion (ten Cate & Imfeld, 1996). Prevalence of erosion in young people in the UK has been measured nationally by the National Child Dental Health survey in 1993 (O'Brien, 1994) and the National Diet and Nutrition survey in 1992-3 (Hinds & Gregory, 1995). In both surveys, erosion proved difficult to assess. In the former survey, over half of five and six year old children had erosion of one or more primary incisors, with a quarter of these progressing into dentine or pulp. In children aged eleven and over, a quarter had

erosion on the palatal surfaces of the permanent upper incisors, although only 2% had progressed into dentine or pulp. The latter survey noted that palatal erosion was present in 19% of the 1½ - 4½ year old children, with dentine or pulp involvement in 8% and the prevalence increasing with age.

Other more local prevalence studies of erosion in UK children have also been carried out. Milosevic, Young & Lennon (1994) examined 1035 children in Liverpool, 30% of whom had exposed dentine, mainly incisally. Eight percent also had exposed dentine on occlusal and/or lingual surfaces. It was of interest that as prevalence of tooth wear increased, levels of deprivation worsened. This was in contrast to a study of 176 four and five year olds carried out in the West Midlands (Millward, Shaw & Smith, 1994) when there was a positive correlation between socio-economic class and prevalence of erosion, in that children from low socio-economic groups had less erosion. Nearly half of the children in this survey showed some erosion, more commonly on the palatal surfaces of maxilliary incisors. Millward *et al.* (1994) also surveyed 101 children attending Birmingham Dental Hospital, 20 of whom had been referred concerning tooth tissue loss with the remaining 81 being chosen randomly from consecutive attenders. Tooth wear was found in over 80% of maxilliary incisors and 30% of primary molars had some dentine exposed.

Surveys of erosion in adults have also been carried out. In the United States, a two centre study of patients in Los Angeles and Boston showed that the prevalence varied not only between the cities but also between tooth types, although overall it was found that 25% of individuals were affected by erosion (Xhonga & Valdmanis, 1983). In a random sample of Swiss adults from two age groups, 8% of the 26-30 age group and 13% of the 46-50 age group showed at least one tooth affected by labial erosion

with involvement of dentine, while 30% of the younger and 43% of the older age groups had erosion into dentine on the occlusal surface of 3 or 4 teeth (Lussi *et al.*, 1991). Ekfeldt (1989) in an epidemiological survey of 585 people found that 65% of 20 year olds showed incisal and occlusal wear of at least one tooth, with this number increasing to an average of 75%-85% in other age groups, confirming that severity and prevalence of wear increases with age. More recent studies have shown that 57% of 11-14 year old school children had tooth wear on more than 10 teeth, although dentine involvement was rare (Bartlett *et al.*, 1998). Investigations into the oral health of young Saudi men found that 28% of maxilliary anterior teeth showed pronounced dental erosion (Johansson *et al.*, 1996). Smith & Robb (1996), in providing baseline data for an epidemiological study, found that 6% of 15-26 year olds and 8% of over 65 year olds had unacceptable levels of tooth wear. They also noted that more men than women showed tooth tissue loss. It has been suggested that the prevalence of erosion is increasing. However, it may also be that the lesion is becoming better recognised by dentists (Jarvinen, Rytomaa & Heinonen, 1991).

1.2.5 Diagnosis and site of erosion

It has long been recognised that it is not always possible to differentiate clinically erosion from either abrasion or attrition (Eccles, 1982a). However, several features indicate that an erosive factor may be involved, such as loss of labial tooth surface, proud restorations and cupping of molar cusps and incisal edges (Eccles, 1982a). Often the palatal surfaces are hollowed out and the incisal edges thin (Eccles, 1982b). Early changes involve diminished enamel lustre, with loss of developmental pits and grooves as erosion progresses, leaving the eroded area smooth and polished with well-defined borders (Bevenius, L'Estrange & Angmar-Månsson, 1988). Once

dentine is exposed, loss of tooth tissue accelerates, particularly in the primary dentition, and especially when abrasion also becomes a factor (Shaw & Smith, 1994). This is noticeable not only on incisors but also molars (Milosevic & Lennon, 1995).

The site of erosion was thought to give some indication as to the aetiology of the condition. For example, dietary factors were thought to be more likely to cause labial erosion, in contrast to intrinsic sources more likely to affect palatal and occlusal surfaces (Eccles, 1982a). However, it seems that it may not be so straightforward, because of individual variation in clearance patterns, salivary flow rates, buffering capacity, as well as concomitant extrinsic and intrinsic causes (Meurman & ten Cate, 1996). The study by Jarvinen, Rytomaa & Meurman (1992) suggested that the cause of dental erosion could not be reliably predicted from the location of the lesion.

1.3 Aetiology

Dental erosion, like dental caries and periodontal disease, has a multifactorial aetiology and although one factor may play a predominant role, many others will contribute to the erosive process (Zero, 1996). A thorough history is required to assess the aetiology, yet in some cases, the aetiology will remain undetermined (Smith & Knight, 1984b).

1.3.1 Endogenous factors

Stafne & Lovestedt (1947) observed that gastrointestinal disorders leading to regurgitation and vomiting could cause dissolution of tooth substance if gastric acids contacted the

teeth several times a week over a period of one or two years. Although the prevalence of erosion from intrinsic acid is unknown, studies by Smith & Knight (1984b) and Jarvinen, Rytomaa & Meurman (1992) suggest that endogenous factors may be involved in a quarter of all erosion cases. Jarvinen, Rytomaa & Heinonen (1991) found the risk of erosion was between 4 and 18 times higher in patients who suffered from regurgitation and chronic vomiting respectively, compared to non-vomiting patients. Acid regurgitation is a symptom of peptic ulcer disease and reflux oesophagitis (Jarvinen *et al.*, 1988). Gastro-oesophageal reflux (GOR) can even be found in children (Aine, Baer & Maki, 1993) and is often asymptomatic (Taylor *et al.*, 1992). However, not all patients with GOR develop erosion (Meurman *et al.*, 1994), again highlighting the importance of individual susceptibility. Reflux may also be associated with certain types of handicap, such as cerebral palsy, with contributory factors including incoordination of swallowing and prolonged recumbence (Shaw & Smith, 1994). Bruxism may combine with erosion to cause severe tooth wear; indeed higher levels of tooth wear have been reported in children suffering from cerebral palsy (Pope & Curzon, 1991).

Gudmundsson *et al.* (1995) investigated the relationship between tooth erosion and GOR by 24-hour intra-oral and intra-oesophageal pH monitoring of 14 erosion patients. They failed to reveal any changes in intra-oral pH during acid reflux. However, the analysis of salivary factors showed lower salivary buffering capacity in erosion patients compared with controls, indicating that salivary defence mechanisms may be highly relevant in the aetiology and progression of erosion. Another study in 1995 also attempted to determine the link between GOR and dental erosion (Schreoder *et al.*, 1995). This investigation did show a relationship, in that 10 out of

12 patients with idiopathic erosion showed reflux on oesophageal pH monitoring. However, it was unable to detect any abnormalities in salivary analyses, including flow rates, pH, buffering capacity, and calcium and phosphate levels.

GOR is not normally under voluntary control, although there has been a case report in the literature describing a voluntary reflux phenomenon, where the patient had the ability to regurgitate his gastric contents. These were held in the buccal pouch before being reswallowed, which eventually caused advanced erosion tooth surface loss (Gilmour & Beckett, 1993). Such human rumination may be linked to psychological problems.

Chronic vomiting can be a manifestation of the eating disorders anorexia nervosa and bulimia nervosa. These psychosomatic illnesses involve self-induced vomiting and the prevalence is about 5% in the Western world (Scheutzel, 1996), with young women being particularly at risk (Bishop, Briggs & Kelleher, 1994). In contrast to anorexic patients who lose weight by a combination of starvation and vomiting, bulimics are of normal weight but indulge in eating binges followed by episodes of vomiting, fasting and laxative abuse (Rytomaa *et al.*, 1998). There have been many reports in the literature concerning erosion as a result of eating disorders (Andrews, 1982; Robb, Smith & Geidrys-Leeper, 1995).

Alcoholism is also a common problem in today's society. As well as causing many other changes, acute alcohol intake can result in gastric reflux (Scheutzel, 1996) while long term alcohol abuse can induce chronic vomiting (Simmons & Thompson, 1987), both contributing to dental erosion. A recent case report has implicated "alcopops" alcoholic soft drinks in the aetiology of dental erosion. These drinks not

only have a low pH, but their alcohol content may also carry an increased risk of vomiting (O'Sullivan & Curzon, 1998). Other possible causes of regular vomiting include metabolic and endocrine disorders or medication side effects (Scheutzel, 1996).

1.3.2 Exogenous factors

Many varying exogenous factors can play a part in the aetiology of dental erosion, with much of the evidence being presented in the form of case reports. Severe erosion was produced in patients with achlorhydria who took dilute hydrochloric acid by mouth (Stafne & Lovestedt, 1947; Knight, 1969). Nowadays, the use of hydrochloric acid in capsules has halted the progress of this form of dental erosion (Smith & Knight, 1984b). The effect of effervescent and chewable vitamin C (ascorbic acid) tablets was examined by Meurman & Murtomaa (1986) who showed that the preparations, all of which had a pH below pH 5.5, caused erosion of bovine teeth *in vitro*. They concluded that in patients with normal salivary flow, the short-term consumption of vitamin C would be harmless, although erosion could occur if the tablets were kept in direct contact with teeth for long periods of time. Hays *et al.* (1992) measured salivary pH while dissolving a chewable vitamin C tablet and showed that, despite the presence of sodium ascorbate as a buffering agent, a significant drop in salivary pH occurred. As there appears to be no topical efficacy of vitamin C they advised that tablets should be swallowed whole. Acetylsalicylic acid (ASA) or aspirin has also been reported to cause erosion (Sullivan & Kramer, 1983). ASA is the drug of choice for treating juvenile rheumatoid arthritis and as they are used three of four times a day for many years, the tablets should again be swallowed whole rather than chewed or sucked (Tanchyk, 1986). A SEM study of

the erosive action of ASA on enamel showed that salicylate solution buffered with calcium carbonate had less erosive potential than an unbuffered solution (Hannig, Rogalla & Albers, 1992). Another acid reported to be involved in erosion is Ecstasy (3, 4, methylenedioxymethamphetamine or MDMA). MDMA causes xerostomia and this, together with the dehydration from over-vigorous activity, is relieved by the consumption of soft drinks, the sugar in which is thought to enhance the absorption of Ecstasy. This increased consumption of soft drinks will be reflected in a higher rate of erosion. The use of such a drug should be considered in young adult patients who present with erosion of unknown origin (Duxbury, 1993). Other acids taken into the mouth may also cause erosion. A case report by Leary & Johnson (1987) recorded the use of nitric acid by a psychologically disturbed patient, highlighting the importance of collaboration with other members of the health field. Mouthwashes have also been implicated in the aetiology of dental erosion because they contain organic acids and calcium chelating agents. Rytomaa *et al.* (1989) compared several available products *in vitro* and found one containing EDTA to be particularly erosive. A study of eleven mouth rinses on the UK market found all of them to contain ethanol and nine to be acidic, which could prevent remineralisation or hasten erosion already present (Bhatti, Walsh & Douglas, 1994).

Industrial acid fumes, such as those present in battery and galvanising factories, have been reported to cause erosion, especially of anterior teeth (Tuominen & Tuominen, 1991). There appeared to be no difference in the amount of tooth surface loss between organic acids and inorganic acids, despite the fact that organic acids are less acidic, and this is possibly because of the contribution of their calcium chelating ability (Tuominen *et al.*, 1991). The incidence of industrial erosion appears to be

declining with improved industrial practice and greater emphasis on safety at work (Smith & Knight, 1984b), although it remains a concern in developing countries (Zero, 1996). Another occupation, which has recently come to light as predisposing towards dental erosion, is winetasting. This appears to be not only because wine has a low pH value of pH 3.2-3.8, but also because of the manner of holding wine in the mouth for several minutes during tasting (Chaudhry, Harris & Challacombe, 1997). In a study of nineteen qualified winetasters, testing between twenty and fifty different wines a day, fourteen were found to have erosion and so it was concluded that erosion was an occupational hazard for winetasters (Wiktorsson, Zimmerman & Angmar-Månsson, 1997).

Environmental factors implicated in the aetiology of dental erosion also include exposure to swimming pools chlorinated by chlorine gas. This results in the formation of hydrochloric acid that requires neutralisation and buffering to maintain the pH in the range pH 7-8 (Zero, 1996). SEM tests by Gabai *et al.* (1988) confirmed that enamel immersed in samples of swimming pool water of various pH values showed signs of erosion. Inadequate buffering and monitoring of pool pH could involve swimmers being exposed to pool water with erosive potential. People involved in sports of any kind, including competitive swimmers, may also be at risk of erosion if they consume large quantities of sports drinks (Milosevic, Kelly & McLean, 1997).

1.3.3 Dietary factors

Dietary factors, although exogenous by nature, have received considerable attention in the aetiology of dental erosion and so an entire section will be devoted to a review

of the literature related to this field. In our multiethnic society, spices, vinegars and other acidic additives may be other contributory factors in erosion, although a recent study failed to show a statistically significant contribution (Bartlett *et al.*, 1998). Linkosalo and Markkanen (1985), in comparing a group of lactovegetarians with a control group, noted the incidence of erosion to be greater in lactovegetarians and found the most important dietary factors to be the consumption of vinegar, vinegar conserves, citrus fruits and acidic berries. Milosevic, Lennon & Fear (1997) studied tooth wear in teenagers and established that eating pickled food other than pickled onions was significantly more frequent in children with wear than in controls. An investigation of those living on a raw food diet showed a significantly higher incidence of erosion in this group compared with controls (Ganss, Schlechtriemen & Klimek, 1999). Other foods with a low pH value such as apple vinegars (Jarvinen, Rytomaa & Heinonen, 1991) and acidic sweets (Bibby & Mundorff, 1975) have also been implicated in the aetiology of erosion but most reports link acidic beverages to the rising prevalence of tooth wear.

Acidic soft drinks have, for many years, been thought to be involved in the erosive process. Early reports by Stafne & Lovestedt (1947) and Holloway, Mellanby & Stewart (1958) linked lemon juice and other fruit drinks to the destruction of enamel. The sales of soft drinks have risen dramatically in the United States (Ismail, Burt & Ekland, 1984) and the United Kingdom (Shaw & Smith, 1994), implicating further soft drinks in the apparent rising prevalence of erosion. A large range of soft drinks is widely available on today's market (Figure 1.3). Case reports have appeared in the literature which also propose a relationship between soft drink consumption and erosion (Touyz, 1986; Asher & Reid, 1987; Mackie & Blinkhorn, 1989). Two early

Figure 1.3
An example of some of the soft drinks available on today's market.

case studies attributed unusual patterns of tooth wear to abnormal drinking habits such as holding drinks in the mouth prior to swallowing (High, 1977) and using a straw or feeder cup placed labial to the dentition (Mackie & Hobson, 1986). In contrast to this, several authors have advocated the use of a straw as being beneficial to the dentition. Birkhed (1984) and Grobler, Jenkins & Kotze (1985) showed by plaque pH studies that use of a straw allowed drinks to avoid contact with teeth. Smith & Shaw (1993) studied the effect of mode of administration on oral clearance and found that straws minimised oral contact of drinks. Videofluoroscopy was employed to visualise fluid distribution by Edwards *et al.* (1998) and demonstrated the correct use of a straw placed well back in the mouth prevented contact of fluid with teeth.

Various epidemiological studies have attempted to establish a relationship between diet and dental erosion. Jarvinen, Rytomaa & Heinonen (1991) proposed there was a greater risk of erosion if soft drinks were drunk daily or sports drinks weekly. Soft drinks were found to be significantly associated with the presence of erosion by Lussi *et al.* (1991). Statistically significant differences were found, in relation to drinking habits, between groups of children graded by severity of erosion (Millward *et al.*, 1994). An increase in the frequency of intake of fruit-based drinks and their consumption last thing at night were strongly associated with the most severe cases of erosion. A study of young Saudi men by Johansson *et al.* (1997) showed a strong correlation between the presence of dental erosion and a high level of consumption of cola-type drinks. Milosevic, Lennon & Fear (1997) found that carbonated drink consumption was of borderline significance as a predictor of tooth wear in teenagers, but believed there was probably a relationship. Another study of tooth wear in

adolescent schoolchildren (Bartlett *et al.*, 1998) failed to establish a link between wear and acidic drink consumption. However, they hypothesised that the consumption of acidic food and drink may provoke GOR and thus the two may have a dual role in the aetiology of tooth wear. They concluded that the relationship between tooth wear and diet, salivary factors and endogenous factors was complex and required further investigation.

The erosive potential of beverages is thought to involve several factors, including low pH and the buffering capacity of the drink. The relative importance of pH versus titratable acidity is still under debate, but may be related to the length of time the drink spends in the mouth (Rugg-Gunn *et al.*, 1998; Larsen & Nyvad, 1999). Soft drinks may contain several different types of acid that contribute to the low pH value. Carbonated beverages will contain carbonic acid formed by carbon dioxide in solution. Even when the carbon dioxide has been blown off and the drinks have become "flat" the pH remains low (Creanor, Ferguson & Foye, 1995). This indicates that soft drinks have inherent acidity due to other acids that are added to stimulate taste and counteract sweetness. These other acids include, for example, phosphoric and citric acids present in cola-type drinks (Sorvari & Rytomaa, 1991). Fruit juices and fruit-flavoured drinks are made from a concentrated source of fruit and consist of organic acids derived from the fruit such as citric acid from oranges, tartaric acid from grapes and malic acid from apples (Touyz, 1994). Added Vitamin C (ascorbic acid) may also contribute to the acidity of soft drinks (Touyz, 1994).

However, it is generally accepted that titratable acidity, that is a measurement of the total acid content, is a more important indicator than actual pH value in determining erosive potential of beverages (Grobler, Jenkins & Kotze, 1985; Grenby *et al.*, 1989).

All of these acids listed above may influence the buffering capacity and, therefore, soft drinks that are strongly buffered are more likely to cause a prolonged pH fall to occur within the mouth (Touyz & Silove, 1993). The situation is complicated further by the competition of these buffers with those of the saliva. The ability of a drink to resist pH changes brought about by salivary buffering may inevitably result in a prolonged period of oral acidity and, therefore, may play an important part in the erosion process (Grobler & van der Horst, 1982).

The laboratory evaluation of drinks has been studied extensively (Grobler, 1983; Grobler & Jenkins, 1983) by measuring pH, fluoride, calcium, phosphorus, buffering capacities and total carbohydrate. The mineral content of the drinks was also assessed in a later study (Grobler & van Wyk 1984). Titrations of frozen fruit juices were compared with fruit juices at room temperature by Touyz & Silove (1983), who showed that frozen juices had an increased buffering capacity and hence could be potentially more damaging to teeth. Grenby *et al.* (1989) showed clear differences between various kinds of drinks and suggested that, theoretically, citrus juices could be more damaging to teeth than cola-type drinks. A similar study was carried out on infant drinks (Grenby, Mistry & Desai, 1990) investigating various properties in an attempt to indicate the relative erosive potential of drinks. Lussi, Jaggi & Scharer (1993) characterised various beverages and foodstuffs, as well as using surface microhardness testing and iodide permeability, in an attempt to estimate erosive potential. A further study (Lussi, Jaeggi & Jaeggi-Scharer, 1995) suggested the possibility of predicting the erosive potential of beverages to within an accuracy of 7 % by using the parameters of baseline pH, buffering capacity, phosphate concentration and fluoride concentration. The ability of drinks to adhere

to enamel may also influence their capacity to cause erosion (Ireland, McGuiness & Sherriff, 1995) in addition to how well saliva may displace the drinks from the tooth surface. Milosevic, Kelly & McLean (1997) analysed the properties of different sports drinks available on the UK market and took the extra variable of viscosity into account. Larsen (1975) found that fruit juices and acidic drinks were undersaturated with respect to HA and FA and believed this fact alone could explain their erosive effects. The acidogenic potential of herbal baby drinks was assessed by Duggal *et al.* (1996), mainly in relation to depression of plaque pH and cariogenicity. However, initial pH and titratable acidity were also taken into account as drinks with low inherent pH and fruit components were thought to have the potential to lead to erosion of the immature enamel of newly erupted teeth. Smith & Shaw (1987) examined baby fruit juices, measuring pH values of ready-to-drink juices before and after dilution and found that even when diluted by a factor of 10, the drinks remained acidic. An *in vitro* study of primary teeth immersed overnight in these juice drinks showed a chalky, opaque appearance with complete loss of surface enamel.

Many other *in vitro* studies have immersed teeth, both human and bovine, in soft drinks for varying amounts of time in an attempt to show the damage that acidic beverages can cause. Human enamel was immersed in various acidic foods and drinks by Conboy & Cox (1971) who concluded that citric acid was damaging to teeth. Grobler, Senekal & Laubscher (1990) used an atomic absorption spectrophotometer to measure the amount of calcium released from enamel immersed in fruit juices and cola drinks for 2,4,5,6 and 40 minutes. Iodide permeability was used by Lussi, Jaggi & Scharer (1993) to detect the early stages of enamel demineralisation, in addition to surface microhardness tests, which have also

been used by many investigators (Andrade *et al.*, 1995; Maupomé *et al.*, 1998; Dodds, Gragg & Rodriquez, 1997). A recent *in vitro* study (Grando *et al.*, 1996) used stereomicroscopy and SEM to show large amounts of enamel erosion on deciduous teeth immersed in soft drinks. Another study confirmed this finding that deciduous enamel is more susceptible than permanent enamel to erosive damage (Johansson *et al.*, 1998).

Bovine teeth have been used extensively to study enamel erosion (Rytomaa *et al.*, 1988; Meurman *et al.*, 1990; Attin *et al.*, 1997). Subsequent deployment of SEM, surface microhardness testing and profilometry allowed the changes in the enamel surface to be assessed. Transverse microradiography has recently been employed to quantify mineral loss from bovine teeth (Amaechi, Higham & Edgar, 1998a) and to measure early mineral loss from human enamel (Hall *et al.*, 1997). Synthetic enamel (sintered HA) (Kanerva *et al.*, 1992) and pulverised HA (Grenby, Mistry & Desai, 1990) have also been placed into various beverages in an attempt to monitor mineral loss. However, human teeth, bovine teeth and powdered HA vary greatly in their dissolution properties. This fact, together with the variations in experimental design and method of substrate preparation, means that direct comparisons between these various studies cannot be made (Zero, 1996). In addition, no account was taken of the many biological modifying factors, such as saliva, pellicle and plaque present *in vivo*.

Animal studies in rats have been widely used to evaluate the erosive effects of beverages (Holloway, Mellanby & Stewart, 1958; Sorvari, 1989; Mistry & Grenby, 1993) but again direct extrapolation of these results to the erosive process in humans remains a problem. There are well established physiological differences between

rats and humans with regard to drinking method, enamel composition and solubility, and salivary pH and buffering capacity (Zero, 1996). Recently, a number of *in situ* models have been developed (West *et al.*, 1998a, 1998b; Rugg-Gunn *et al.*, 1998; Hall *et al.*, 1999) in the hope of studying erosion under controlled *in vivo* conditions.

Attention has been devoted to modifying the composition of drinks to reduce their erosive potential, as it has been shown that added calcium and phosphate can protect against enamel erosion (Hay *et al.*, 1962). Sorvari, Kiviranta & Luoma (1988) added fluoride and magnesium to sport drinks and showed a protective effect. Calcium lactate was added to *Coca-Cola* (Beiraghi *et al.*, 1989), significantly reducing tooth erosion in rats. The substitution of citric acid with malic acid reduced the HA dissolving effect *in vitro* of certain sports drinks (Meurman *et al.*, 1990). Recent work by Hughes, West & Addy (1997) suggested that adding 1ppm fluoride to drinks reduced erosion *in vivo* by 50%, while further work by Hughes *et al.* (1999) indicated that added calcium reduced the erosive potential of a blackcurrant drink. Larsen & Nyvad (1999) showed that a drink with added calcium and phosphate, intended to counteract osteoporosis, also appeared to prevent enamel erosion, while a recent *in vitro* study described the protective effect of fluoride and xylitol against enamel erosion from orange juice (Amaechi, Higham & Edgar, 1998b).

Several workers have measured fluoride content of soft drinks, not necessarily in relation to erosion. With a shift away from drinking tap water in favour of consumption of other beverages, concern has been expressed that people living in optimally fluoridated areas may be missing out on its benefits when they substitute water for other soft drinks (Enno, Craig & Knox, 1976). However, if drinks are manufactured using fluoridated water, the beneficial effects will be retained (Schulz,

Epstein & Forrester, 1976). Children who consumed fluoridated beverages daily for 3 years showed a reduced incremental caries rate compared with children who consumed pure citrus beverages (Gedalia *et al.*, 1981), showing that fluoride in drinks has an anti-caries effect, as well as a potentially reduced erosive effect. Concern, however, has been expressed that children who drink excessive amounts of juice drinks prepared with fluoridated water may in fact be predisposed to fluorosis (Kiritsy *et al.*, 1996), highlighting the importance of strict monitoring of fruit juice intake in children.

1.4 Saliva

The role of saliva in the aetiology of dental erosion is an area that has received considerable attention and yet still requires greater clarification.

1.4.1 Salivary composition

Buffering of acids, from both dietary and bacterial sources, is an important salivary function, which minimises pH changes in the oral cavity. The substances in saliva which have been thought to be involved in buffering are bicarbonate, calcium phosphate, protein, mucoid and ammonium (Lilienthal, 1955). Ammonium, rather than buffering the acid, actually neutralises it (Lilienthal, 1955). In resting saliva, phosphates (Tenovuo, 1997) and peptides (Mandel, 1987) are the major buffers, while bicarbonate is thought to be the principal buffer in stimulated saliva (Lilienthal, 1955; Edgar, 1992). At low pH values of 4 – 4.5, salivary proteins display some buffering action (Tenovuo, 1997).

Saliva is supersaturated with respect to tooth mineral at normal intra-oral pH levels, which maintains the solid phase of tooth enamel (Lilienthal, 1955). When the pH falls, the hydrogen ion concentration rises, and these react with the phosphate groups. The saliva is then no longer supersaturated with respect to the mineral, so dissolution of enamel occurs. As the pH rises again, this reaction is reversed and mineral is redeposited on the defects by the process of remineralisation as the degree of supersaturation returns (Edgar, 1992). Although saliva is supersaturated with respect to tooth mineral, spontaneous precipitation of salts is prevented, probably due to ion binding components in the saliva, such as pyrophosphate, statherin (a tyrosine-rich protein) and proline-rich proteins. The proline-rich proteins are also adsorbed to the pellicle, possibly inhibiting crystal growth at that site (Tenovuo, 1997). Many factors may affect the buffering capacity of saliva, and these include time of day, diet, hormonal effects and smoking (Hays *et al.*, 1992).

Unstimulated resting saliva is slightly acidic but, with stimulation and an increase in flow rate, the pH rises (Dawes, 1996). When acidic food enters the mouth, it stimulates a rapid flow of alkaline saliva (Jenkins, 1966). This stimulated saliva has a higher concentration of bicarbonate and so has increased buffering capacity (Dawes, 1970). Salivary flow rate may also be altered by many exogenous factors, including drugs such as antidepressants and diuretics, special diets (Bevenius, L'Estrange & Angmar-Månsson, 1988), impaired salivary gland function, strenuous exercise, diseases such as diabetes or arthritis (Sorvari & Rytomaa, 1991) and Sjögren's disease (Pullon & Miller, 1985). Each of these may be considered as potential contributing factors to the development of erosion lesions (Bevenius, L'Estrange & Angmar-Månsson, 1988). There is a clear relationship between

reduced flow rate and a lowered ability to clear dietary acids from the mouth (Zero, 1996). However, Billings (1993) suggested that low flow rate alone would not affect susceptibility to oral disease (and thus erosion) but should be considered in combination with other factors.

1.4.2 Salivary analysis

Flow rate and buffering capacity have been studied in populations, without any reference to dental erosion. Mazengo *et al.* (1994) studied groups of rural and urban people in Tanzania and found those living in rural areas to have higher salivary buffering capacity. They concluded that this was due to a diet rich in grain fibre. They also found no association between flow rate and buffering capacity. This was in contrast to a study by Wikner & Soder (1994), who found a positive correlation between secretion rate and buffering capacity. Those individuals with low flow rates also had low buffering values, whilst those with high flow rates could have either low or high buffering capacity. Sugar consumption between meals was thought to play a significant role in the development of low buffering values. Saliva produced at low flow rates has a lower bicarbonate level, and so a lower pH and hence lower buffering capacity than that produced at higher flow rates (Zero, 1996).

Many individuals have investigated various salivary factors in an effort to determine their individual or combined importance in the aetiology of dental erosion. Salivary citrate levels were measured by Zipkin & McClure (1949) in an attempt to relate this to enamel solubility. Mannerberg (1963) compared erosion subjects with those who had no erosion and found the salivary mucin content to vary. Fluoride concentrations in stimulated saliva were measured by West & Milosevic (1996), who

found significantly higher concentrations of fluoride in the saliva of those with wear, compared with controls. Tooth wear had progressed in these individuals despite this higher fluoride concentration. Unstimulated salivary flow rate and buffering capacity have been found to be directly associated with dental erosion. Jarvinen, Rytomaa & Heinonen (1991) concluded that those with low unstimulated salivary flow rates were at greater risk of developing erosion. There were similar findings by Bevenius & L'Estrange (1990) and Woltgens *et al.* (1985), the latter also revealing higher concentrations of calcium and phosphate in the saliva of erosion-susceptible patients. Patients with gastrointestinal disorders were investigated by Jarvinen *et al.* (1988) who found that some of those with erosion had lower stimulated salivary flow rates. They concluded that unstimulated flow rates should also have been assessed, as this is the rate at which saliva flows for most of the day. Other studies of patients with GOR have also investigated salivary factors, with somewhat conflicting results. Meurman *et al.* (1994) found higher numbers of patients with low stimulated salivary buffering capacity among those with erosion than among those without, although this was not statistically significant. However, a significant relationship was found by Gudmundsson *et al.* (1995), in that those with erosion had low salivary buffering capacity. No salivary abnormalities were detected by Schroeder *et al.* (1995).

Salivary parameters have also been investigated in groups of bulimic patients. Some studies have found no differences in salivary buffering capacity between patients with eating disorders and controls (Milosevic & Slade, 1989), while others have shown a relationship. Rytomaa *et al.* (1998) found that the number of subjects with low unstimulated salivary flow rate was three times higher among bulimics than among controls. A recent study by Milosevic & Dawson (1996) showed

significantly lower stimulated salivary flow rates among bulimics compared with healthy subjects. Lactovegetarians are another example of a group of people with interesting dietary habits. A lactovegetarian diet contains a high proportion of coarse fresh food, citrus fruits and acidic drinks (Linkosalo & Markannen, 1985). This study showed not only a lower incidence of erosion in a control group, but also significantly higher stimulated buffering capacity in controls compared with erosion cases. Another investigation (Linkosalo, Syrjanen & Alakuijala, 1988) was carried out to show that diet could in fact influence salivary composition. A third study (Linkosalo, Halonen & Markkanen, 1988) suggested the protective role of salivary amylase, but advised further investigation. Winetasters were the subject of a study by Wiktorsson, Zimmerman & Angmar-Månsson (1997) and, as well as having a higher occurrence of erosion, they also had low flow rates and buffering capacities for both unstimulated and stimulated saliva.

Other investigators have included salivary analysis while examining their chosen population. Tuominen & Tuominen (1991) took salivary samples during their study of erosion in factory workers but failed to show any correlation between tooth tissue loss and salivary parameters. Salivary buffering capacity was measured in 24 children with erosion, which revealed that 14 had low and 5 had medium buffering capacity, indicating that tests on saliva may help to identify children at risk of dental erosion (O'Sullivan & Curzon, 1995). A recent prevalence study of tooth wear in adolescents (Bartlett *et al.*, 1998) included analysis of stimulated salivary flow rate and buffering capacity and, although there was no evidence of a relationship between tooth wear and salivary factors, a link between buffering capacity and symptoms of GOR was identified. This perhaps indicates that, in trying to take many factors into

account, investigators are recognising the complex, multifactorial aetiology of dental erosion.

1.4.3 Salivary investigations

Various investigations have been carried out to study the effects of acidic drinks on salivary pH. Tenovuo and Rekola (1977) found that after a 70 mL rinse for two minutes, acidic drinks caused a drop in salivary pH. A cola drink caused a greater drop than either orange juice or a carbonated orange drink. They believed this was because cola drinks stimulated the salivary flow rate less than orange drinks, thus resulting in a lower salivary buffering response. These results were in contrast to Frostell (1970), who found that fruit juices caused a sharper fall in plaque pH than other drinks, although recovery was also quicker. Imfeld & Muhlemann (1978) used telemetry to monitor salivary pH changes and found that there was a drop following intake of dietary acids. Large amounts of acids were neutralised slowly by salivary buffers. However, Bibby (1983) proposed that acidic juices may stimulate a more favourable salivary response than other food and drinks, so leading to more rapid elimination. An increase in salivary flow rate after vitamin C consumption was concluded to be due to direct acidic stimulation by Meurman & Murtomaa (1986).

Further studies by Imfeld (1983), using telemetry to measure intra-oral pH, showed that acids had the immediate and marked effect of lowering the pH of oral fluid. Drinking caused a less pronounced fall in pH than rinsing and allowed a quicker recovery. Drinks with a high titratable acidity slowed pH recovery, so Imfeld concluded that drinks with a high acid content reflected this by causing greater pH changes in the mouth. This was in agreement with Jenkins (1981) who stated that

the pH values of food and drink influence, not only the rapidity of pH drop, but also the lower value reached. Salivary flow rate and buffering capacity are also important, as smaller changes in pH occur during eating and drinking in those who have high salivary buffering capacity (Grobler & van der Horst, 1982). However, the buffering capacity of the drinks themselves should also be taken into account, as drinks with a high buffering capacity will be less influenced by salivary buffering. For example, it takes saliva longer to neutralise orange juice than a cola drink.

Changes in pH of the tongue surface were measured by Meurman *et al.* (1987). After rinsing with 100 mL of an acidic drink for 1 min, tongue pH dropped significantly but returned to baseline within several minutes. Bashir and Lagerlöf (1996) investigated the effect of citric acid on salivary pH. After a 5 sec rinse with citric acid, salivary samples were taken and pH measured. A significant drop in pH was noted after 1 min but generally readings were back to baseline after 5 min, although there were large individual variations. The investigators noted the influence of other factors, such as degrees of saturation and clearance patterns. The pH at the tooth surface was measured by Millward *et al.* (1997), who used an electrode embedded in a vinyl splint. One hundred mL of citric acid was drunk by glass, straw and feeder cup, all of which caused a sharp fall in pH, which again recovered rapidly. This led them to advocate continuous drinking rather than sipping (which could cause repeated falls in pH).

1.4.4 Pellicle and plaque

Pellicle formation is the process involving adsorption of salivary glycoproteins, and other proteins from bacteria and gingival crevicular fluid, onto the tooth surface

(Edgar, 1992). The salivary mucins are mainly from the submandibular and sublingual glands (Imfeld, 1996b). It has long been recognised that this organic layer can protect the outer enamel surface from chemical insults (Jenkins, 1966) by acting as a diffusion barrier and displaying ionic permeability (Zahradnik, Morena & Burke, 1976). Meurman & Frank (1991a) examined the influence of acquired pellicle on enamel erosion *in vitro*. Bovine blocks were immersed in a cola beverage and then examined using SEM. The pellicle was found to have a protective effect, even although the pellicle did not cover the entire surface of the tooth. Various studies have tried to take the effect of pellicle into account when studying erosion *in vitro*. An investigation by Nieuw Amerongen, Oderkerk & Driessen (1987) indicated that pellicle inhibited demineralisation by citric acid, but Rytomaa *et al.* (1988) failed to show any protective mechanism. However, recent investigations (Balz & Hannig, 1997; Medrano *et al.*, 1997) confirmed that pellicle seems to be an important factor in protecting enamel against erosion, although this may depend on the length and nature of the acid exposure (Tucker *et al.*, 1996). In addition, a recent study showed both qualitative and quantitative differences in pellicles produced by erosive and non-erosive individuals (Bell, Creanor & Foye, 1998).

In an epidemiological study of four-year-old children from varying socio-economic backgrounds, erosion was found to be less common in those of lower social class (Millward, Shaw & Smith, 1994). It was concluded that poorer oral hygiene and the protective effect of mature pellicle were responsible for this observation. Pellicle acts as a substrate for colonisation by bacteria and hence the formation of plaque (Edgar, 1992). It is recognised that plaque acts as an extremely good buffer (Jenkins,

1966), confirming the clinical finding that erosion tends to occur in areas that are free from dental plaque (McIntyre, 1992).

1.4.5 Salivary clearance

A further factor that may also be involved in the development of dental erosion is the complex manner in which substances are eliminated from the oral cavity. Work by Weatherell *et al.* (1986) on fluoride distribution following rinsing showed that substances could spread very slowly around the oral cavity and that the movement was influenced not only by salivary secretion and swallowing, but also by individual variation. Similar studies using glucose as a marker (Weatherell *et al.*, 1989) confirmed site-specific differential patterns in oral clearance. Salivary clearance in children was examined by Watanabe (1992) and found to follow a similar pattern to that of adults. Clearance rates also vary depending on the mode of drinking, with drinks from a feeder cup taking longer to be eliminated than those taken from a glass or through a straw (Smith & Shaw, 1993). Citric acid clearance was found to vary between individuals by Bashir, Ekberg & Lagerlöf (1995) and a further study (Bashir, Gustavsson & Lagerlöf, 1995) confirmed that citric acid concentrations were higher on upper incisors than on lower incisors or sublingual sites. This pattern seemed to explain the clinical distribution and high prevalence of enamel erosion on upper incisors.

In conclusion, saliva has many functions in the oral cavity, summarised by Zero (1996) as follows:

- It dilutes and clears potentially erosive agents
- It neutralises and buffers dietary acids
- The calcium and phosphate in saliva maintain a supersaturated state next to the tooth
- Salivary pellicle protects the tooth surface from demineralisation
- Saliva provides calcium, phosphate and fluoride for remineralisation

1.5 Treatment

The treatment of erosion lesions is complicated and, as is often the case, prevention is better than cure.

1.5.1 Prevention

Once the main aetiological factor or factors have been identified, appropriate preventive advice can be given. A thorough clinical history will elicit information about the relevant factors (Lussi, 1996). Those patients with erosion from a dietary source should be given counselling about reducing the frequency of the erosive products, changing the method of consumption and avoiding erosive foods and drinks between meals and before bedtime, when the salivary flow rate is virtually nil (Bevenius, L'Estrange & Angmar-Månsson, 1988; Nunn, Shaw & Smith, 1996). Where erosion is thought to be due to intrinsic acids, appropriate referral to a medical practitioner is required (Shaw & Smith, 1994). Sodium bicarbonate (Bevenius, L'Estrange & Angmar-Månsson, 1988) or magnesium hydroxide (Nunn, Shaw & Smith, 1996) placed in an occlusal splint helps to neutralise the effects of gastric acids. A recent study by Attin *et al.* (1997) showed that, following an acidic

challenge, further enamel was lost from toothbrush abrasion, with a significant decrease in enamel hardness. Oral hygiene instruction should, therefore, include advice that brushing be avoided immediately after erosive insults, to reduce enamel loss through abrasion (Imfeld, 1996b). Likewise, acidic mouthwashes and abrasive toothpastes should be avoided (Lussi, 1996). However, application of desensitising toothpastes will help reduce sensitivity (Nunn, Shaw & Smith, 1996), while bicarbonate-containing toothpastes buffer and neutralise acids taken into the mouth (Imfeld, 1996b). Antacid tablets, used in the treatment of gastric reflux, are effective buffers and have been shown to counteract the drop in oral pH following the consumption of an acidic drink (Meurman *et al.*, 1988). The use of chewing gum to stimulate salivary flow, and hence neutralisation and remineralisation, may also be beneficial and investigations into the addition of neutralising agents such as sodium bicarbonate and urea are ongoing (Imfeld, 1996b).

1.5.2 Remineralisation

The remineralisation of carious lesions has been studied extensively, but less is known in relation to the erosive process, where the term "repair" may actually be more appropriate (Imfeld, 1996b). The consumption of products with high calcium and phosphate content, such as milk and cheese, is thought to help counteract erosion. An *in situ* study by Gedalia *et al.* (1991a) showed that enamel softened by a cola beverage was rehardened when exposed to milk and saliva, and this was presumed to be due to the deposition of organic and mineral material onto the enamel surface. A similar study showed that hard cheese could reharden enamel surfaces (Gedalia *et al.*, 1991b). Although a reduction in porosity occurred, the enamel did not return to its former morphological state. In this study, parafilm stimulated saliva

did not cause significant rehardening, which may have been due to the lack of repeated stimulation and the short time after which rehardening was measured. Another study failed to show that saliva could reduce the depth of enamel erosion *in vitro* (Rytomaa *et al.*, 1988), even when fluoride was added to the saliva at a level which had previously been shown to be effective in the remineralisation of caries (2 ppm). It was concluded that the role of fluoride in the prevention of demineralisation and repair by remineralisation may not function as well as it does in the caries process. However, a recent *in situ* study showed that fluoride and saliva reharden enamel previously exposed to citric acid (Stosser & Nekrashevych, 1998). The authors suggest that other protective agents such as calcium and phosphate should also be used to enhance remineralisation.

Remineralising solutions used in caries research were tested with regard to erosion lesions (Kelly & Smith, 1988) and found to have a small but statistically insignificant effect at reducing the amount of erosion *in vitro*. The fact that there are fundamental differences between the chemistry of erosion and caries may account for this finding. An investigation into the effects of brushing acid-etched enamel with and without dentifrice showed some interesting results (Kuroiwa *et al.*, 1994). Brushing with paste caused abrasion of acid-eroded enamel while brushing without paste resulted in the formation of a thick pellicle. The acid-etched enamel was being remineralised by precipitation of minute crystallites from etched enamel and minerals from the saliva. Recently, a buffering lozenge has been developed by Tenovuo *et al.* (1997) which releases, amongst other ingredients, calcium, phosphate, buffering compounds and fluoride. It was shown to elevate salivary pH and buffering capacity and may, therefore, be of benefit to those prone to erosion, especially if flow rates are low.

Further research is ongoing to analyse chemically the long-term efficacy of such a lozenge.

1.5.3 Fluoride

There is much more known about the role of fluoride in the progress of caries rather than erosion lesions. Despite this, the use of neutral fluoride mouthwashes has been advocated in the control of erosion (McIntyre, 1992; Nunn, Shaw & Smith, 1996; Lussi, 1996). The effect of adding fluoride to soft drinks has been discussed previously. However, some studies have used fluoride in other forms and these will now be reviewed. A fluoride varnish (*Duraphat*) and a sodium fluoride solution were used to treat enamel specimens prior to their immersion in a cola beverage (Sorvari *et al.*, 1994). Surface microhardness testing showed an increase in enamel hardness and subsequent inhibition of enamel softening. SEM confirmed that the varnish component had remained on the surface even after immersion in acetone, which was used to remove the *Duraphat*. This suggested that the varnish itself may act as a barrier against erosion. The authors were keen to emphasise that, although topical fluoride inhibited initial erosion, it could not prevent erosion completely. The structure of the enamel surface and the presence of prismatic and aprismatic enamel were further modifying factors in the progress of erosion. However, it was concluded that topical fluoride could be effective, especially as erosion etching of the enamel increases the surface reaction area, allowing topically applied fluoride to accumulate in the enamel and provide protection against further erosive challenges.

Low, rather than high concentrations of fluoride, applied frequently are thought to be more effective in the remineralisation of carious lesions. However, in the case of

erosion, fluoride is applied to stop the progression of the lesion by reducing the acid solubility of the surface. The application of high concentrations of fluoride may, therefore, be more suited to this purpose (Imfeld, 1996b). An investigation into the effect of fluoride in toothpaste was carried out by Bartlett, Smith & Wilson (1994). The erosion/abrasion model used in this trial and a previous one (Kelly & Smith, 1988) indicated that wear due to erosion was greater than that due to abrasion, while a combination of erosion and abrasion produced more wear than either component did separately. In the 1994 study, the authors were interested mainly in the abrasive component of an erosion/abrasion regime. The results showed that the use of a fluoride toothpaste during the abrasive component reduced enamel solubility during the erosive episode. An *in vitro* investigation by Attin, Zirkel and Hellwig (1998) into the abrasion of eroded enamel showed sodium fluoride applications significantly reduced tissue loss.

McIntyre (1992) recommended a combination treatment of fluoride mouthwashes and antacid rinses. Theoretically, by using the mouthwash first, fluoride would be incorporated into the softened tooth, stimulating remineralisation, before the alkaline antacid rinse completed rehardening of enamel. However, it was noted that fluoride would not totally prevent erosion because at pH values below 4.5, even fluorapatite dissolves. A recent *in vitro* study showed that treatment of enamel with titanium tetrafluoride inhibited enamel softening (Buyukyilmaz, Ogaard & Rolla, 1997). In conclusion, it seems that fluoride does play a role in the prevention of dental erosion, although more research is needed to determine the mechanisms involved (Rugg-Gunn, 1993).

1.5.4 Restorative options

Various restorative approaches are available to repair the function and aesthetics of the dentition. However, various authors emphasise that definitive restorations should not be placed until the cause of the erosion has been identified and eliminated (Bevenius, L'Estrange & Angmar-Månsson, 1988; ten Cate & Imfeld, 1996; Nunn, Shaw & Smith, 1996). Loss of enamel leads to an increase in tooth transparency (Eccles & Jenkins, 1974), with continued wear leading to fractures of enamel and shortening of teeth (Eccles, 1982b). The visibility of the underlying dentine increases with enamel loss, producing a yellow tooth colour (Bishop, Briggs & Kelleher, 1994). At this stage, composite or porcelain veneers are the treatment of choice to improve aesthetics (Lambrechts *et al.*, 1996). Adhesive techniques avoid the need for additional tooth preparation and so are preferable to conventional methods. The use of palatal veneers has been reported by several authors (Milosevic, 1990; McLundie, 1991; Reid, Simpson & Taylor, 1991). As wear progresses, dentine becomes exposed, leading to sensitivity and even pulpal involvement (Bishop, Briggs & Kelleher, 1994). Interocclusal space is also lost, necessitating orthodontic treatment (or the use of intrusion appliances in adults) to recreate space prior to restorative treatment (Bishop, Briggs & Kelleher, 1994; Reid, Simpson & Taylor, 1991). Full coverage crowns are sometimes required when tooth tissue loss is extensive (McIntyre, 1992; Lambrechts *et al.*, 1996). As with many aspects of dental erosion, there is no consensus on the best method of treatment. However, it is clear that the widespread availability of adhesive dentistry gives practitioners many options, allowing treatment to be tailored to the needs of the individual patient.

1.5.5 Monitoring erosion

Patients who have received treatment for erosion should be followed up to allow preventive advice to be reinforced and restorations to be monitored (Bevenius, Evans & L'Estrange, 1994). Study models taken at regular intervals and used in conjunction with a silicone rubber index allow the progression of gross wear to be assessed (Nunn, Shaw & Smith, 1996). It has been suggested that circles of unfilled resin can been bonded to the tooth surface to act as marker points and determine whether erosion is continuing or has been controlled on that surface (McIntyre, 1992). Metal disks, cemented onto eroded enamel surfaces, have also been recommended, with subsequent analysis using profilometry (Bartlett, Blunt & Smith, 1996). The use of a replica impression technique has been proposed by Millward, Shaw & Smith (1995) to allow microscopic examination and comparison of erosion lesions *in vivo*. This would permit accurate lesion monitoring during the clinical management of patients. Whitehead *et al.* (1997) also used a replica impression technique and examined changes in surface enamel using profilometry, again allowing erosion to be monitored *in vivo*. Digital terrain modelling, where a computer generates a 3-D picture, has recently been described as an accurate and reproducible technique of monitoring progress of erosion (Chadwick & Mitchell, 1996).

The fact that the treatment of dental erosion is complicated highlights the importance of prompt intervention. Erosion should be diagnosed and treated at an early stage to allow preventive measures to be taken and to minimise any operative treatment required.

1.6 Aims of the Present Study

Dental erosion is a vast subject and there has been a great deal of research conducted in this field already. There does seem to be an individual susceptibility (Moss, 1998) and so the current research was planned with this in mind. The first stage was to attempt to rank various soft drinks in order of erosive potential, as a purely *in vitro* study. Acid/base titrations were carried out to assess the buffering capacity of a range of soft drinks. Saliva was then introduced as the main biological modifying factor present in the mouth. Saliva was used as a biological base in acid/base titrations to test the efficacy of saliva as a neutralising and buffering agent. The intention was also to test the results from the previous study and see if the ranking order would be maintained in a biological model. Saliva from normal and erosive subjects was used to try and determine differences in salivary buffering ability between those with and those without erosion.

The next aim was to investigate what, if any, variations occurred *in vivo* during drinking. Normal and erosive volunteers were used once again to study variations in salivary buffering between the two groups. Various drinks were also used to continue the investigation into the proposed differences between the erosive potential of different drinks. Finally, a study of the organic elements of saliva was carried out. The organic component of saliva is believed to be involved in buffering at extremes of pH and formation of acquired pellicle, which may protect against enamel erosion. Analysis of salivary proteins was performed on a range of subjects from normal and erosive groups to see if there were any quantitative or qualitative variations between the two populations.

2 MATERIALS AND METHODS – GENERAL

2.1 Introduction

Aqueous solutions can be described as acidic or basic (alkaline). In a neutral solution, hydrogen ion concentration equals hydroxyl ion concentration. Where the concentration of hydrogen ions exceeds that of hydroxyl ions, a solution is said to be acidic. Aqueous solutions that are alkaline have a greater concentration of hydroxyl ions than hydrogen ions. The acidity of a solution can be described by giving its hydrogen ion concentration.

2.1.1 The pH scale and pK values

Hydrogen ion concentration can be expressed using molar values (M), but this may involve the use of small fractions, e.g. 10^{-14} M. The pH scale is one method widely used to assess hydrogen ion concentration, using a logarithmic scale to give a convenient way of expressing acidity. The hydrogen ion concentration is inversely proportional to the pH value, so as hydrogen ion concentration decreases, pH rises. In addition, as the pH increases by one unit, hydrogen ion concentration decreases by a factor of 10. Solutions with a pH value greater than 7 are alkaline, whereas solutions with a pH value below 7 are acidic.

Acids are described as weak or strong, depending on their ability to dissociate in water, with weak acids only dissociating partially in water. Each acid has an equilibrium constant (K_a). This acid dissociation constant describes the extent to

which the acid dissociates in solution. Acids with more than one hydrogen ion, such as phosphoric acid, have a K_a value for each dissociation step.

2.1.2 Measurement of pH

In this project, pH was measured using a glass combination microelectrode (Microelectrodes Inc., Bedford, NH, USA), connected to an Ionanalyser EA940 (Orion Research, Forest Row, East Sussex, UK), as seen in Figure 2.1. The electrode was calibrated at the start of each experiment using standard buffer solutions of pH 7 and pH 4, which spanned the expected pH values. These standard solutions were made up by dissolving buffer tablets (Merck, Poole, UK) of the required pH value in 100 mL of deionised water. The electrode was calibrated first against the pH 7 buffer, as pH 7 is the isopotential point of the electrode. Fresh buffer solutions were used at the beginning of each session. The electrode was washed in a stream of deionised water between solutions and all solutions were stirred with a non-heating magnetic stirrer (Bibby Sterelin Ltd., Stone, Staffs, England) during pH measurement (Figure 2.1). The pH was measured once the reading became stable.

The electrode was cleaned at the end of each session in a standard cleaning solution (Electrode Cleaning Solution for Biological Materials, Whatman International Ltd., Maidstone, Kent), as recommended by the manufacturers. The solution contained pepsin and hydrochloric acid to remove any protein deposits accumulated during the experiment. When not in use, the electrode was stored in a glass tube containing a sponge moistened with buffer pH 4, to prevent the membrane from drying out.

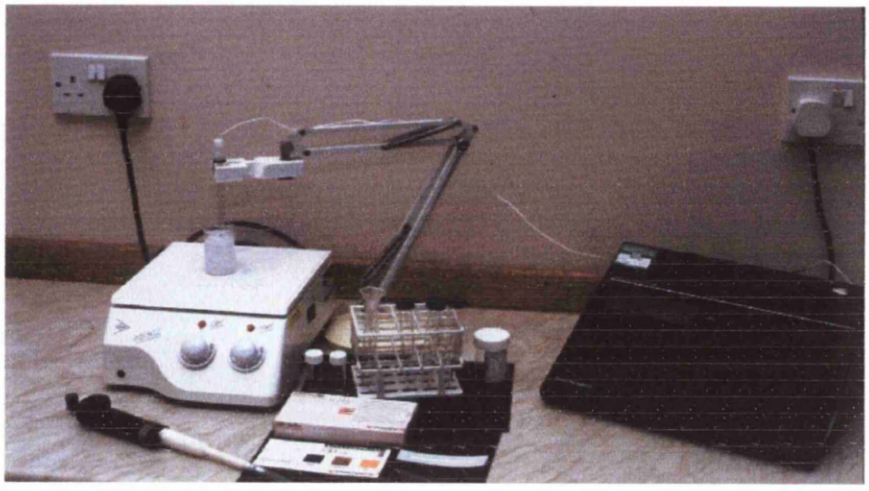

Figure 2.1
Laboratory equipment used to measure pH and collect saliva.
A glass microelectrode connected to an Ionanalyser was used to measure pH. Samples of saliva were collected via a funnel into graduated test tubes. Salivary buffering capacity was assessed using the reactive pads of the *Dentobuff* kit.

2.1.3 Titrations

Acidity or alkalinity titrations determine the total amount of acid or base in a sample. For example, by measuring the volume of base required to react with a known volume of acid solution, it is possible to determine the concentration of acid in that solution. Sodium hydroxide is a frequently used base. A 1M solution is created using 40g (molecular weight of NaOH) of sodium hydroxide pellets (Merck, Poole, England), made up to one litre by adding deionised water. A 2M solution requires 80g of sodium hydroxide pellets, etc.

To determine the amount of titratable acid in a solution, sodium hydroxide can be added to bring the pH to neutrality. The amount of sodium hydroxide added can be converted to give the equivalent acid in the solution (Bhatti, Walsh & Douglas, 1994).

2.1.4 Buffers and buffering capacity

A buffer is a solution where the pH remains nearly constant despite the addition of moderate amounts of acid or base (Hearst & Ifft, 1976). Many biological buffers exist, including those in blood and saliva, which play an important role in maintaining homeostasis. Buffers contain two species, one to react with hydrogen ions, the other with hydroxyl ions, although the two must not react with each another (Masterton, Slowinski & Stanitski, 1981). Typically, a buffer is a mixture of a weak acid and its conjugate base, and this can be described as a conjugate acid/base pair.

The capacity of a buffer describes the amount of acid or base that a solution can absorb without a significant change in pH. The term buffering capacity is defined as the number of moles per litre of base that must be added in order to increase the pH by one unit (Hearst & Ifft, 1976). The greater the amount of base needed to increase the pH by one unit, the greater the buffering capacity. A graph of pH plotted against added base gives a visual demonstration of buffering capacity, with the smallest slope showing the maximal buffering capacity and the greatest slope minimal buffering capacity.

2.2 Salivary Collection

All salivary samples were collected at least one hour after the last intake of food. Where possible, most samples were taken between 10 a.m. and 2 p.m., to minimise Circadian variation.

2.2.1 Unstimulated saliva

Individuals were asked to sit quietly, with their heads bowed forwards. Prior to collection of the sample, they were requested to swallow any residual saliva present in the mouth. The volunteers were then asked to allow saliva to collect under the tongue, which was then expectorated into a graduated test tube with a funnel (Figure 2.1). To allow flow rates to be measured, the test was carried out over a 5 min period, the total volume measured and the final result expressed in mL per min.

2.2.2 Stimulated saliva

Salivary flow can be stimulated by mechanical and gustatory means. The use of sorbitol gum in the present study fulfilled both of these roles. Volunteers were given two coated gum pellets (Wrigley's *Extra*, The Wm. Wrigley Junior Co. Ltd., Plymouth, England) and asked to chew and swallow normally for 2 min, to allow the coating to be removed from the gum. They were then asked to swallow all the saliva present in the mouth before the test period commenced. The individuals continued to chew for 2 min while spitting any saliva produced into a graduated test tube. The final volume was divided by two to give a result in mL per min.

2.2.3 Storage of saliva

There are various components present in saliva that play a role in salivary buffering capacity, including the electrolyte bicarbonate. The concentration of bicarbonate may be affected as carbon dioxide diffuses out when saliva is exposed to atmospheric air. Collection of saliva under liquid paraffin lowers the diffusion of carbon dioxide (Söderling, 1989). For the same reason, when the pH of small volumes of saliva was being measured, *Clingfilm* was used to seal the plastic tube and so reduce evaporation (Creanor *et al.*, 1994). Saliva samples were analysed as soon as possible after collection to minimise any changes which may have occurred.

2.2.4 Salivary buffering capacity.

Salivary buffering capacity is regulated by carbonic acid/bicarbonate, phosphate and salivary proteins. One straightforward, commercially available method used to

measure salivary buffering capacity at the chairside is the *Dentobuff* kit (Frostell, 1980), or *Vivacult BC* system (Ivoclar Vivadent, Meridian, Leicester, UK). A sample of saliva is placed on an absorbent reactive pad, as seen in Figure 2.1, and the colour change observed after 5 min. The result gives an indication of the final pH of the saliva and, thus, whether the salivary buffering capacity is low (pH <4), medium (pH 4.5-5.5) or high (pH >6). If salivary mucins cause the colour change to be uneven, the lowest value is taken, as recommended by the manufacturers.

2.3 Clinical Methods

Clinical work mainly involved salivary sampling. However, in some cases, a clinical assessment of erosion was also required.

2.3.1 Assessment of erosion

A modified Eccles index of erosion was used throughout the research, as referred to previously (Section 1.2.2).

I Enamel erosion only

II Dentine involved, but for less than 1/3 of surface

III Obvious pulpal involvement or extensive dentine exposure

2.3.2 Assessment of caries

To gain some insight into the relationship between caries and erosion, a guide to an individual's caries experience was also used. The DMF index allows the number of

teeth decayed, missing and filled as a result of caries, to be counted. The use of DMFT gives a broad indication of the treatment experience or need by counting only entire teeth, regardless of the number of surfaces involved. DMFS describes all tooth surfaces and is, therefore, more precise although it is not specific (Whelton & O'Mullane, 1997). The DMF index is widely used in epidemiological studies of dental caries.

3 BUFFERING CAPACITIES OF SOFT DRINKS *IN VITRO*

3.1 Introduction

The rising prevalence of erosion has been linked to the increasing consumption of soft drinks, the UK sales of which have risen seven-fold since 1950 (Shaw & Smith, 1994). The sorts of drinks that may cause erosion are those with intrinsically low pH, and include ones such as carbonated beverages, fruit juices, diluting juices, squash drinks and flavoured mineral waters. The erosive potential of beverages is thought to involve several factors, as discussed previously (Section 1.3.3), including low pH and titratable acidity. The ability of a drink to resist pH changes brought about by salivary buffering may inevitably result in a prolonged period of oral acidity and, therefore, may play an important part in the erosion process (Grobler & van der Horst, 1982).

The aims of this study were, therefore, to measure the initial pH of several widely available soft drinks and to determine their ability to maintain a low pH, by measuring their buffering capacities.

3.2 Materials and Methods

Several groups of drinks were tested and, where available, "diet" or "light" versions of drinks were tested with still mineral water as control.

3.2.1 Groups of drinks

Five groups of drinks were tested. There were six varieties of fruit juices (FJ) in the first group including apple, grape, pineapple, grapefruit, and orange juice, as well as freshly squeezed orange juice. Six carbonated drinks (CD) such as *Coca-Cola, Pepsi* and *Irn Bru* and their diet equivalents were chosen to make up the next group. A further group consisted of six fruit-based carbonated drinks (FBCD), for example *Fanta, 7-Up* and *Lilt* together with the diet versions. Six flavoured spring waters (FW) were also selected. These included *Rio Tropical, Rio Light, Caledonian Clear* (contains fructose), *Perfectly Clear* (contains aspartame sweetener) and *Strathmore Twist* (no sweetening agent), as well as an "own brand" variety from *Marks & Spencer (M&S)* of peach water (contains aspartame sweetener). Finally six plain carbonated mineral waters (PW) were tested, which included *Perrier, Strathmore* and some supermarket own labels from *Safeway, M&S* and *Boots*. The still mineral water used as a control was *Evian*

3.2.2 pH measurement

The initial pH of each drink was measured using a pH electrode connected to an Orion EA940 Ionanalyser, as described in Chapter 2 (Section 2.1.2). One hundred mL of the freshly opened drink, which was at room temperature, was placed in a beaker and stirred using a non-heating magnetic stirrer until a stable reading was obtained. Several readings were taken of the drinks from each group to give a mean measurement for that drink.

3.2.3 Buffering capacity

One hundred mL of each drink was titrated with 1 M sodium hydroxide (NaOH), added in 0.5 mL increments, until the pH reached 10 (Touyz, 1994) to assess the total titratable acidity - a measure of the drink's own buffering capacity. The samples were again stirred using a non-heating magnetic stirrer until a stable pH reading was obtained after each addition of NaOH. Titrations were repeated in triplicate for several drinks from each group to ensure reproducibility and to give a mean value for that drink. Drinks that were at either extreme of their group following the first titration were tested again, as well as any drinks giving an unusual result.

Assessment of buffering capacity data was carried out in two ways. Initially, a graph was plotted of pH against added millilitres of sodium hydroxide. The slope of the resultant curve allowed comparison of differing buffering capacities. In addition, the total volume of sodium hydroxide required to raise the pH to 10 was noted, as well as to pH 5.5 and pH 7. These amounts for the various groups of drinks were then used for statistical analysis.

3.2.4 Data analysis

To enable the readings from the separate types of drinks within each of the five main groups to be combined and treated as a single homogeneous sample, it had to be ascertained that there were no significant differences between the separate types of drink within the five groups. The amounts of sodium hydroxide required to titrate the drinks to pH 10 were compared within each of the five groups. When it was clear that

no differences existed within each group, the data were combined into five main groups. The mean amounts of NaOH required to raise the pH of each group of drinks were then compared using Mann Whitney tests.

3.2.5 Other investigations

In order to investigate the contribution of carbonic acid to the pH and buffering capacity of soft drinks, some titrations were carried out on drinks which had been allowed to go "flat". By leaving the cans open on the bench for some time, it was assumed that the carbon dioxide, formed from carbonic acid in solution, had been lost to the atmosphere. Titrations were then carried out in the normal manner and the slopes obtained were compared with the titration curves of the equivalent fresh drink

3.3 Results

The results showed that there were clear differences between each of the five groups of drinks.

3.3.1 pH measurement

There was a large range of initial pH values for the soft drinks tested. Table 3.1 shows examples of drinks with the highest and lowest pH values in each group. These range from pH 2.48 for *Pepsi Cola* to pH 5.51 for *Perrier* water, with the control still mineral water *Evian* having a pH of 7.47. The mean pH and standard deviation (SD) values obtained once the six drinks in each group were combined are

Group	Drink	pH	SD (if mean shown)
FJ	Apple juice	3.14	0.06
	Freshly squeezed orange juice	3.86	
CD	*Pepsi Cola*	2.48	0.03
	Diet Coke	3.16	
FBCD	*Lilt*	2.7	0.09
	Diet Fanta	2.91	0.07
FW	*Caledonian Clear*	2.88	0.09
	Strathmore with a twist of lime	4.86	
PW	*Highland Spring (Safeway)*	4.98	
	Perrier	5.51	0.03
SW	*Evian*	7.47	0.1

Table 3.1
Drinks with the lowest and highest initial pH values in each group. Where mean values are displayed, 1 SD is also shown.

Key:

FJ	Fruit juices
CD	Carbonated drinks
FBCD	Fruit-based carbonated drinks
FW	Flavoured waters
PW	Plain sparkling mineral water
SW	Still water
SD	Standard deviation

shown in Table 3.2. The mean initial pH was lowest for the carbonated drinks (2.81; SD=0.27) and highest for plain mineral waters (5.23; SD=0.21).

3.3.2 Buffering capacity reproducibility

Several drinks from each group were titrated repeatedly to give a measure of the reproducibility of the methodology. The slopes of the curves were compared after the drinks had been titrated on various occasions. Figures 3.1 a-f show examples of one drink from each group titrated at three different times and indicate that, especially in the early part of the titration, the soft drink behaved almost identically on all three occasions. These examples are typical of the results obtained from other drinks and thus indicate that the method of buffering capacity assessment used was reproducible.

3.3.3 Buffering capacity of fruit juices

Figure 3.2 displays the titration curves of the six varieties of pure fruit juice tested. Where triplicate measures were taken, the mean titration curve is shown. For most fruit juices, between 10 and 15 mL of NaOH were required to bring the drink to pH 10. However, grapefruit juice required 22 mL to raise its pH value. Apple juice required, on average, only 10 mL to reach pH 10 despite the fact that the initial pH of apple juice was the lowest in the fruit juice group. The numbers of mL of NaOH required to raise the pH of the fruit juices to 10 are detailed in Table 3.3. Where a mean value is displayed, 1 standard deviation is also given

Group (n=6)	Mean pH	SD
FJ	3.45	0.29
CD	2.81	0.27
FBCD	2.83	0.08
FW	3.33	0.76
PW	5.23	0.21
SW	7.47	0.10

Table 3.2
Mean initial pH value plus 1 SD for each of the groups of drinks.

Key:		
	FJ	Fruit juices
	CD	Carbonated drinks
	FBCD	Fruit-based carbonated drinks
	FW	Flavoured waters
	PW	Plain sparkling mineral water
	SW	Still water
	SD	Standard deviation
	n	number

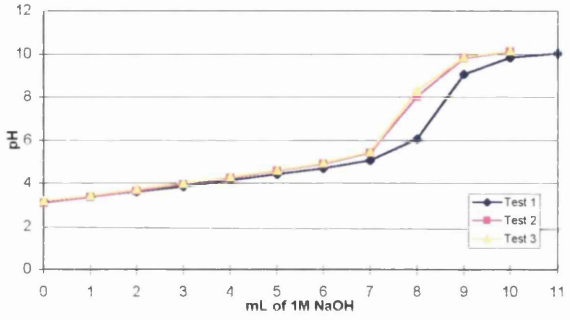

Figure 3.1 a
Repeat titrations of apple juice to ensure reproducibility.

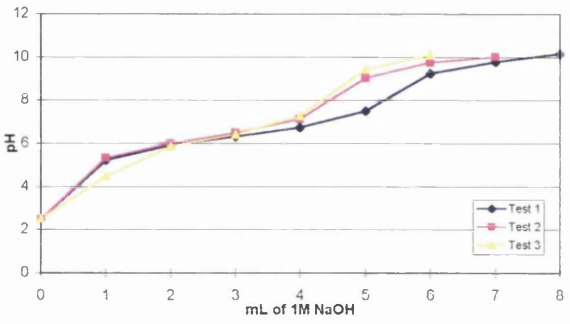

Figure 3.1 b
Repeat titrations of *Coca-Cola* to ensure reproducibility.

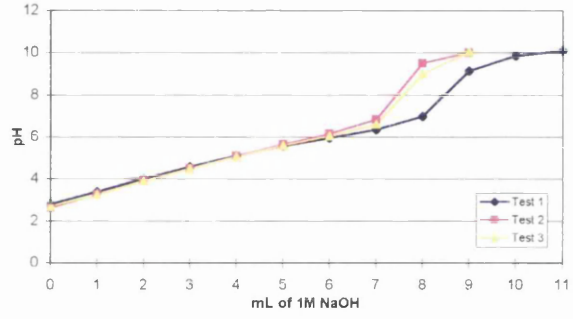

Figure 3.1 c
Repeat titrations of *Lilt* to ensure reproducibility.

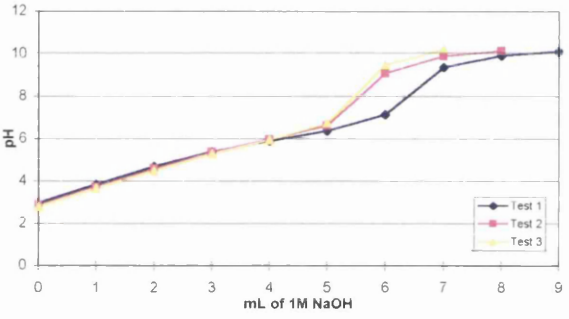

Figure 3.1 d
Repeat titrations of *Caledonian Clear* to ensure reproducibility.

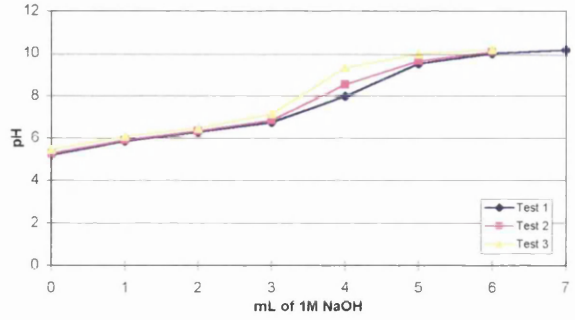

Figure 3.1 e
Repeat titrations of *Brecon Careg* to ensure reproducibility.

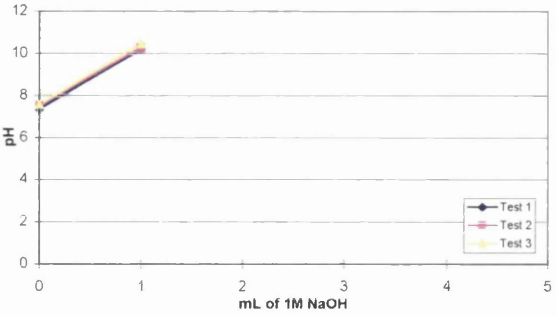

Figure 3.1 f
Repeat titrations of *Evian* to ensure reproducibility.

Figure 3.1
Repeat titrations to ensure reproducibility, with one drink from each group being shown as an example.

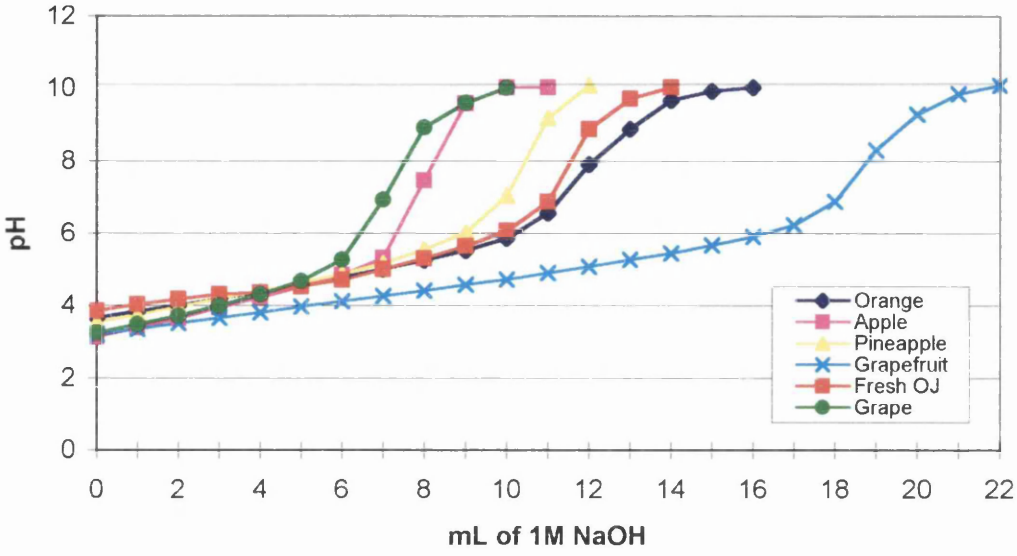

Figure 3.2
Titration curves for the six fruit juices tested.

Key: OJ Orange juice

Drink	mL of NaOH	SD (if mean shown)
Apple juice	9.83	0.58
Grape juice	10	
Pineapple juice	12.5	
Freshly squeezed orange juice	13.5	
Orange juice	14.83	0.76
Grapefruit juice	21.5	0.5

Table 3.3
The number of mL of NaOH (sodium hydroxide) required to raise the pH of the fruit juices to 10. Where mean values are displayed, 1 SD (standard deviation) is also shown.

3.3.4 Buffering capacity of carbonated drinks

The titration curves for the six carbonated drinks that were not fruit-flavoured are shown in Figure 3.3. The carbonated drinks required approximately 5 - 8 mL of base to raise their pH to 10. The mean number of mL of NaOH needed to bring the pH to 10 for this group was 6.64 mL. There were no large differences between *Coca-Cola* and *Diet Coke,* or *Irn Bru* and *Diet Irn Bru*. This suggests that the presence or absence of sugar does not affect the buffering capacity of a drink. There was a discrepancy between *Pepsi* and *Diet Pepsi,* but this was not significant when compared using the Mann Whitney test. Table 3.4 displays the mean number of mL of NaOH required to bring each drink in the group to pH 10.

3.3.5 Buffering capacity of fruit-based carbonated drinks

The fruit-based drinks *Lilt*, *Fanta* and *7-Up*, plus their diet equivalents, required between 6 and 10 mL of NaOH to be brought to pH 10, with an average of 8.7 mL. The titration curves are displayed in Figure 3.4. Table 3.5 shows the number of mL required to raise the pH of each drink. One SD is shown where the mean number of mL is quoted. There were no differences observed between regular and diet versions of the same drink. The drinks *7-Up* and *Light 7-Up* behaved in a similar fashion, as did *Lilt* and *Diet Lilt*. *Fanta* and *Diet Fanta* showed a larger discrepancy, but again this was not significant when compared using the Mann Whitney test.

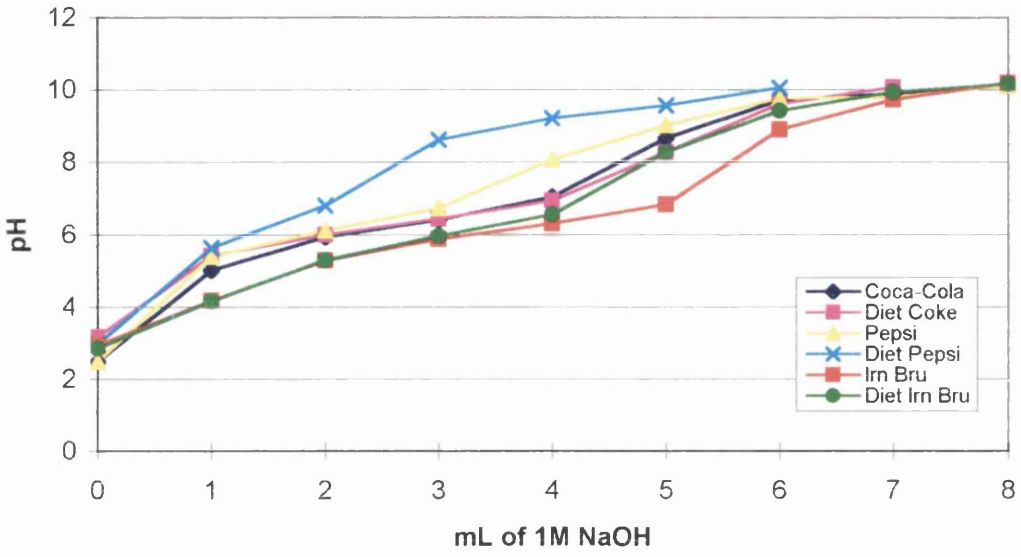

Figure 3.3
Titration curves for the six carbonated drinks without fruit flavouring.

Drink	mL of NaOH	SD (if mean shown)
Diet Pepsi	4.67	2.02
Pepsi Cola	6.5	1.32
Coca-Cola	6.83	1.04
Diet Irn Bru	6.83	1.04
Diet Coke	7	
Irn Bru	8	

Table 3.4
The number of mL of NaOH (sodium hydroxide) required to raise the pH of the carbonated drinks to 10. Where mean values are displayed, 1 SD (standard deviation) is also shown.

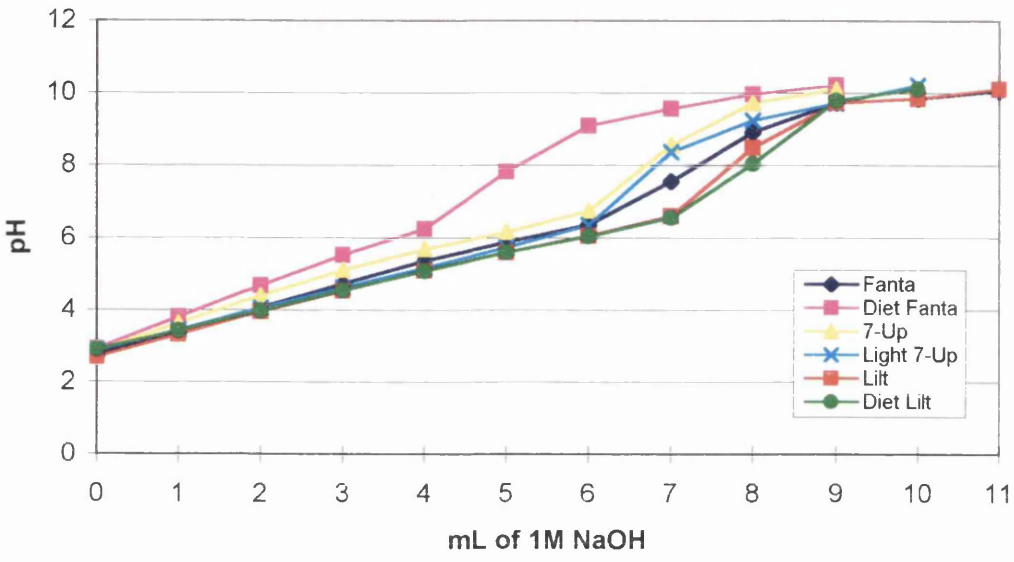

Figure 3.4
Titration curves for the six fruit-based carbonated drinks.

Drink	mL of NaOH	SD (if mean shown)
Diet Fanta	6.67	1.61
7 Up	8.67	0.29
Light 7 Up	8.83	1.89
Fanta	9.17	1.26
Lilt	9.33	1.04
Diet Lilt	9.5	

Table 3.5
The number of mL of NaOH (sodium hydroxide) required to raise the pH of the fruit-based carbonated drinks to 10. Where mean values are displayed, 1 SD (standard deviation) is also shown.

3.3.6 Buffering capacity of flavoured waters

The flavoured waters group included drinks consisting of a mineral or spring water base with added fruit flavouring. All drinks were sweetened with either sugar or artificial sweeteners except *Strathmore with a twist of lime*. The presence or absence of sugar did not affect the comparable buffering capacity of equivalent regular and light drinks, with no significant differences being noted between *Rio Tropical* and *Rio Light*. This group, like the fruit-based carbonated drinks also required between 6 and 10 mL of NaOH to take the pH to 10. The titration curves are shown in Figure 3.5, while Table 3.6 lists the number of mL required to raise the pH.

3.3.7 Buffering capacity of plain mineral waters

The titration curves for the six plain sparkling mineral waters are shown in Figure 3.6, with the control, *Evian*, also shown. Between 4 and 6 mL of NaOH were required to raise the pH of the plain mineral waters. The amounts needed for each drink are shown in Table 3.7, with 1 SD being shown where appropriate.

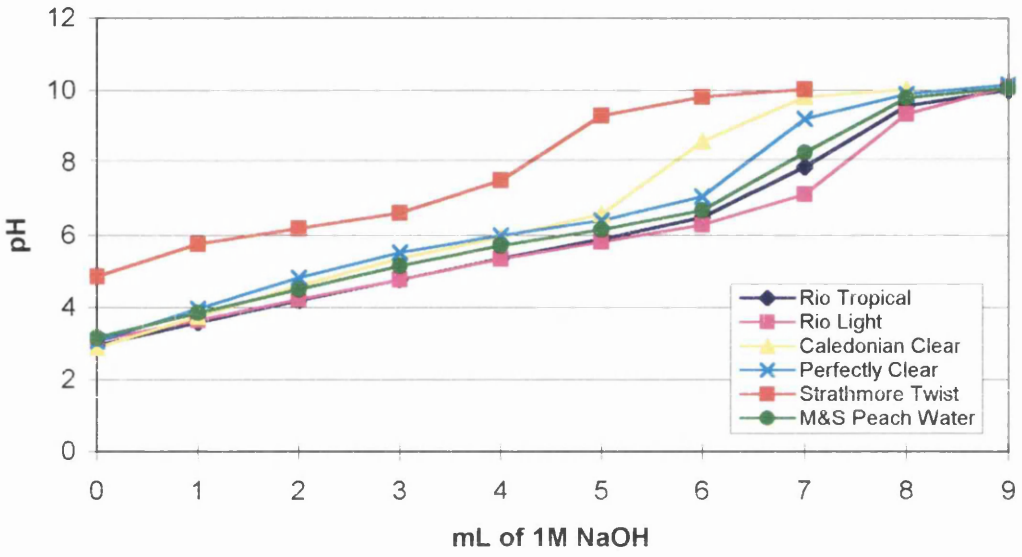

Figure 3.5
Titration curves for the six flavoured waters.

Key: *M&S* *Marks & Spencer*

Drink	mL of NaOH	SD (if mean shown)
Strathmore with a twist of lime	6.5	
Caledonian Clear	7.67	0.76
Perfectly Clear	8.5	
M&S Peach water	8.5	
Rio	8.67	1.04
Rio Light	9.17	0.29

Table 3.6
The number of mL of NaOH required to raise the pH of the flavoured waters to 10. Where mean values are displayed, 1 SD is also shown.

Key:

M&S	*Marks & Spencer*
NaOH	Sodium hydroxide
SD	Standard deviation

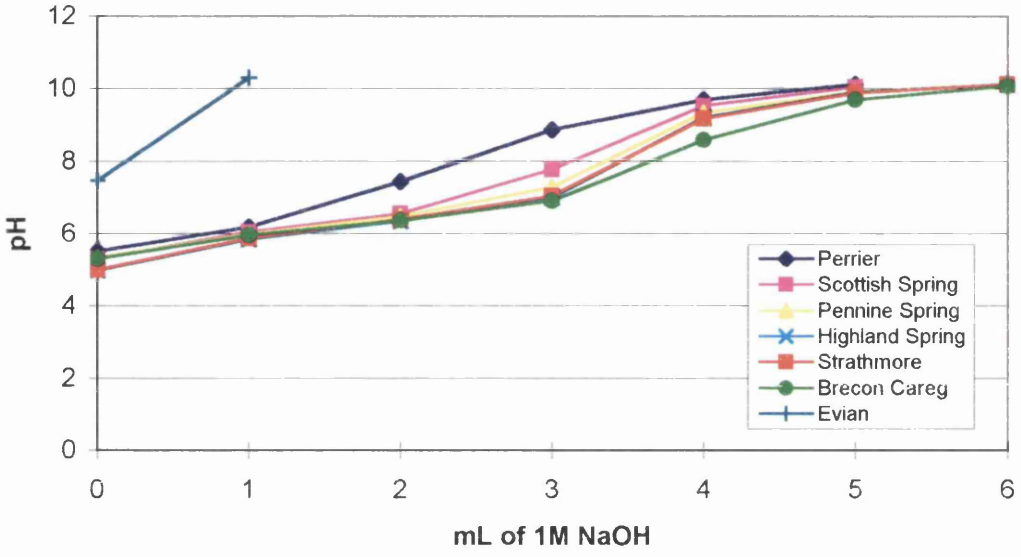

Figure 3.6
Titration curves for the six plain mineral waters and the still mineral water control.

3.3.8 Buffering capacity of groups of drinks

To enable statistical analysis to be carried out comparing the five main groups, the readings from the separate types of drinks within each of the main groups had to be combined. Prior to this, it had to be ascertained that there were no significant differences between the separate types of drink within the five groups. The amounts of sodium hydroxide required to titrate the drinks to pH 10 were noted for the individual drinks that had been analysed repeatedly. These figures were than compared within each of the five groups using Mann Whitney tests. No significant differences were found between the drinks with triplicate readings within any one group. The data in each group were, therefore, combined to give a data set for each of the five main groups of drinks and the groups compared as a whole with one another. Figure 3.7 summarises the mean titration curves for each of the five main groups, showing the differences between the slopes obtained from the various groups. Figure 3.8 shows a histogram of the differing mean amounts of NaOH required by each group to reach pH 10, with error bars indicating 1 SD. The greater the height of the bar, the higher the buffering capacity for that group.

The mean amounts of NaOH required to raise the pH of each group of drinks were then compared using Mann Whitney tests. Table 3.8 shows the p values for the individual comparisons made between each group of drinks for the number of mL of NaOH necessary to bring the pH to 10. Similar comparisons were also made between the number of mL required to raise the pH to 5.5 and 7. These results are shown in Tables 3.9 and 3.10 respectively. The fruit-based carbonated drinks and flavoured mineral waters were significantly different from the carbonated drinks and plain mineral waters respectively at each of the above pH reference points. There was no

Drink	mL of NaOH	SD (if mean shown)
Perrier	4.2	1.0
Scottish Spring (M&S)	5	0.5
Highland Spring (Safeway)	5.5	
Strathmore	5.5	
Pennine Spring (Safeway)	5.5	
Brecon Careg (Boots)	6	0.5
Evian (Control)	0.5	0

Table 3.7
The number of mL of NaOH required to raise the pH of the plain mineral waters to 10. Where mean values are displayed, 1 SD is also shown.

Key:

M&S	*Marks & Spencer*
NaOH	Sodium hydroxide
SD	Standard deviation

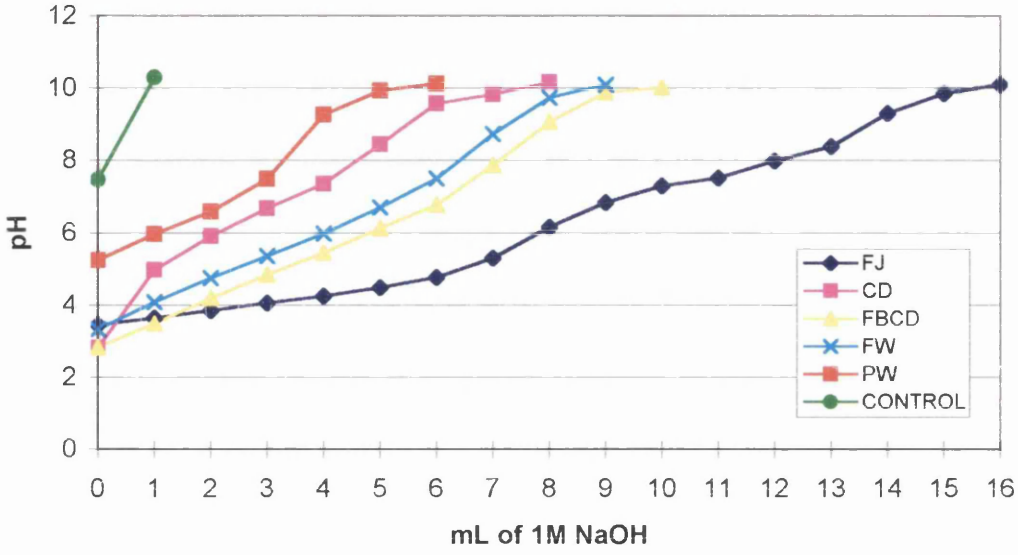

Figure 3.7
Mean titration curves for the five groups of drinks and the control.

Key:	FJ	Fruit juices
	CD	Carbonated drinks
	FBCD	Fruit-based carbonated drinks
	FW	Flavoured waters
	PW	Plain sparkling mineral water

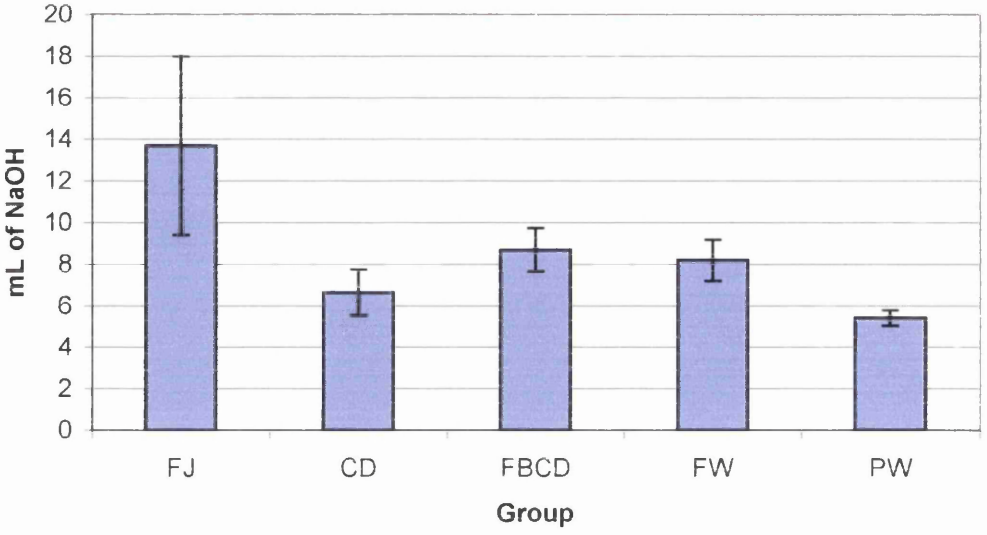

Figure 3.8
Mean number of mL NaOH required to raise pH of groups of drinks to 10. Error bars show 1 SD.

Key:		
	FJ	Fruit juices
	CD	Carbonated drinks
	FBCD	Fruit-based carbonated drinks
	FW	Flavoured waters
	PW	Plain sparkling mineral water
	SD	Standard deviation
	NaOH	Sodium hydroxide

significant difference between the fruit-based carbonated drinks and the flavoured mineral waters at the three pH levels. There were significant differences between all of the other groups, the only exception being that there was no significant difference between carbonated drinks and flavoured waters at pH 5.5. As the results of the comparisons at the three pH levels were so similar, the actual number of mL of NaOH required to reach pH 5.5 and pH 7 are not displayed.

3.3.9 Buffering capacity of flat drinks

Titrations were carried out on *Pepsi Cola*, *Coca-Cola*, *7-Up* and *Fanta*, which had been allowed to go flat. Figures 3.9 a and 3.9 b display the titration curves for some flat drinks and their fresh equivalents. The initial part of the slope is very similar, suggesting that carbonic acid contributes only weakly to the buffering capacity of a drink, even though the two curves do separate more in the later stages of the titration.

3.4 Discussion

The results of this study using an *in vitro* system indicate that the drinks within any one group behave in the same fashion due to their acid content. The repeated titrations were found to be reproducible not only for individual drinks but also for drinks within each group, emphasising their similarities. There were no differences found between regular and diet versions of the same drink, despite the fact that the presence of refined carbohydrate gives the potential for more acid production in the mouth, a feature which this study did not attempt to address. Consistent differences were maintained between the groups of drinks when analysed at pH 5.5, 7 and 10.

Group	CD	FBCD	FW	PW
FJ	0.005	0.005	0.005	0.005
CD	-	0.031	0.05	0.045
FBCD	-	-	NS	0.005
FW	-	-	-	0.005

Table 3.8
Mann Whitney test p values comparing the number of mL of NaOH (sodium hydroxide) necessary to bring the pH to 10.

Key:

FJ	Fruit juices
CD	Carbonated drinks
FBCD	Fruit-based carbonated drinks
FW	Flavoured waters
PW	Plain sparkling mineral water
NS	Not significant

Group	CD	FBCD	FW	PW
FJ	0.005	0.005	0.005	0.005
CD	-	0.005	NS	0.005
FBCD	-	-	NS	0.005
FW	-	-	-	0.005

Table 3.9
Mann Whitney test p values comparing the number of mL of NaOH (sodium hydroxide) necessary to bring the pH to 5.5.

Key:

FJ	Fruit juices
CD	Carbonated drinks
FBCD	Fruit-based carbonated drinks
FW	Flavoured waters
PW	Plain sparkling mineral water
NS	Not significant

Group	CD	FBCD	FW	PW
FJ	0.005	0.016	0.005	0.005
CD	-	0.008	0.031	0.045
FBCD	-	-	NS	0.005
FW	-	-	-	0.005

Table 3.10
Mann Whitney test p values comparing the number of mL of NaOH (sodium hydroxide) necessary to bring the pH to 7.

Key:		
	FJ	Fruit juices
	CD	Carbonated drinks
	FBCD	Fruit-based carbonated drinks
	FW	Flavoured waters
	PW	Plain sparkling mineral water
	NS	Not significant

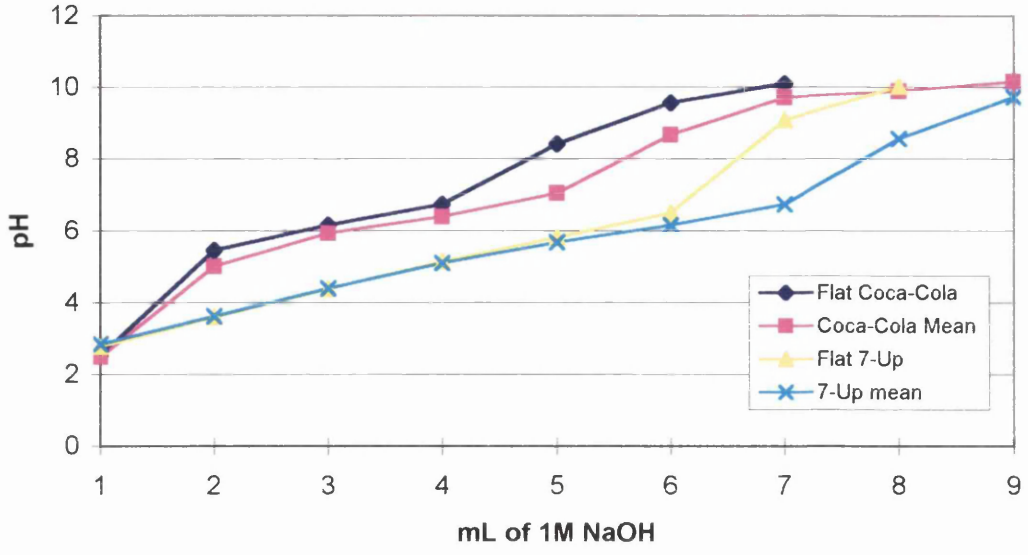

Figure 3.9a
Titration of *Coca-Cola* and *7-Up*, compared with titration of similar flat drinks.

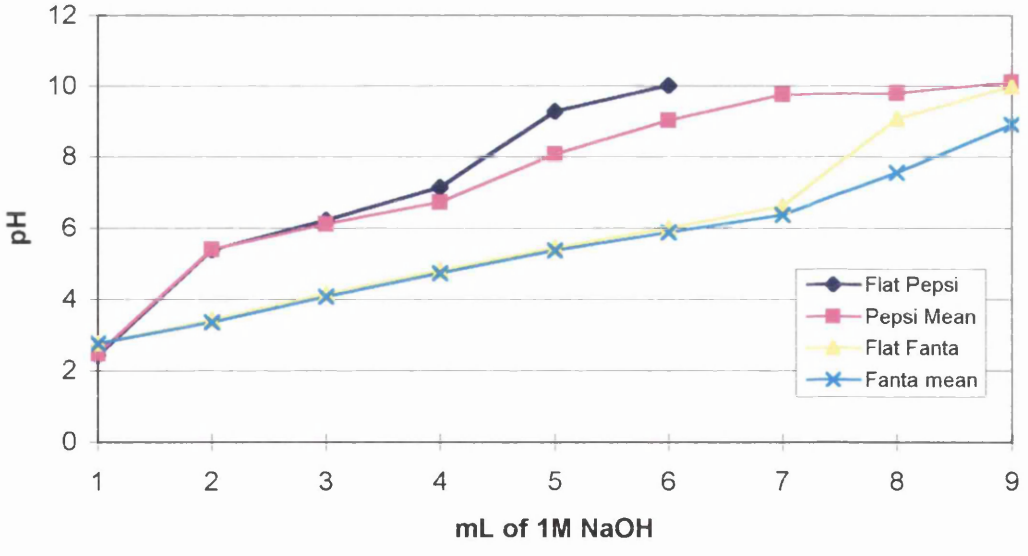

Figure 3.9b
Titration of *Pepsi Cola* and *Fanta*, compared with titration of similar flat drinks.

Figure 3.9
Titrations of fresh drinks compared with titrations of flat drinks.

The buffering capacities of soft drinks tested *in vitro* can therefore be ranked as follows:

fruit juices > fruit-based carbonated drinks including flavoured mineral waters > other fizzy drinks such as *Coca-Cola* and *Irn Bru* > sparkling mineral waters > still mineral water.

The results highlight the role of fruit-based acids in determining the buffering capacity of soft drinks. The fruit-based carbonated drinks and flavoured mineral waters were significantly different from the carbonated drinks and plain mineral waters respectively, emphasising the importance of acids derived from the fruit in enhancing the drink's own buffering capacity. The flavoured mineral waters were also significantly more buffered than the carbonated drinks, again stressing the relevance of fruit-based acids. The fact that there was no significant difference between the fruit-based carbonated drinks and the flavoured mineral waters further underlines this fact. There was, however, one flavoured mineral water that behaved differently from the others, although there was no statistical difference. *Strathmore with a twist of lime,* with no sweetening agent, had lower buffering capacity than the other drinks in the group and showed a greater similarity to the plain carbonated mineral water group. This feature may be attributed to the lack of sweetness in this drink, as additional acid is often added to beverages to counteract sweetness (Sorvari & Rytomaa, 1991).

With regard to the actual acid content of the groups of drinks, the regular cola drinks contained phosphoric acid, with their diet counterparts having both phosphoric acid and citric acid. Diet and regular *Irn Bru* also contained citric acid. Citric acid was

again present amongst the ingredients of all the fruit-based carbonated drinks. In addition, other acids were listed, quite apart from the fruit flavouring. Malic acid was present in *7-Up*, *Light 7-Up* and *Diet Lilt*, tartaric acid in *Diet Lilt* and Vitamin C in *Fanta*. The flavoured waters were carbonated and contained fruit flavouring, citric acid (*Rio Tropical, Rio Light, Caledonian Clear* and *Perfectly Clear*) and Vitamin C (*Rio Tropical* and *Rio Light*). Fruit flavoured drinks are obviously a complex mixture of acids, which buffer over varying ranges and hence resist a rise in pH.

It was also interesting to note that initial pH value gave no indication of the underlying buffering capacity and therefore the erosive potential of the drink. Generally, the pure fruit juices had a higher initial pH than the carbonated drinks but required much more NaOH to raise the pH. This study agrees broadly with those already found in the literature (Grobler & van der Horst, 1982; Grenby *et al.*, 1989) that fruit juices have greater erosive potential. However, the current investigation goes on to compare other fruit flavoured drinks with various beverages on the UK market and a clear trend is evident. It was thought important to include the flavoured mineral waters as these drinks are relatively new to the consumer and are seen by many as "designer" drinks. The public may well perceive that these waters are healthier than other canned drinks because they contain mineral water with only a hint of pure fruit to flavour it, and often have no added sugar. This study shows that the mere addition of fruit flavouring has a marked effect on the total acidity.

Another point of interest was the difference between carbonated and still mineral water. The addition of carbon dioxide, forming carbonic acid in solution, clearly lowers the pH and enhances the buffering capacity. However, the initial pH of the

plain mineral waters was around that of 5.5 and so the influence of sparkling mineral waters on salivary pH and potential dental erosion must be investigated further. As far as other carbonated drinks are concerned, the study showed that carbonic acid might contribute little to the overall acidity of these drinks. Flat drinks showed similar titration curves to fresh drinks, suggesting that other acids present in the drinks continue to maintain a low pH. Flat or still drinks, therefore, may not be considered necessarily "safer" for teeth, as the influence of the fruit flavouring on the total acid content of a drink is significant.

All of these drinks need to be tested *in vivo* to ascertain if the increased buffering properties of fruit-based drinks have a greater potential to lower the pH of the oral cavity. Many factors will be involved in the mouth, not least the ability of a drink to promote increased salivary flow due to gustatory stimulation.

3.5 Conclusions

In conclusion, it has been found that, *in vitro*, pure fruit juices have significantly greater buffering capacity than other soft drinks. Fruit-based carbonated drinks have more erosive potential than other carbonated drinks, with flavoured waters having the same erosive potential as fruit-based carbonated drinks. Carbonated drinks with no fruit flavouring have considerably less buffering capacity than other flavoured drinks tested and, therefore, may have reduced erosive potential.

4 BUFFERING CAPACITY OF SALIVA *IN VITRO*

4.1 Introduction

The previous chapter has indicated that the laboratory evaluation of soft drinks can rank them in order of titratable acidity and, therefore, erosive potential. In the oral cavity, however, saliva becomes a major modifying factor as it buffers and neutralises acidic substances. This study had three aims. The first aim was to use saliva as a biological base, rather than sodium hydroxide. By carrying out titrations with saliva, the intention was to take account of biological variations that occur in the oral cavity. Another aim of the study was to investigate the effectiveness of saliva at coping with the acidic challenge from soft drinks, while a further aim was to determine if there were differences between the buffering capacity of saliva from normal and erosive individuals.

4.2 Materials and Methods

Titrations were carried out by adding the chosen drink to saliva and the drop in pH measured.

4.2.1 Drink selection

Six drinks, which were deemed to be representative of the groups determined by the previous study, were chosen: orange juice (OJ) from the fruit juice group, *Lilt* and *Diet Lilt* from the fruit-based carbonated drinks group, *Coca-Cola* and *Diet Coke* from the carbonated drinks group and *Perrier* water from the sparkling waters group.

All drinks were stored and used at room temperature. Each drink was freshly opened and the pH measured at the start of each titration.

4.2.2 Salivary collection

Ten mL of sorbitol gum-stimulated saliva was collected from volunteers as described in Section 2.2.2. Each titration required 4 mL of stimulated saliva, so by collecting 10 mL of saliva on each occasion, 2 drinks could be tested. Saliva was collected and stored under paraffin in a graduated test tube and used as soon as possible after collection.

4.2.3 Subject selection

Eleven volunteers who had no evidence of dental erosion were chosen to test each of the six drinks in a random order, as determined by a random number table, with repeat titrations carried out on two randomly selected drinks. In addition, any readings that seemed to be unusually high or low were repeated to ensure reproducibility. The final number of titrations carried out for each individual was, therefore, at least eight. Five individuals with diagnosed dental erosion were also asked to give salivary samples on repeated occasions to enable all six drinks to be tested and repeat titrations for reproducibility to be carried out, again resulting in at least 8 titrations for each person. Saliva was also obtained on one occasion from 18 individuals with dental erosion, which enabled two random drinks to be tested. For every 3 erosion patients who gave a saliva sample, all six chosen drinks were tested. The final numbers testing each drink were, therefore, 11 normal and 11 erosive volunteers.

4.2.4 Titration protocol

Four mL of saliva was added, under paraffin, to a plastic specimen pot. The initial pH was measured while the saliva was stirred by a magnetic stirrer, as detailed previously in Section 2.1.2. The drink chosen was then added in increments and the pH measured once the reading became stable. The drink was added firstly in100 μL increments, to detail the initial fall in pH. After 1 mL of drink had been added, the increments were increased as follows:

0.5 mL increments until 5 mL of drink added

1 mL increments until 10 mL of drink added

2 mL increments until 20 mL of drink added

5 mL increments until 50 mL of drink added.

The addition of the above increments brought the pH of the saliva/drink mixture as close as possible to the initial (i.e. intrinsic) pH of the drink.

4.2.5 Other investigations

It is recommended that saliva is collected and stored under paraffin to prevent evaporation and loss of bicarbonate to the atmosphere, which is believed to be the main buffering agent in saliva (Söderling, 1989). By collecting and storing saliva without a protective paraffin seal, it could be assumed that some bicarbonate might have been lost. Some additional salivary samples, therefore, were collected and stored in this way prior to analysis, to give a crude estimate of the importance of paraffin use and bicarbonate buffering.

In a similar fashion, to test the robustness of the methodology, it was decided to investigate the effects of freezer storage on saliva. Some samples were frozen and defrosted prior to final analysis and this enabled any possible changes that might have occurred during these processes to be identified.

4.2.6 Data analyses

Graphs of salivary pH against mL of drink added were produced for each titration. In the first instance, charts were plotted for each individual testing all six drinks. The numbers were then amalgamated and one data set constructed for each of the six drinks, which included the results from all 22 subjects. These data were subsequently subdivided into normal and erosive groups. Charts were drawn up for both groups testing each of the drinks, resulting in 12 graphs.

To investigate what differences might be present between drinks, initially data from the sixteen common subjects were examined together. Analyses were then carried out on the data from the two subgroups, again to investigate potential differences between the drinks. Comparisons were made between the six drinks by examining the change in the pH of the saliva sample (delta pH) caused by the test drink. The changes in pH were calculated after the following standard amounts of drink had been added: 0.1 mL, 0.5 mL, 1 mL, 5 mL and 10 mL. A repeated measures analysis of variance (ANOVA) was used to compare all 6 drinks at each volume separately. If this was significant, follow up Bonferroni comparisons were used to compare pairs of drinks.

The mean baseline pH values of the stimulated salivary samples were analysed using the two-sample t test, to see if there were differences between the normal and erosive groups. Each drink was then taken individually and the data examined for differences between normal and erosive individuals. The pH of the saliva was noted after the standard 0.1 mL, 0.5 mL, 1 mL, 5 mL and 10 mL of drink had been added (actual pH) and the figures used for analysis. In addition, the change in pH after the above increments of drink had been added (delta pH) was also recorded and used in subsequent data analysis. Prior to using the two-sample t test, a repeated measures ANOVA was carried out to see if there was significant interaction between volume of drink and group (normal/erosion). A significant interaction would indicate that the size of the difference between the two groups changes with the addition of increasing volumes of drink.

4.3 Results

In most cases, the addition of 20 mL of drink was enough to bring the pH down to the desired value of the intrinsic pH of the drink, with a plateau being reached in the later stages of the titration. The non fruit-based carbonated drinks, *Coca-Cola* and *Diet Coke,* however, required 50 mL to reach a plateau, reflecting not only their lower initial pH but also their lower titratable acidity.

4.3.1 Reproducibility

Repeat titrations were carried out to ensure reproducibility. Figure 4.1 displays graphs of saliva from both normal and erosive individuals being tested with a range of drinks. There were no gross differences observed within any one individual's

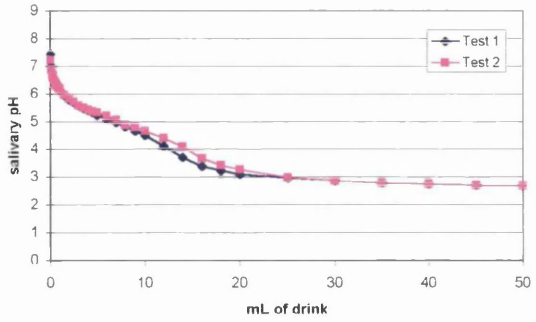

Figure 4.1 a
Reproducibility of erosive saliva testing *Coca-Cola* on 2 occasions.

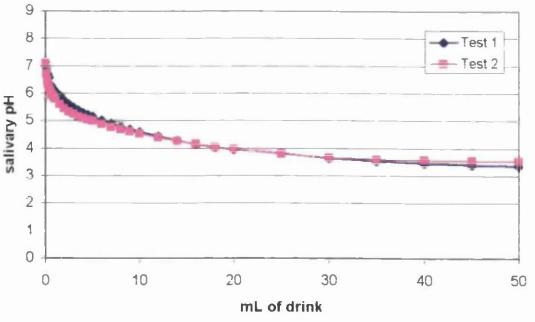

Figure 4.1 b
Reproducibility of normal saliva testing *Diet Coke* on 2 occasions.

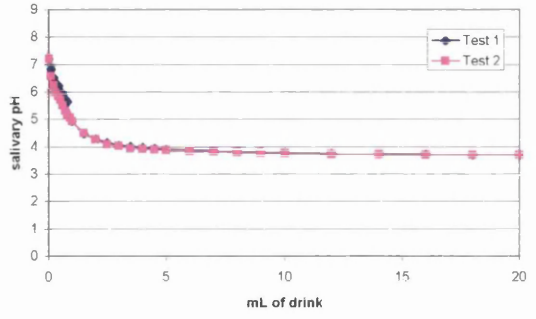

Figure 4.1 c
Reproducibility of erosive saliva testing orange juice on 2 occasions.

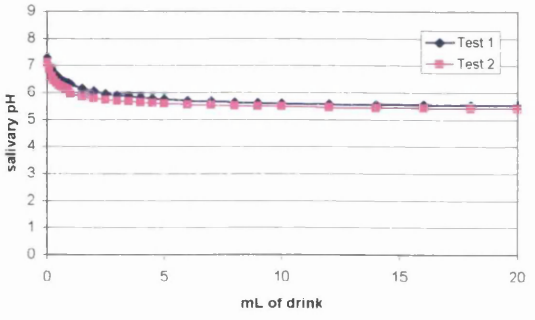

Figure 4.1 d
Reproducibility of normal saliva testing *Perrier* on 2 occasions.

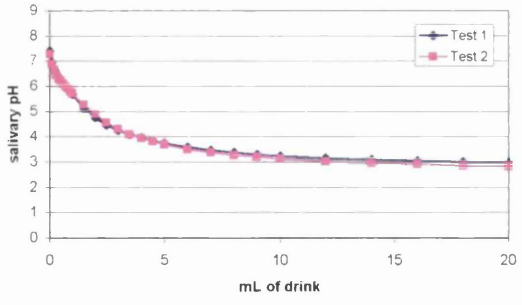

Figure 4.1 e
Reproducibility of normal saliva testing *Lilt* on 2 occasions.

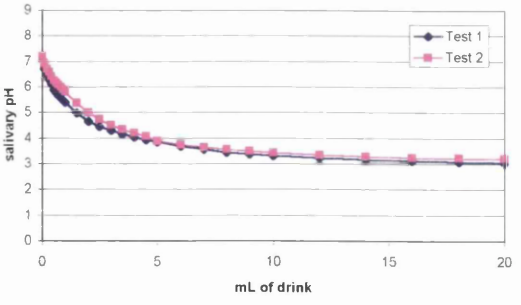

Figure 4.1 f
Reproducibility of erosive saliva testing *Diet Lilt* on 2 occasions.

Figure 4.1
Reproducibilty of methodology, with examples of the six drinks being tested with the saliva from normal and erosive subjects.

salivary buffering ability for a given drink and so the study was deemed reproducible. One set of readings was chosen, therefore, for inclusion and subsequent data analysis.

4.3.2 Differences between drinks for any one individual

Figure 4.2 shows graphically the results of the saliva from a normal individual being tested with all six drinks. There are clear differences observed in the rapidity of the pH drop when different drinks are added, shown by the slope of the curve. This is particularly evident during the initial stages of the titration. A similar graph is displayed in Figure 4.3 for an erosive individual and again shows clear differences in the way in which saliva buffers various acidic soft drinks. Table 4.1 details the drop in pH (delta pH) measured after the standard amounts of drink had been added to the saliva, with four individuals (2 normal and 2 erosive) being chosen as examples.

4.3.3 Differences between drinks for all subjects

The data for the 16 subjects (11 normal and 5 erosive) who tested the six drinks were amalgamated. As the greatest changes in pH occurred early in the titration, the delta pH data collected after the 0.5 mL additions were analysed and a mean value for each drink displayed in a histogram (Figure 4.4). The drinks all have a different titratable acidity, as shown in the previous study (Chapter 3), and this fact is reflected in the height of the bars on the histogram. After the addition of 0.5 mL of drink, orange juice caused an average fall in pH of 1.6 units, compared with *Lilt* and *Diet Lilt,* which induced a fall in pH of around 1.2 units. The cola-based drinks and mineral water caused the pH to fall by 0.8 and 0.7 pH units respectively.

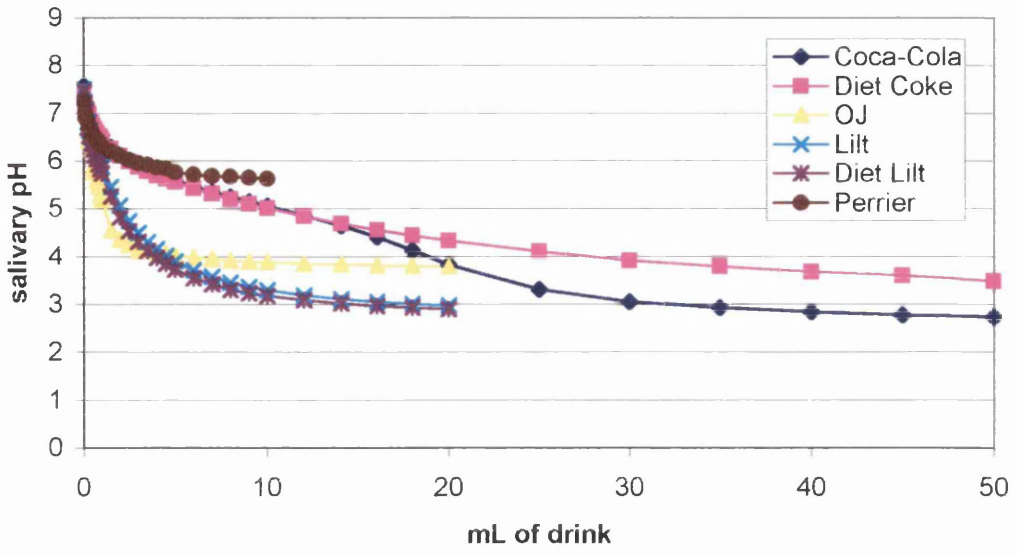

Figure 4.2
All six test drinks being added to the saliva from a normal individual.

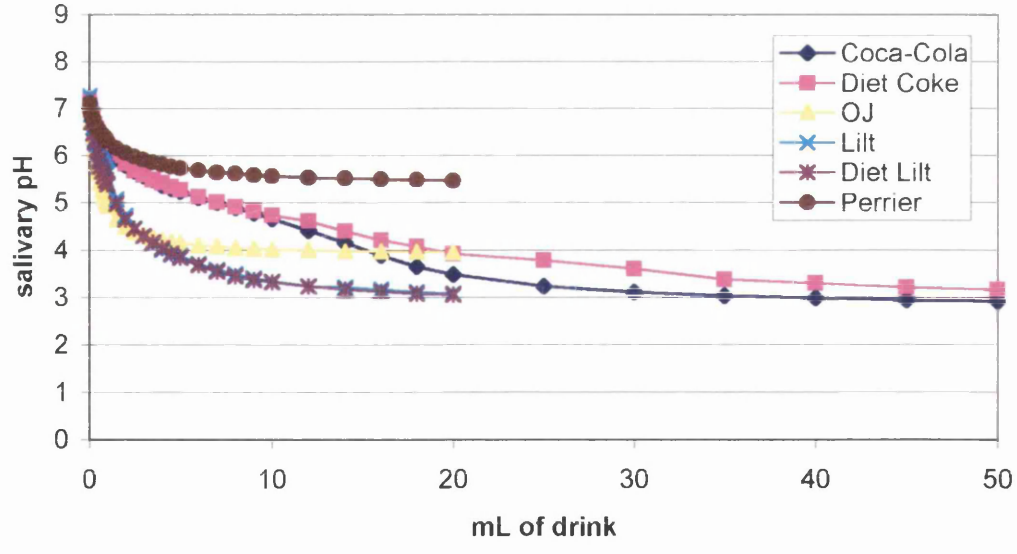

Figure 4.3
All six test drinks being added to the saliva from an erosive individual.

Normal GC	**Initial pH**	**Delta pH at: 0.1 mL**	**0.5 mL**	**1 mL**	**5 mL**	**10 mL**
Coca-Cola	7.58	0.34	0.83	1.03	1.99	2.52
Diet Coke	7.46	0.23	0.71	1.01	1.9	2.45
Orange Juice	7.42	0.46	1.57	2.23	3.39	3.54
Lilt	7.51	0.45	1.14	1.63	3.6	4.21
Diet Lilt	7.47	0.46	1.23	1.73	3.74	4.29
Perrier	7.25	0.24	0.68	0.92	1.49	1.62
Normal IG						
Coca-Cola	7.52	0.2	0.76	1.11	2.01	2.53
Diet Coke	7.36	0.36	0.92	1.19	1.97	2.44
Orange Juice	7.56	0.46	1.41	2.3	3.46	3.63
Lilt	7.2	0.36	1.07	1.6	3.53	4.02
Diet Lilt	7.4	0.45	1.19	1.64	3.57	4.21
Perrier	7.13	0.14	0.48	0.71	1.27	1.43
Erosive NW						
Coca-Cola	7.27	0.44	1.07	1.39	2.42	3.52
Diet Coke	7.22	0.29	0.89	1.2	2.26	2.8
Orange Juice	7.23	0.52	1.88	2.63	3.36	3.46
Lilt	7.05	0.4	1.18	1.77	3.63	3.99
Diet Lilt	7.26	0.41	1.35	2.12	3.93	4.29
Perrier	7.19	0.32	0.8	1.09	1.66	1.77
Erosive MG						
Coca-Cola	7.31	0.42	0.95	1.23	2.15	2.8
Diet Coke	7.19	0.29	0.77	1.11	2.15	2.7
Orange Juice	7.2	0.62	1.5	2.27	3.32	3.44
Lilt	7.1	0.49	1.33	1.99	3.79	4.13
Diet Lilt	7.19	0.53	1.32	1.94	3.8	4.14
Perrier	7.25	0.25	0.83	1.19	1.8	1.98

Table 4.1

Changes in salivary pH induced by each of the test drinks. Examples are given of the delta pH in the saliva of 2 normal and 2 erosive volunteers. The change in salivary pH from its initial value is given at each of the standard increments.

The volunteers are identified by their initials. GC and IG are normal volunteers, while NW and MG are erosive subjects.

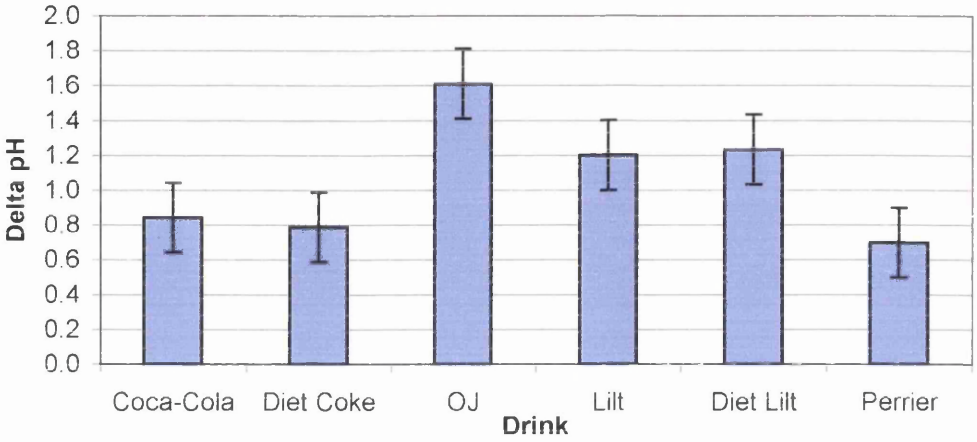

Figure 4.4

The mean changes in pH induced by the initial 0.5 mL of the six test drinks when added to saliva. Error bars show 1 SD (standard deviation).

Key: OJ Orange juice

The mean changes in pH (delta pH) caused by the six drinks when added to the saliva of the 16 volunteers are shown in Figure 4.5. The regular and diet versions of the same drink behaved in a similar fashion and so the lines are superimposed on the graph. The addition of orange juice caused the greatest change in salivary pH, followed closely by *Lilt/Diet Lilt*. In contrast, the addition of *Coca-Cola/Diet Coke* and *Perrier* water resulted in smaller changes in salivary pH, shown by slopes that are less steep than those of the fruit-based drinks. The largest change caused in the pH of saliva by addition of the drinks occurred initially, as shown in the steeper part of the curve. Latterly, a plateau was reached as the drink approached its intrinsic pH. The fact that orange juice has a higher initial pH than *Lilt/Diet Lilt* explains why the lines for the two drinks cross after 4 mL of drink have been added.

A repeated measures ANOVA was significant ($p<0.001$) at each of the standard volumes (0.1, 0.5, 1, 5 and 10 mL). Follow up Bonferroni comparisons showed differences at 0.1 mL between the fruit-based drinks (orange juice, *Lilt, Diet Lilt*) and the non fruit-based drinks (*Coca-Cola, Diet Coke, Perrier*). After both 0.5 mL and 1 mL had been added, the orange juice had caused a significantly greater fall in pH than *Lilt* or *Diet Lilt*, which in turn had caused a significantly greater fall in pH than either *Coca-Cola, Diet Coke* or *Perrier*. Significant differences were seen between the cola-based drinks and the mineral water after 5 mL and 10 mL had been added, with the cola-based drinks causing the pH of saliva to fall to a lower level. *Lilt* and *Diet Lilt* were shown to cause a significantly greater fall in pH than orange juice after these additions, but this is a reflection of the lower intrinsic pH of the *Lilt* drinks. After 5 mL of orange juice had been added to saliva, the actual pH value of orange juice had already been reached whereas *Lilt*, with its lower pH value, continued to

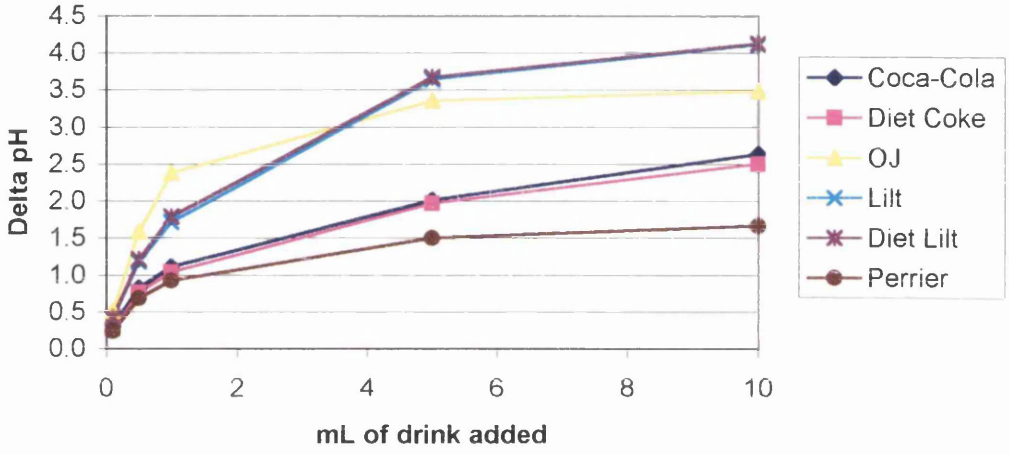

Figure 4.5
The mean changes in salivary pH induced by the six test drinks at each of the standard points.

cause the pH of the saliva to fall until its own intrinsic pH was reached. These results are summarised in Table 4.2.

Prior to investigating any variation between normal and erosive groups, differences between the drinks within each of the two groups were analysed. Graphs similar to Figure 4.5 were constructed for each of the normal and erosive groups (Figures 4.6 and 4.7 respectively). There continued to be clear differences between the drinks reflecting their differing total titratable acidity, regardless of the group testing the drink.

4.3.4 Differences in baseline pH between normal and erosive groups

Each of the 11 normal and 5 erosive individuals who took part in the full study had six baseline measurements of stimulated salivary pH from which to calculate a mean initial pH for each subject. These stimulated salivary pH values and 1 standard deviation (SD) are shown in Table 4.3. The difference between the mean initial salivary pH values for the normal (7.36, SD=0.12) and erosive (7.21, SD=0.03) subjects was statistically significant when analysed using a two-sample t test ($p=0.002$). The remaining 18 erosive volunteers had two baseline salivary pH values. When these mean values were also included, the statistical difference between the normal and "erosive" stimulated salivary pH remained significant ($p=0.024$).

After addition of:	Drinks in order of lower pH induced
0.1 mL	OJ, L, DL < C, DC, P
0.5 mL	OJ < L, DL < C, DC, P
1 mL	OJ < L, DL < C, DC, P
5 mL	DL, L < OJ < C, DC < P
10 mL	DL, L < OJ < C, DC < P

Table 4.2
Differences in pH between drinks after each of the standard values had been added. The drinks causing a lower pH are shown by "<".

Key:		
	OJ	Orange juice
	L	*Lilt*
	DL	*Diet Lilt*
	C	*Coca-Cola*
	DC	*Diet Coke*
	P	*Perrier*

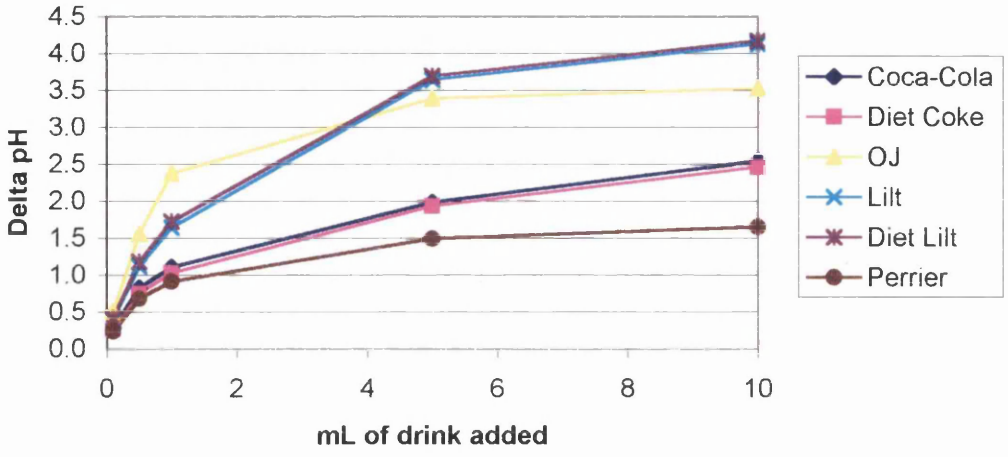

Figure 4.6
The mean changes in normal saliva pH induced by the six test drinks at each of the standard points.

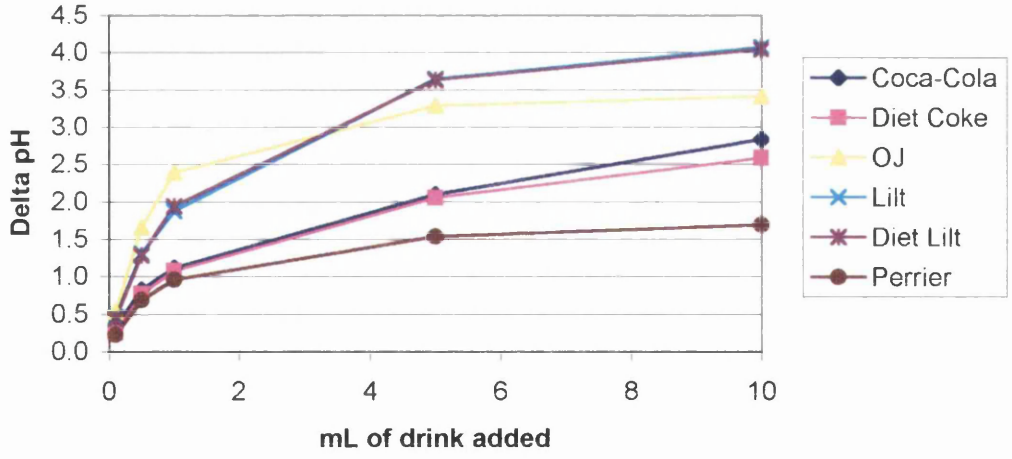

Figure 4.7
The mean changes in "erosive" saliva pH induced by the six test drinks at each of the standard points.

Normal Mean initial pH	SD	Erosive Mean initial pH	SD
7.35	0.05	7.17	0.07
7.45	0.11	7.25	0.15
7.36	0.09	7.20	0.08
7.36	0.17	7.22	0.18
7.63	0.09	7.21	0.07
7.32	0.14		
7.43	0.08		
7.27	0.05		
7.33	0.20		
7.26	0.13		
7.17	0.11		

Table 4.3
Mean initial salivary pH for 11 normal and 5 erosive volunteers, with 1 SD (standard deviation) shown.

4.3.5 Differences in actual pH between normal and erosive groups

The data were examined for differences between normal and erosive groups at each of the standard values. At the chosen points, the actual measured pH values were noted for analysis for each of the drinks. Actual pH was considered to be potentially the pH of the mouth during an acidic challenge. Repeated measures ANOVA were significant for the drinks *Coca-Cola*, orange juice, *Lilt* and *Diet Lilt*, indicating that the patterns of changes in pH with increasing volumes were different for the two groups. When each drink was analysed individually, it was of interest to note that the differences between baseline values for the two groups were not always significantly different, despite the overall difference seen in Section 4.3.4. This was probably due to random variation and also to the changing mix of subjects in the erosion group. There were no differences in baseline pH noted between the two groups when testing *Diet Coke*, orange juice, *Lilt* and *Perrier*.

Significant differences in actual pH between the two groups were seen at several of the chosen standard points for all drinks except orange juice. However, further analysis of this drink showed a significantly lower pH of 0.19 units in the erosive group after the addition of 0.3 mL of orange juice to saliva ($p=0.04$). Table 4.4 summarises the differences in actual pH value at baseline and each of the standard points and also indicates the p values when analysed using the two-sample t test. The significant results are highlighted in bold. As the pH scale is logarithmic, relatively small changes in pH value correspond to larger differences in hydrogen ion concentration. For example, after 0.5 mL of *Diet Lilt* had been added to saliva, the mean pH of the erosive group saliva was 0.32 units lower than that of the normal group, equating to twice the number of hydrogen ions being present in the erosive

Drink		Baseline	0.1 mL	0.5 mL	1 mL	5 mL	10 mL
Coca-Cola	Difference in pH	**0.13**	**0.18**	**0.18**	**0.18**	**0.25**	**0.38**
	p value	**0.05**	**0.002**	**0.004**	**0.008**	**0.001**	**0.004**
Diet Coke	Difference in pH	0.1	**0.12**	**0.16**	**0.18**	**0.2**	**0.2**
	p value	0.15	**0.05**	**0.02**	**0.01**	**0.02**	**0.03**
OJ	Difference in pH	0.09	0.11	0.2	0.11		
	p value	0.15	0.06	0.11	0.35		
Lilt	Difference in pH	0.11	**0.16**	**0.26**	**0.31**	0.07	0.04
	p value	0.12	**0.01**	**0.005**	**0.01**	0.42	0.57
Diet Lilt	Difference in pH	**0.22**	**0.24**	**0.32**	**0.39**	0.13	0.07
	p value	**0.004**	**0.001**	**0.001**	**0.002**	0.12	0.21
Perrier	Difference in pH	0.09	0.13	**0.15**	**0.18**	0.15	0.14
	p value	0.15	0.09	**0.04**	**0.03**	0.07	0.08

Table 4.4
Differences between normal and erosive groups in actual pH at each standard point.
Values noted show amount by which the mean pH of erosive saliva is lower.
Bold marks significant results.

group saliva. Figures 4.8 and 4.9 show graphically the differences between the mean titration curves for normal and erosive groups, with the pH of "erosive" saliva falling more quickly to lower values. There was, however, wide variation within each group, as well as overlap between the two groups, which can be seen in the two examples shown in Figure 4.10.

4.3.6 Differences in delta pH between normal and erosive groups

These results were analysed for each drink individually. Analysis of the change in pH was relevant particularly for the two drinks *Coca-Cola* and *Diet Lilt*, where there were significant differences in baseline pH between the normal and erosive groups. The changes in pH following the addition of 1 mL or 5 mL of *Coca-Cola* to normal and "erosive" saliva were not significantly different. After 10 mL of *Coca-Cola* had been added, the mean pH of "erosive" saliva fell by 2.79 units, compared with a 2.55 unit fall in normal saliva, and this was statistically significant ($p=0.042$). In the case of *Diet Lilt*, significant differences were seen after 0.5 mL and 1 mL of drink were added to saliva. The addition of 0.5 mL caused a greater fall of 0.1 pH unit in "erosive" saliva ($p=0.04$), while the addition of 1 mL of *Diet Lilt* caused a greater fall of 0.19 units ($p=0.03$).

Although there were no differences observed between the baseline values for the other drinks, *Lilt* also caused a significantly larger delta pH in "erosive" saliva than in normal saliva. After the addition of 1 mL of *Lilt* there was, on average, a 1.85 unit drop in "erosive" saliva compared with a 1.65 unit drop in normal saliva ($p=0.017$). There was no significant difference after 5 mL of *Lilt* had been added. Table 4.5 summarises the average changes in pH induced by each drink after the addition of

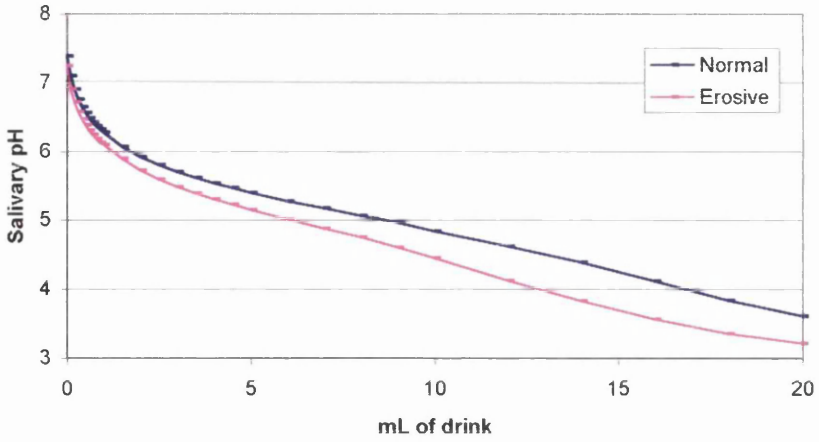

Figure 4.8 a
Mean titration curves of normal and 'erosive' saliva when *Coca-Cola* added.

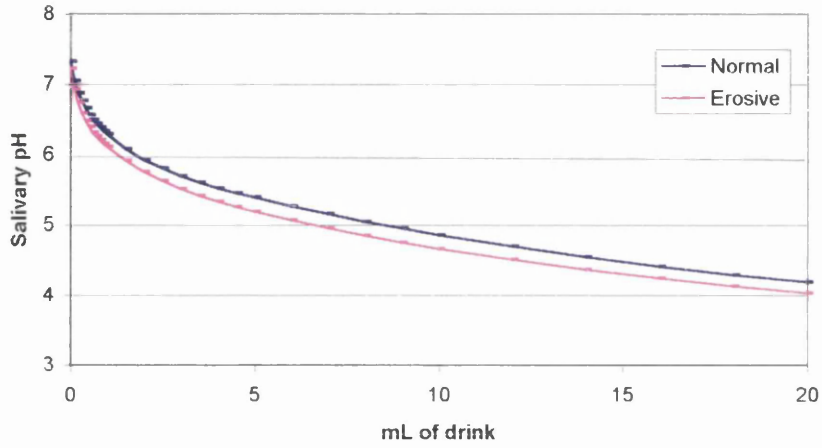

Figure 4.8 b
Mean titration curves of normal and 'erosive' saliva when *Diet Coke* added.

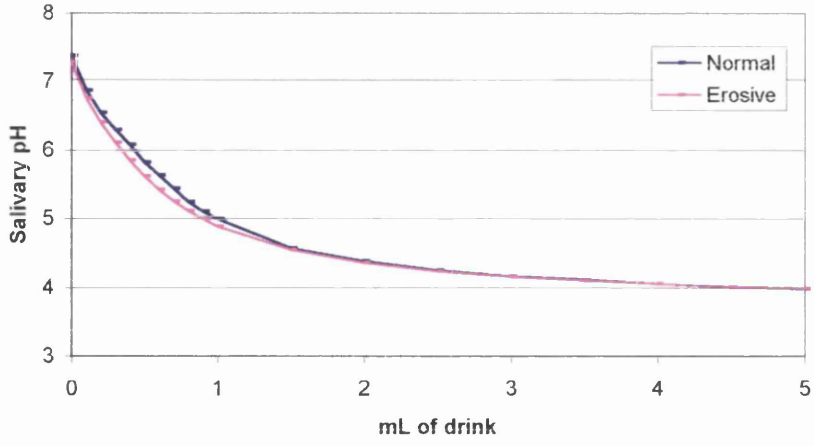

Figure 4.8 c
Mean titration curves of normal and 'erosive' saliva when orange juice added.

Figure 4.8
Mean titration curves for normal and erosion groups when carbonated drinks and fruit juice added.

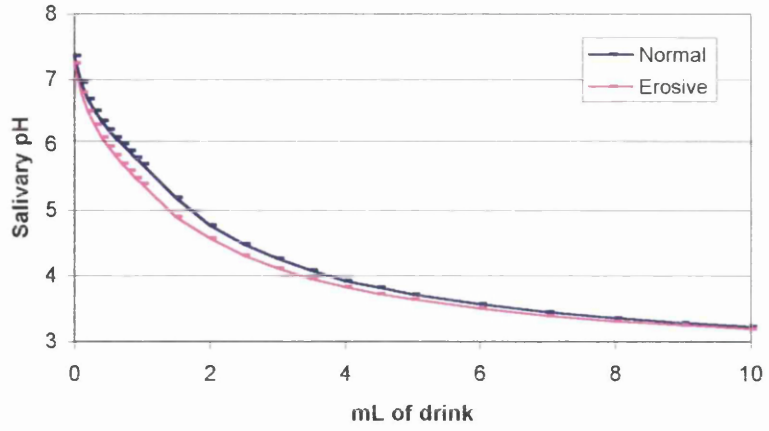

Figure 4.9 a
Mean titration curves of normal and 'erosive' saliva when *Lilt* added.

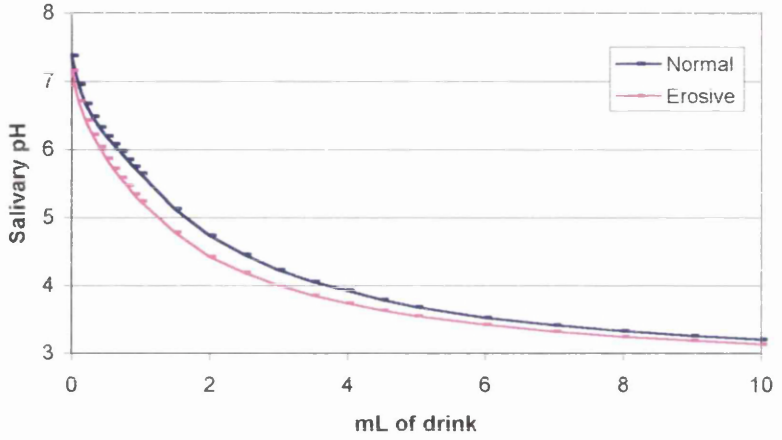

Figure 4.9 b
Mean titration curves of normal and 'erosive' saliva when *Diet Lilt* added.

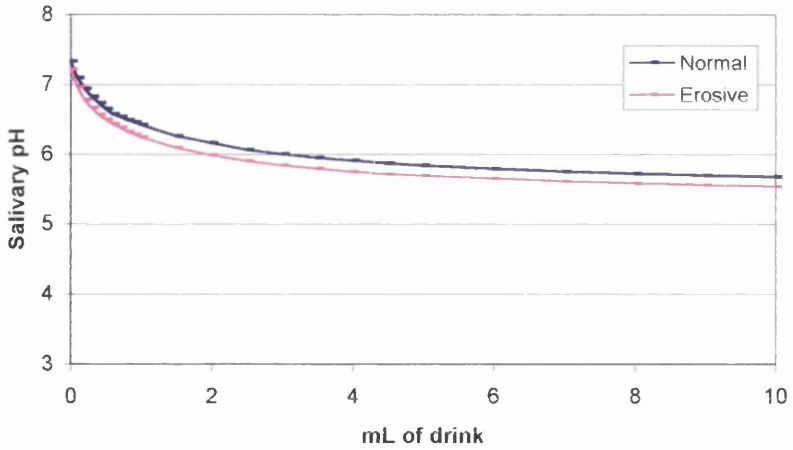

Figure 4.9 c
Mean titration curves of normal and 'erosive' saliva when *Perrier* added.

Figure 4.9
Mean titration curves for normal and erosion groups when fruit-based carbonated drinks and mineral water added.

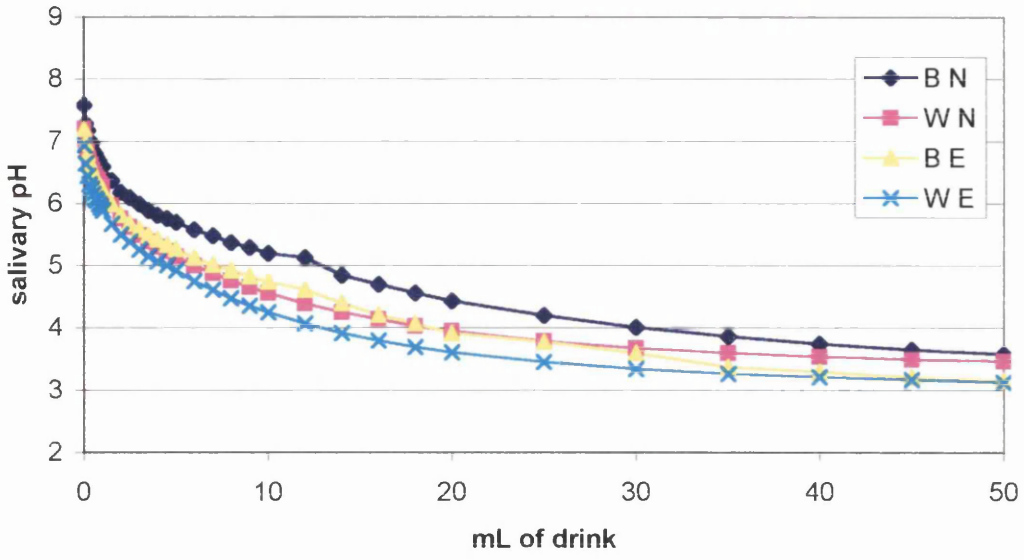

Figure 4.10 a
Examples of the best and worse case scenaria from normal and erosive groups testing *Diet Coke.*

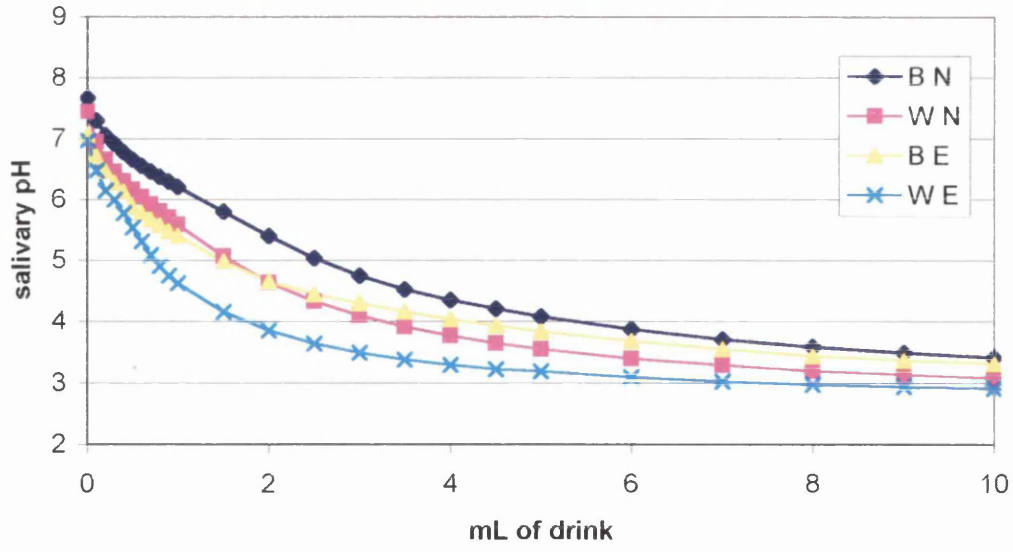

Figure 4.10 b
Examples of the best and worse case scenaria from normal and erosive groups testing *Diet Lilt.*

Key	BN	Best Normal
	WN	Worst Normal
	BE	Best Erosive
	WE	Worst Erosive

Drink		Delta pH at: 0.1 mL	0.5 mL	1 mL	5 mL	10 mL
Coca-Cola	N Mean	0.29	0.82	1.11	1.98	**2.55**
	E Mean	0.34	0.86	1.15	2.09	**2.79**
	p value					**p=0.042**
Diet Coke	N Mean	0.27	0.76	1.03	1.93	2.46
	E Mean	0.29	0.82	1.11	2.03	2.57
OJ	N Mean	0.50	1.55	2.38	3.39	3.53
	E Mean	0.53	1.67	2.40	3.29	3.42
Lilt	N Mean	**0.40**	**1.12**	**1.65**	3.64	4.13
	E Mean	**0.46**	**1.28**	**1.85**	3.61	4.06
	p value	**p=0.03**	**p=0.004**	**p=0.017**		
Diet Lilt	N Mean	0.42	**1.18**	**1.73**	3.70	4.17
	E Mean	0.44	**1.29**	**1.91**	3.61	4.02
	p value		**p=0.004**	**p=0.03**		
Perrier	N Mean	0.24	0.68	0.91	1.49	1.65
	E Mean	0.25	0.72	0.97	1.52	1.67

Table 4.5
The mean changes in pH for normal and erosive groups with the 6 test drinks at each of the standard increment points. The pH of erosive saliva was lower following the addition of *Coca-Cola, Lilt* and *Diet Lilt.* Bold marks significant results.

Key:
OJ Orange juice
N Normal
E Erosive

the chosen standard volumes. The significant results are highlighted in bold, with the p values noted underneath.

4.3.7 The effect of collection and storage of samples under paraffin

Titrations were carried out on saliva collected and stored without paraffin. Two drinks were tested on each of two occasions, with saliva from a normal individual on the first occasion and an erosive individual on the second. The results are displayed graphically on Figure 4.11 for the normal subject and Figure 4.12 for the erosive subject. For the sake of comparison, the titrations carried out under paraffin using the same individual's saliva are also displayed. The initial pH of the saliva was higher, as would be expected after loss of bicarbonate. The pH also fell more quickly in the initial stages of the titration, but the changes appear relatively minor. The pH did, however, take longer to become steady, probably as a result of fewer ions moving across the electrode membrane.

4.3.8 The effect of freezing on salivary samples

Titrations of two normal and two "erosive" saliva samples that had been frozen at –20° C were carried out. The resultant graphs are shown in Figures 4.13 and 4.14 for normal and "erosive" saliva respectively, again showing titrations of fresh saliva from the individual for comparison.

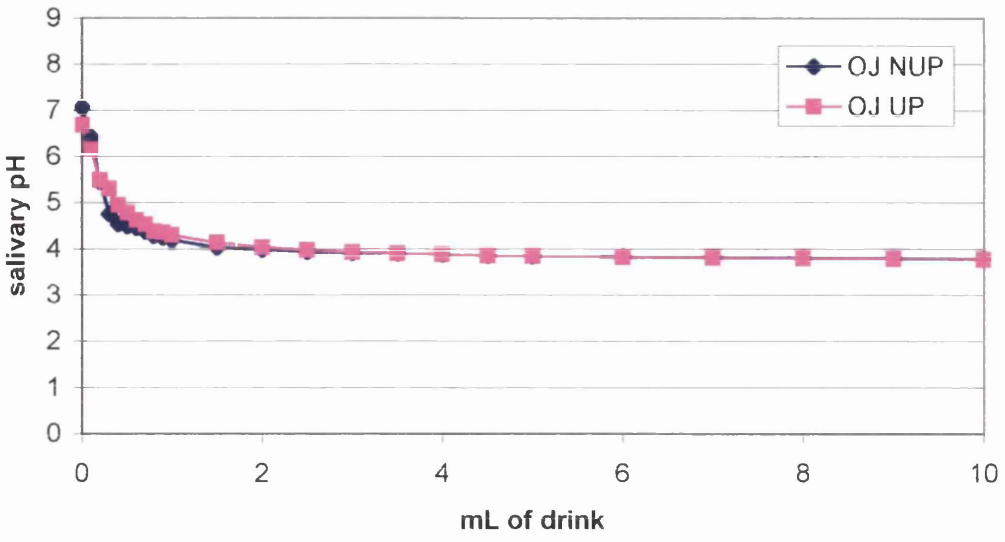

Figure 4.11
Normal saliva tested with orange juice under paraffin (UP) and not under paraffin (NUP).

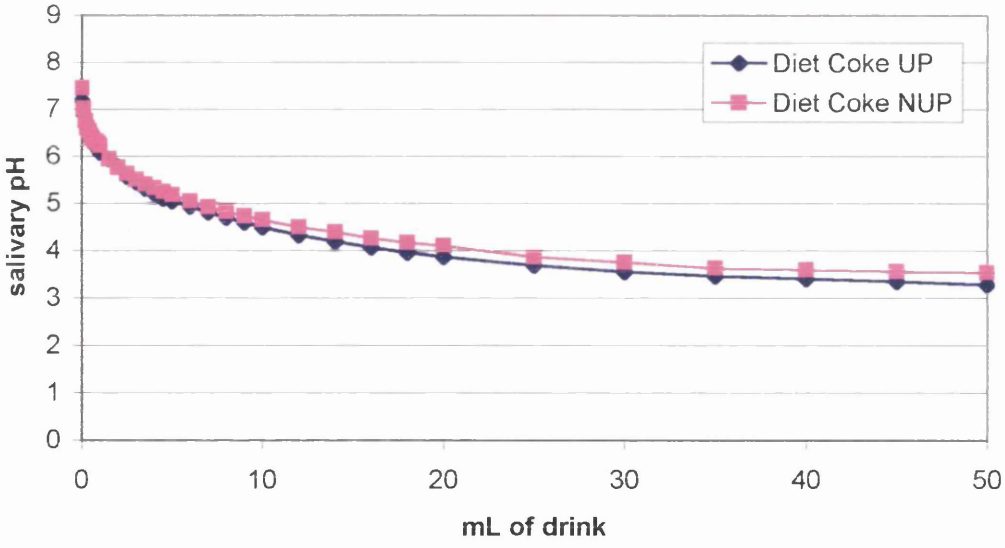

Figure 4.12
"Erosive" saliva tested with *Diet Coke* under paraffin (UP) and not under paraffin (NUP).

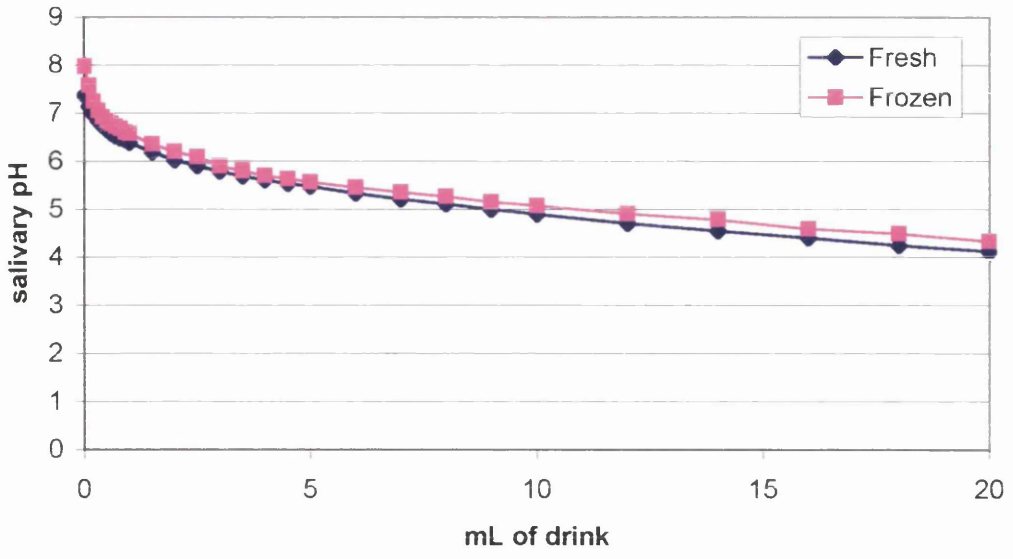

Figure 4.13
Comparison of fresh normal saliva with saliva from same subject following storage in freezer. Drink being tested is *Diet Coke*.

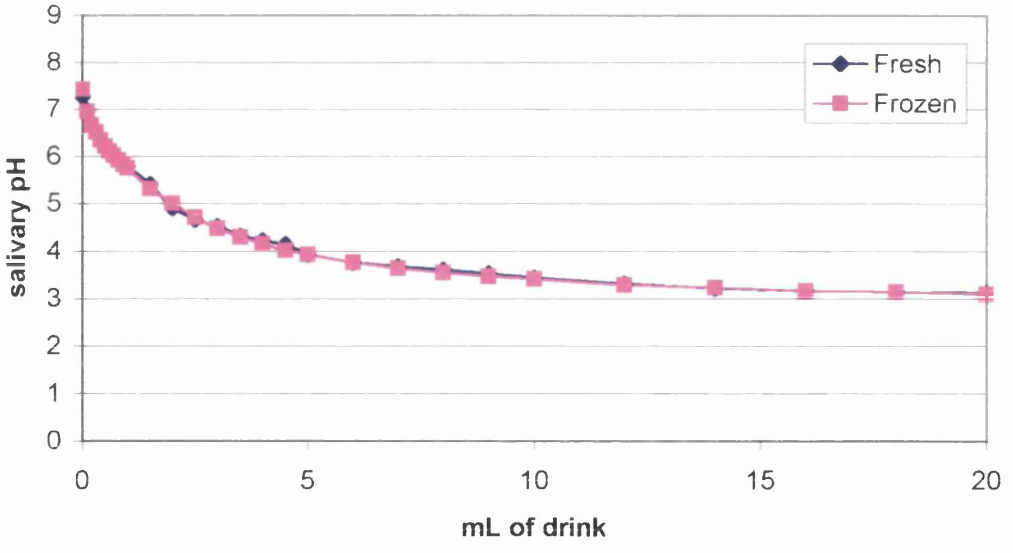

Figure 4.14
Comparison of fresh "erosive" saliva with saliva from same subject following storage in freezer. Drink being tested is *Lilt*.

4.4 Discussion

The results were of great interest as they revealed not only differences between the drinks, but also variation between the buffering ability of normal and "erosive" saliva *in vitro*. The original methodology of this experiment appeared to be robust, as no gross differences were observed during minor alterations to the protocol, such as freezing of salivary samples or the employment of paraffin. It appears, therefore, that the use of fresh saliva or of a protective paraffin seal are not strictly necessary for the tests carried out in this study.

Differences in the buffering capacity of various acidic drinks had been shown in the previous study (Chapter 3). Sodium hydroxide was used as the base and it is, therefore, difficult to extrapolate the results directly to the oral cavity. The use of saliva *in vitro* gives an insight into the buffering ability of this biological base. The results of the first study ranked soft drinks in order of erosive potential, depending on the total amount of acid present in the drink. When saliva was used as the base in the titration, this ranking was preserved, indicating that the intrinsic acidity of drinks may indeed reflect their intra-oral erosive potential. Fruit-based drinks, such as orange juice and *Lilt*, caused a significantly greater fall in salivary pH than *Coca-Cola* and *Perrier* water. The inclusion of saliva in the study means that these results can be extrapolated to the oral cavity with more confidence than results from purely *in vitro* chemical analysis, although caution should still be exercised.

The amount of saliva present in the oral cavity at any one time varies with many factors, but is generally believed to be about 1 mL, in contrast to the 4 mL used in this study. This *in vitro* study does represent only a static situation, as no further

saliva was added to the model. In the oral cavity, continued salivary stimulation and production increases neutralising and buffering of acidic substances. Despite this limitation, the current study still showed saliva to be an immensely powerful buffering agent. The addition of 50 mL of *Coca-Cola* was required to lower the pH of just 4 mL of saliva to the initial pH of *Coca-Cola*.

It was of interest to note that, although *Lilt* and *Diet Lilt* behaved in an identical fashion, *Coca-Cola* and *Diet Coke* showed differences. The initial pH of *Diet Coke* is higher than that of *Coca-Cola*, which is probably a reflection of the difference in acid content. *Coca-Cola* contains phosphoric acid, whereas *Diet Coke* contains phosphoric acid and citric acid. The titration curves produced *in vitro* when sodium hydroxide was added appeared broadly similar, but small differences existed around the points of inflection at the pK values of the two acids. These differences appear to be highlighted in the latter stages of the titrations when saliva is used as the base (Figure 4.15).

The current study highlights that differences exist between the saliva of those with and those without erosion. The results show that the stimulated salivary pH of those with erosion is lower, which agrees with a recent study (O'Sullivan & Curzon, 1998). The salivary pH of those with erosion will thus require a smaller fall to achieve an intra-oral pH level at which tooth mineral becomes unstable.

The salivary buffering ability of subjects with erosion may also be reduced. The actual pH measurements from erosive subjects were lower than those from normal subjects at each of the chosen points, although this may be in part a reflection of the different baseline pH values. The actual pH readings were considered important

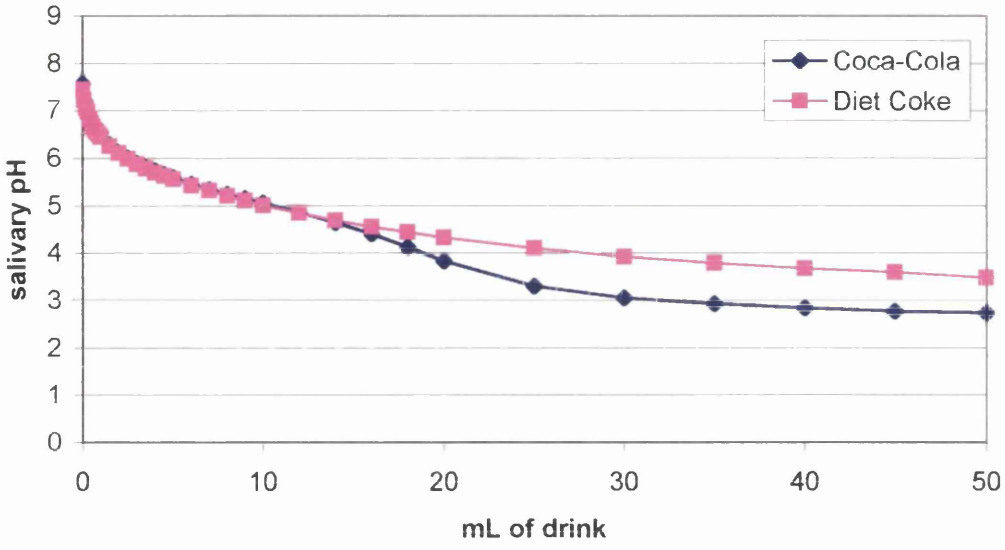

Figure 4.15
Titration of *Coca-Cola* and *Diet Coke* showing separation of the two drinks after 15 mL had been added to saliva, reflecting the different acid content.

because pH value gives a measurement of hydrogen ion concentration. In addition, in some instances, the pH of "erosive" saliva fell more sharply (delta pH). The pH at which tooth mineral becomes unstable, therefore, may be reached more quickly, especially when the lower initial pH of "erosive" saliva is taken into account. Actual pH and delta pH should be considered together, as a fall from pH 7 to pH 6 is not directly comparable with a fall from pH 5 to pH 4, because of the difference in hydrogen ion concentration. The results from the analysis of both these measures agree broadly and back up each another. Differences in actual pH at the standard points would be expected if the baseline values are also significantly different, and indeed this effect was observed for *Coca-Cola* and *Diet Lilt*. However, it was interesting to see that significant differences also occurred between normal and erosive groups during the titration of other drinks. Where baseline values were different (*Coca-Cola* and *Lilt*), significant variations between normal and erosive groups were seen in the delta pH as the titration progressed, confirming that the pH of "erosive" saliva fell more quickly when subjected to an acidic challenge.

In the same way, multiple analyses were carried out to show differences between normal and erosive groups for any one drink, to take account of known variables. The total titratable acidity of each drink is not the same and so it was anticipated that differences might be seen at various standard points. The fact that statistically significant differences in delta pH were seen early in the titration with *Lilt* and *Diet Lilt* (0.1-1 mL) but later with *Coca-Cola* (10 mL) underlines this variation in total titratable acidity. There were few differences noted between the normal and "erosive" saliva when titrated with orange juice and this may be due to the high titratable acidity of pure fruit juices. A significant difference was seen in the actual pH after

0.3 mL had been added, in line with the early differences seen with the fruit-based carbonated drinks, which have the next highest titratable acidity. It may be that the magnitude of the acidic insult from a pure fruit juice is too intense for even normal saliva to counteract, and thus differences are only seen in the initial stages of the titration.

There was variation in salivary buffering ability within each of the normal and erosive groups, and also some crossover between the two groups. The aetiology of dental erosion is known to be multifactorial and, therefore, the influence of salivary pH can be considered as only one element in the progress of erosion. Despite this, it is hoped that this protocol might form the basis for a predictive test for susceptibility to dental erosion. The results from a range of normal individuals could be used for comparison. If the pH of the saliva under investigation fell more quickly than the salivary pH of controls when challenged by acidic drinks, this could indicate a deficiency in salivary buffering ability. Patients could then be given appropriate, tailored preventive advice. There does appear to be a degree of individual susceptibility in developing dental erosion. If such individuals were identified at an early stage, progress of dental erosion may be slowed or even prevented altogether.

For the sake of completeness, the stimulated saliva samples were also tested using the *Dentobuff* (*Vivacult BC*) test strip, which gave an estimate of high, medium or low salivary buffering capacity. It was of interest to note that for the best and worst of the normal individuals, as seen in Figure 4.10, there was good correlation between the results of the *Dentobuff* test. Individuals with high *Dentobuff* results showed better salivary buffering capacity using the methodology described here than subjects whose saliva had medium *Dentobuff* results. However, no such correlation was

found within the erosion group. The saliva from some subjects with a high *Dentobuff* result showed a poorer ability to buffer the acidic drinks than those who had a medium *Dentobuff* result. The same was also true for some erosive individuals whose saliva had a medium *Dentobuff* result, yet performed better than those with a high *Dentobuff* result. This suggests that there may be inherent differences in the salivary buffering mechanisms of erosive individuals, which warrants further investigation. The variation in results also suggests that the *Dentobuff* test may be a poor predictor of those who are prone to dental erosion and so the results of the *Dentobuff* strip should be interpreted with caution.

4.5 Conclusions

In conclusion, there are clear differences in the way in which saliva buffers various soft drinks, reflecting the intrinsic acid content of the drink. The saliva from subjects with erosion appears to cope less well with an acidic challenge, with the pH falling more quickly to a lower value.

5 SALIVARY BUFFERING *IN VIVO* WHILE REPEATEDLY SIPPING SOFT DRINKS

5.1 Introduction

The ability of a drink to resist pH changes brought about by salivary buffering may inevitably result in a prolonged period of oral acidity and, therefore, may play an important part in the erosion process (Grobler & van der Horst, 1982). It has been seen in the previous studies that drinks vary widely in their buffering capacity and so their erosive potential, as drinks with a high buffering capacity will be influenced less by salivary dilution or buffering. In addition, there appear to be differences between individuals in their salivary buffering ability, which may be particularly marked between those who suffer from dental erosion and those who do not have erosion.

Various investigations have been carried out to study the effects of acidic drinks on salivary pH (Section 1.4.3). All show that soft drinks have the potential to lower intra-oral pH, although there are significant variations in the methodology of the different studies. There were several aims in this current study. In the first instance, it was hoped to develop a reproducible, simple methodology to allow an insight into events occurring to oral pH while consuming acidic drinks. Changes in salivary pH following repeated sips of a soft drink were monitored, thereby providing information about the effectiveness of the intraoral buffering system. The second aim of the investigation was to assess whether individuals with diagnosed dental erosion would show a similar pattern of pH changes to non-erosive individuals, while a third aim was to assess the influence of the intrinsic acidity of a drink on salivary pH.

5.2 Materials and Methods

Nine volunteers, four of whom had diagnosed dental erosion, were recruited for this study. There were 4 males and 5 females, in the age range 18 to 41. All volunteers were in good health and all, including the four with erosion, admitted to drinking soft drinks occasionally. None had a medical history which indicated that gastric-oesophageal reflux might have been a complicating factor. Ethical approval for this study was sought and given by the Local Ethics Committee. Volunteers were asked not to consume any food or drink in the hour before the test and to refrain from tooth cleaning within the previous 3 hours. On the morning of the test, each was requested to brush his/her teeth thoroughly and to use interdental cleaning aids. Testing was usually carried out between 11a.m.and 3 p.m. to minimise diurnal variation. Each volunteer tested *Coca-Cola*, *Diet Coke* and pure orange juice on two separate occasions, in a random order at least several weeks apart, to ensure reproducibility of the methodology and results.

5.2.1 Preliminary investigations

Prior to this study, a pilot investigation was carried out in an attempt to ascertain what a near "normal" drinking pattern, in relation to the volume of a sip, might be. This was established by asking 20 individuals working in the Dental Hospital to drink a standard 330 mL can of carbonated drink. The number of sips taken and the time required to drink the can were noted and a protocol drawn up. It was decided that each volunteer would take sips of 25 mL, measured into a 50 mL plastic container, with each sip 1 min apart. The volume of each can would, therefore, be consumed in approximately 13 min.

5.2.2 Protocol

The drinks tested were freshly purchased and used at room temperature. A baseline salivary sample was obtained by asking each volunteer to drool for 1 min into a clean tube before starting the 1 hour experiment. The volunteers were then asked to take repeated sips of the chosen soft drink, the pH of which had been measured. Each sip was swallowed immediately and neither held in nor rinsed around the mouth. A single spit sample was taken 15 sec after each sip into a clean tube to enable pH measurement. Once the drink was finished, samples were taken at 14, 15, 17, 20, 25, 30, 35, 40, 50 and 60 min to monitor pH recovery. After each test, volunteers were offered a fluoride mouthwash and advised not to brush their teeth for approximately 1 further hour.

5.2.3 pH measurement

The pH of the samples was measured immediately as described previously (Section 2.1.2). The pH measurement of the sample was taken once the reading became stable and the samples were measured under *Clingfilm* to minimise evaporation.

5.3 Results

5.3.1 Reproducibility

Each individual tested the three test drinks on two occasions to ensure reproducibility. If the two tests give different results, a further exposure was undertaken. The two more similar results were kept and the aberrant readings discarded. The reproducibility of the study was acceptable, as shown by examples of the salivary profiles from three

volunteers, each testing a different drink (Figures 5.1 a-c). Therefore, one set of results (the worst case scenario, when the pH fell lower) for each subject was chosen for analysis and presentation. To ensure that this did not introduce any error into the study, statistical analyses of 2 key points were also carried out using the best case scenaria data. Results of the two-sample t test analysis of "lowest pH reached" and "time spent below pH 6" using the other data set were in complete agreement, further confirming the reproducibility of the study. Two-sample t tests were used throughout the analysis when differences between the normal and erosive groups were being compared. In contrast, when comparisons were being made between drinks, the paired t test was used instead.

5.3.2 Initial pH of the drinks

The pH of *Coca-Cola* ranged from 2.21 - 2.74 (mean = 2.44, SD=0.15), *Diet Coke* from 2.8 - 3.23 (mean = 3.07, SD=0.13) and orange juice from 3.6 - 3.93 (mean = 3.73, SD=0.09). These figures are in the range of those obtained in Chapter 3.

5.3.3 Baseline unstimulated salivary pH

Mean baseline unstimulated salivary pH measurements for the nine subjects were between 6.2 and 6.85. The mean baseline measurement for the normal group was 6.54 and for the erosive group 6.5, which were not significantly different. Mean baseline values were calculated also for each drink series, for use in the analysis of change in pH. Once again, no differences were found between any of the drink series, or the normal and erosive groups.

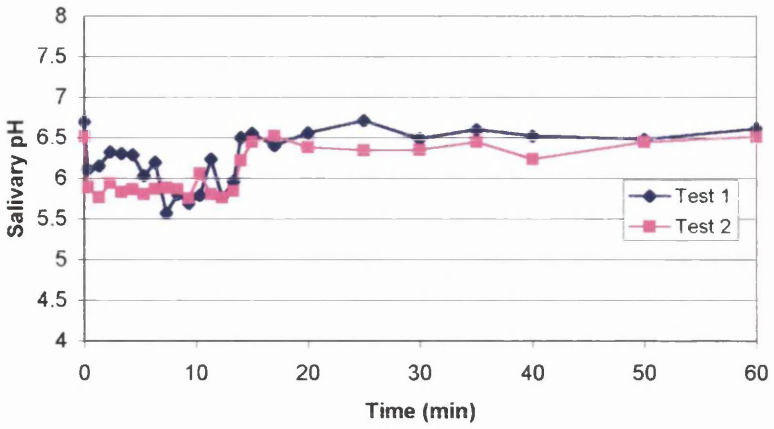

Figure 5.1 a
Normal volunteer consuming *Coca-Cola* on 2 occasions, to show reproducibilty.

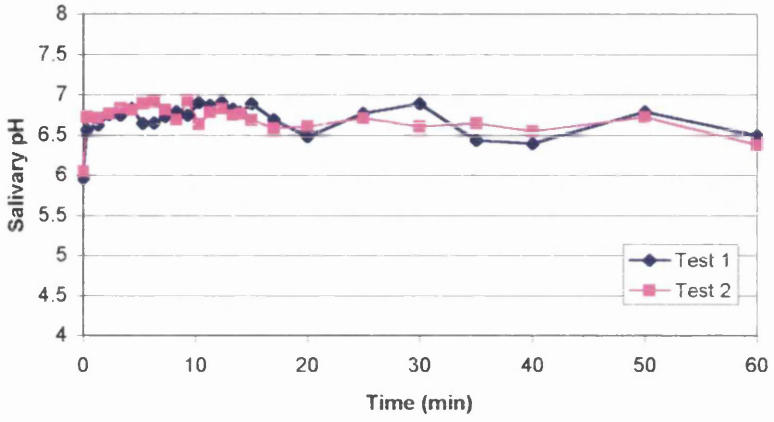

Figure 5.1 b
Normal volunteer consuming *Diet Coke* on 2 occasions, to show reproducibilty.

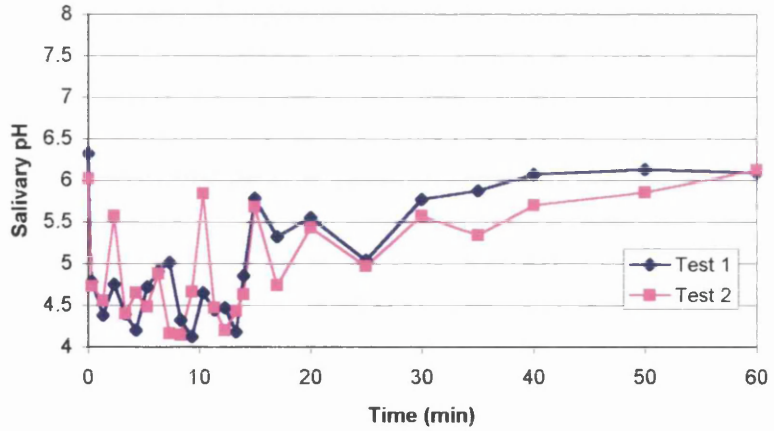

Figure 5.1 c
Erosive volunteer consuming orange juice on 2 occasions, to show reproducibilty.

Figure 5.1
Salivary profiles of 3 volunteers, each testing chosen drink on 2 occasions, to show reproducibilty. Figures a, b and c refer to *Coca-Cola*, *Diet Coke* and orange juice respectively.

5.3.4 Differences between drinks

The total titratable acidity of the orange juice has already been shown to be greater than that of the cola drinks (Chapter 3). The differences between the drinks will, therefore, be studied prior to investigating variations between individuals. The results for all 9 subjects testing *Coca-Cola*, *Diet Coke* and orange juice are displayed in Figures 5.2 a-c. Error bars showing 1 SD are included. The plots for each drink were then placed onto one graph, which is shown for clarity without error bars, in Figure 5.3. *Coca-Cola* caused salivary pH to drop further than *Diet Coke* and also induced a prolonged recovery. *Diet Coke* caused pH to fall slightly, followed by a reflex rise in pH after consumption had stopped. Orange juice, with the highest total titratable acidity, induced the greatest fall in salivary pH.

To compare differences between drinks, the overall average salivary pH of every individual during each test was calculated. The 9 readings for each drink were then compared using the paired t test. The mean salivary pH value while drinking *Coca-Cola* was 6.31, while consuming *Diet Coke* was 6.52 and while testing orange juice 5.91. Significant differences were found between *Coca-Cola* and *Diet Coke* ($p=0.02$), *Coca-Cola* and orange juice ($p=0.007$) and *Diet Coke* and orange juice ($p=0.003$).

5.3.5 Salivary profiles

Three distinct salivary profiles were observed for each of the three drinks tested. One group showed a fall in salivary pH, the second a marked fall and the third a clear rise. These three patterns were observed regardless of the drink being tested.

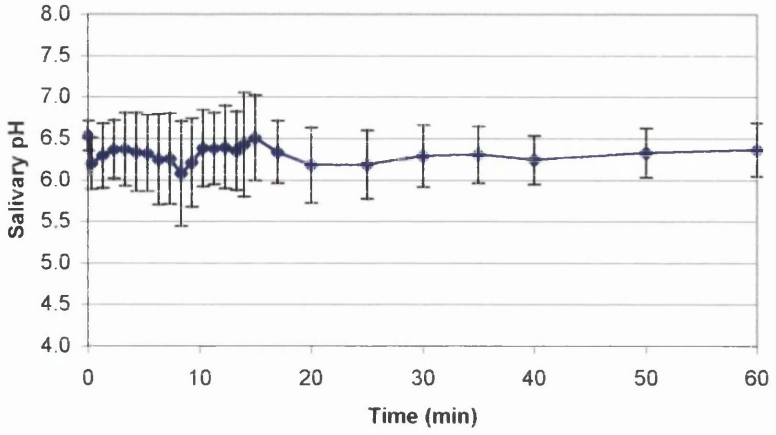

Figure 5.2 a
Mean salivary profile of 9 subjects testing *Coca-Cola* .

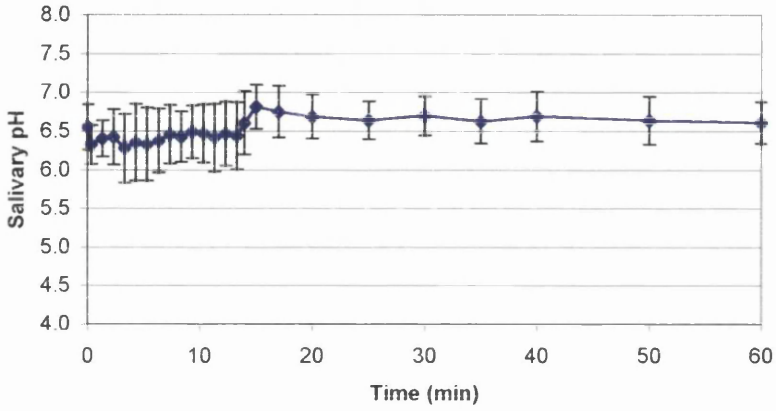

Figure 5.2 b
Mean salivary profile of 9 subjects testing *Diet Coke*.

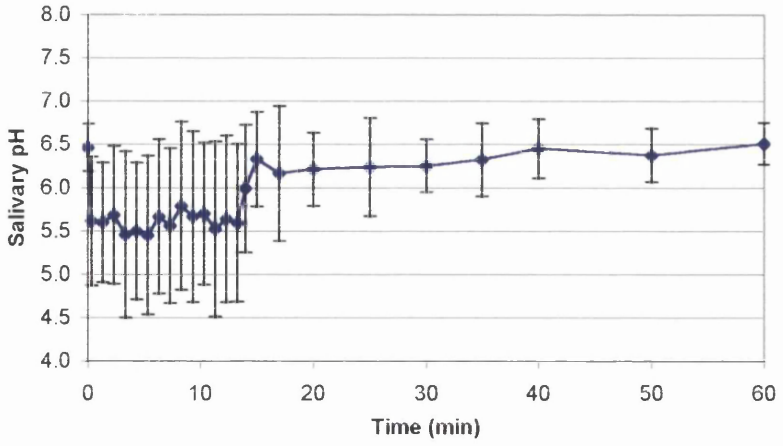

Figure 5.2 c
Mean salivary profile of 9 subjects testing orange juice.

Figure 5.2
Mean salivary profile of 9 subjects testing each drink in turn, with error bars showing 1 SD (standard deviation). Figures a, b and c refer to *Coca-Cola*, *Diet Coke* and orange juice respectively.

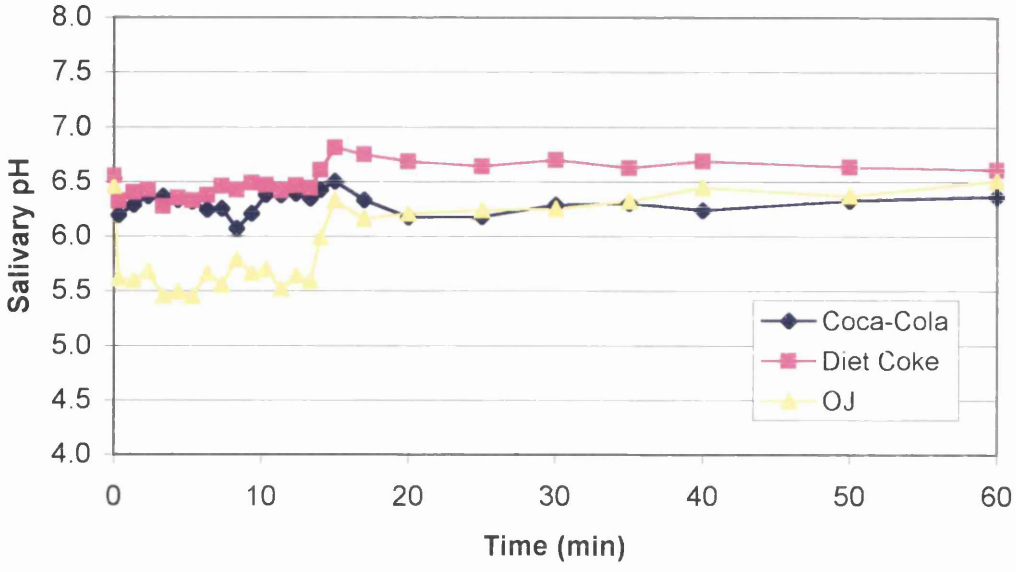

Figure 5.3
Mean salivary profiles of all subjects consuming each of the 3 test drinks, *Coca-Cola, Diet Coke* and orange juice (OJ).

Examples of the three patterns obtained for each drink are shown in Figures 5.4 a-c. In general, the erosive volunteers showed a marked and prolonged fall in salivary pH. Their salivary profiles fell further and remained acidic for longer than those of the non-erosion group. In contrast, there were two subjects in the non-erosion group who experienced a rise in pH during drinking, which gradually fell back to baseline. The remaining three subjects within the non-erosion category showed a fall in salivary pH during drinking but this was neither as long nor as prolonged as in the erosion group.

5.3.6 Differences between normal and erosion subjects

To investigate differences between normal and erosion individuals, the data from each group of subjects were combined. The values plus 1 SD are shown in Table 5.1 using *Coca-Cola* as an example, while charts for each of the drinks are displayed in Figures 5.5 a-c. The mean pH of the saliva from the erosion group always fell to a lower value than that of the normal group. The error bars show that there is not only variation within each group but also overlap between the two.

For statistical analysis, two-sample t tests were used to compare the average pH over the test period for normal and erosive subjects, consuming each test drink in turn. When normal and erosive subjects were compared, the erosive group showed a significantly lower pH than the normal group while drinking *Coca-Cola* ($p=0.04$). However, no significant differences were found between the average pH of normal and erosive groups when testing *Diet Coke* ($p=0.09$) or orange juice ($p=0.15$).

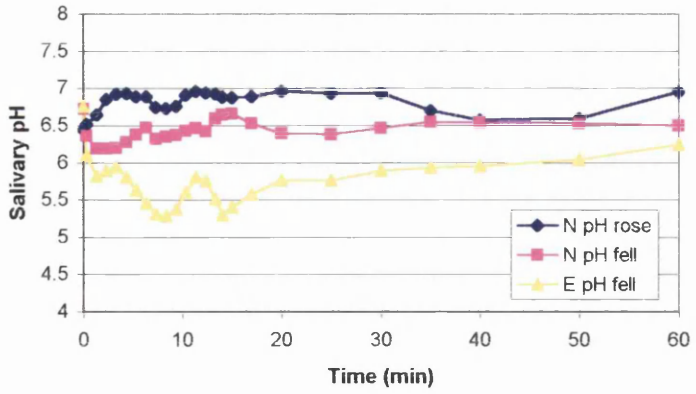

Figure 5.4 a
Three salivary profiles observed with *Coca-Cola* for normal (N) and erosive (E) subjects.

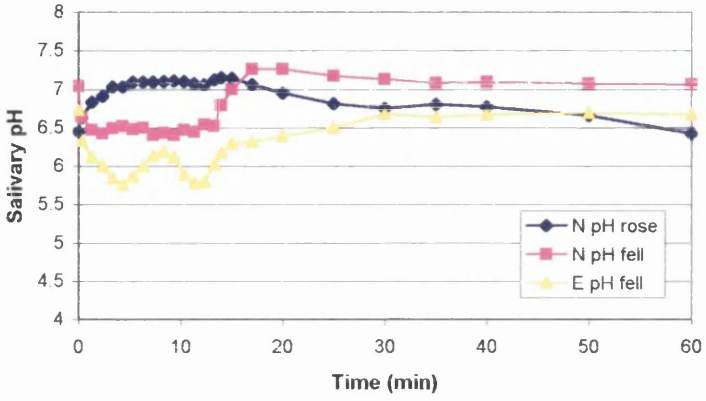

Figure 5.4 b
Three salivary profiles observed with *Diet Coke* for normal (N) and erosive (E) subjects.

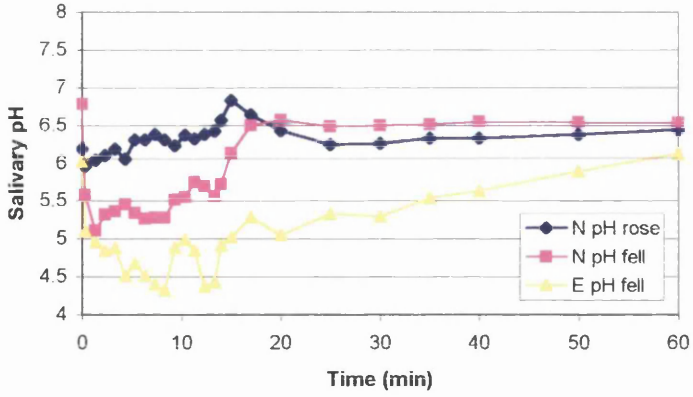

Figure 5.4 c
Three salivary profiles observed with orange juice for normal (N) and erosive (E) subjects.

Figure 5.4
Examples of the 3 salivary profile patterns observed with each drink for normal (N) and erosive (E) subjects. Figures a, b and c refer to *Coca-Cola, Diet Coke* and orange juice respectively.

Time (min)	Normal mean	Normal SD	Erosive mean	Erosive SD
0	6.57	0.16	6.48	0.21
0.33	6.32	0.33	6.04	0.22
1.33	6.38	0.46	6.18	0.31
2.33	6.46	0.41	6.26	0.28
3.33	6.53	0.53	6.17	0.23
4.33	6.55	0.46	6.07	0.39
5.33	6.49	0.43	6.10	0.45
6.33	6.47	0.39	5.97	0.63
7.33	6.50	0.44	5.95	0.57
8.33	6.39	0.48	5.68	0.62
9.33	6.47	0.47	5.89	0.47
10.33	6.66	0.40	6.03	0.28
11.33	6.44	0.48	6.29	0.41
12.33	6.56	0.53	6.18	0.42
13.33	6.54	0.47	6.10	0.40
14	6.76	0.34	6.01	0.69
15	6.77	0.20	6.17	0.62
17	6.63	0.18	5.96	0.08
20	6.53	0.21	5.74	0.16
25	6.46	0.35	5.83	0.04
30	6.52	0.19	5.99	0.34
35	6.56	0.19	5.99	0.15
40	6.45	0.20	5.98	0.14
50	6.55	0.15	6.04	0.10
60	6.53	0.28	6.16	0.28

Table 5.1
Example of mean salivary pH for normal and erosive groups testing *Coca-Cola*, with 1 SD (standard deviation) being shown.

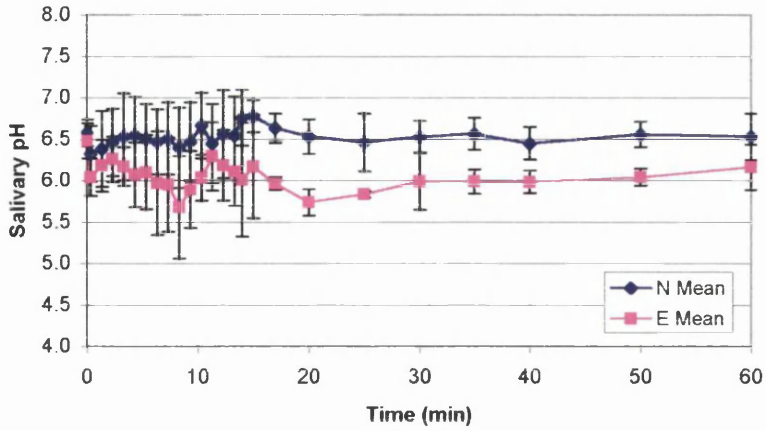

Figure 5.5 a
Salivary profiles of normal (N) and erosive(E) groups testing *Coca-Cola*. Error bars show 1 SD (standard deviation).

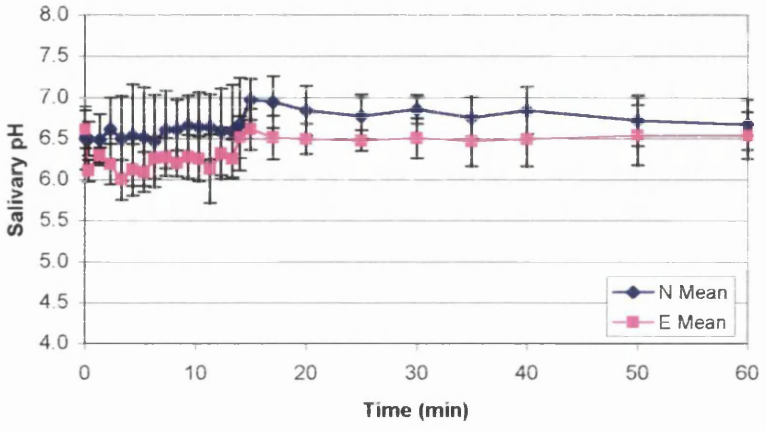

Figure 5.5 b
Salivary profiles of normal (N) and erosive (E) groups testing *Diet Coke*. Error bars show 1 SD (standard deviation).

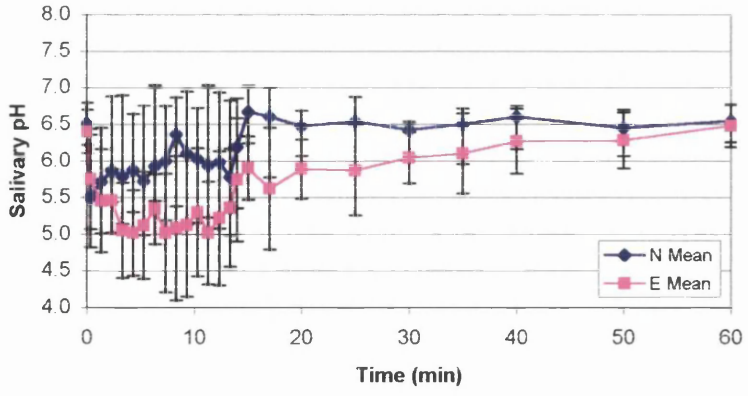

Figure 5.5 c
Salivary profiles of normal (N) and erosive (E) groups testing orange juice. Error bars show 1 SD (standard deviation).

Figure 5.5
Salivary profiles of normal (N) and erosive (E) groups testing each drink in turn. Figures a, b and c refer to *Coca-Cola, Diet Coke* and orange juice respectively.

The average pH for each subject was also taken for the 0-13 min drinking period and the 14-60 min recovery time. These figures were then subjected to statistical analysis. Once again, only *Coca-Cola* showed statistical significance and even then, only during the recovery period. The erosive group had a mean pH of 5.99 during recovery, compared with the normal group value of 6.58 (p=0.002). A corrected average, rather than a biased average, was also calculated for each recovery period, to give more equal weight to readings taken over a longer time period. The results from these analyses were the same as previously noted, with only *Coca-Cola* reaching significance (p=0.0007).

5.3.7 Actual pH value after first sip

In general, the salivary pH fell even after one sip of the test drink. While testing *Coca-Cola*, the initial pH of saliva fell to 6.2. With *Diet Coke* the pH fell to 6.32, and with orange juice to 5.61. When analysed using the paired t test, salivary pH following the first sip of orange juice was significantly lower than following either one sip of *Coca-Cola* (p=0.04) or one sip of *Diet Coke* (p=0.03), as shown in Table 5.2.

Normal and erosion groups were then compared using the two-sample t test. Baseline values and pH values after one sip are noted in Table 5.3, as well as the fall in pH. A significant difference was found between normal and erosive groups following one sip of *Diet Coke*, using the two-sample t test (p=0.01) when the pH dropped to 6.5 for the normal group and to 6.11 for the erosive group. However, no difference was found while drinking *Coca-Cola*, when pH for the normal group fell to 6.32 compared with the erosion group pH of 6.04 (p=0.19). It was interesting to

Drink	Baseline Mean salivary pH	First sip Mean salivary pH	Fall in pH
Coca-Cola	6.55	6.20	0.35
Diet Coke	6.55	6.32	0.23
Orange Juice	6.46	5.61	0.85

Table 5.2
Mean salivary pH values at baseline and following first sip of each test drink. The difference in pH between these 2 points is also noted.

Drink	Baseline Mean salivary pH	First sip Mean salivary pH	Fall in pH
	Normal group		
Coca-Cola	6.61	6.32	0.29
Diet Coke	6.51	6.50	0.01
Orange Juice	6.51	5.50	1.01
	Erosion group		
Coca-Cola	6.48	6.04	0.44
Diet Coke	6.61	6.11	0.50
Orange Juice	6.40	5.75	0.65

Table 5.3
Mean salivary pH values for normal and erosive groups at baseline and following first sip of each test drink. The difference in pH between these 2 points is also noted.

note that after one sip of orange juice, the mean salivary pH of the normal group had fallen to a lower value (pH 5.5) than the erosive group (pH 5.75), although this was not significant (p=0.65).

5.3.8 Change in salivary pH after one sip

As well as analysing the actual pH value following one sip, the change in pH from baseline to first sip was also subjected to statistical testing. Values for the fall in pH value are shown in Table 5.2. The difference from baseline of 6.55 to 6.20 after one sip of *Coca-Cola* was significant using the paired t test (p=0.01). After one sip of orange juice the pH fell from 6.46 to 5.61 and this change was also found to be significant (p=0.005).

The differences in pH for normal and erosion groups are shown in Table 5.3. Using the paired t test, for the normal group there was a significant fall in pH after one sip of orange juice from pH 6.51 to 5.50 ($p<0.05$). The erosive group showed a significant fall in pH after consuming one sip of *Diet Coke*, when salivary pH fell from 6.61 to 6.11 (p=0.02). However, the fall in salivary pH from 6.40 to 5.75 when drinking orange juice was not significant (p=0.07).

5.3.9 Lowest pH reached

The mean lowest pH reached for all subjects testing each of the drinks in turn is shown in Table 5.4. Significant differences were found between all 3 drinks in relation to the mean lowest pH achieved, when compared using the paired t test. The mean lowest pH of 5.82 achieved while drinking *Coca-Cola* was found to be

Drink	Normal subjects					Erosive subjects				Mean	SD
	1	2	5	6	7	3	4	8	9		
Coca-Cola	6.24	6.36	5.99	5.75	6.3	5.82	5.07	5.28	5.56	5.82	0.45
Diet Coke	6.44	6.39	6.28	5.5	6.42	5.95	5.61	5.87	5.98	6.05	0.35
Orange Juice	6.58	5.82	4.94	4.03	4.99	5.87	4.14	4.07	4.53	5.00	0.91

Table 5.4
Lowest salivary pH values reached by each of the 9 subjects consuming the 3 test drinks in turn. The mean value for each drink and 1 SD (standard deviation) are also shown.

significantly different from the lowest pH of 6.05 achieved when drinking *Diet Coke* (p=0.03). The mean lowest pH of 5.00 attained during orange juice consumption was significantly lower than that found while drinking *Coca-Cola* (p=0.006) or *Diet Coke* (p=0.002).

The mean lowest pH values achieved by normal and erosive groups during consumption of each test drink are shown in Table 5.5. Within the normal group, a difference was found using the paired t test between the lowest pH achieved during consumption of orange juice and *Diet Coke* (p=0.04). The lowest pH reached by the normal group drinking *Diet Coke* was 6.21, compared with 5.27 while consuming orange juice. Within the erosion group, a significant difference (p=0.05) was found in the lowest pH achieved with *Diet Coke* (5.85) and orange juice (4.65). A significant difference (p=0.03) was also found between the lowest pH attained by *Coca-Cola* (5.43) and *Diet Coke* (5.85). There was no significant difference between *Coca-Cola* and orange juice (p=0.07).

Normal and erosion groups were then compared using the two-sample t test. The lowest pH achieved by the normal group (6.13) compared with the erosion group (5.43) while drinking *Coca-Cola* was significantly different (p=0.009). However, no differences were found between the normal and erosion groups for *Diet Coke* (p=0.15) or orange juice (p=0.35).

5.3.10 Fall in salivary pH required to reach lowest pH

The differences between "pH after the first sip" and "lowest pH reached" for each of the drinks are noted in Table 5.6. In all cases, a further fall in pH occurred between

Drink	Normal subjects					Mean	SD
	1	2	5	6	7		
Coca-Cola	6.24	6.36	5.99	5.75	6.3	6.13	0.25
Diet Coke	6.44	6.39	6.28	5.5	6.42	6.21	0.40
Orange Juice	6.58	5.82	4.94	4.03	4.99	5.27	0.97
	Erosive subjects						
	3	4	8	9			
Coca-Cola	5.82	5.07	5.28	5.56		5.43	0.33
Diet Coke	5.95	5.61	5.87	5.98		5.85	0.17
Orange Juice	5.87	4.14	4.07	4.53		4.65	0.84

Table 5.5
Lowest salivary pH values reached by each subject in the normal and erosion groups, consuming the 3 test drinks in turn. The mean value for each drink and 1 SD (standard deviation) are also shown.

Drink	First sip Mean salivary pH	Lowest pH Mean salivary pH	Fall in pH
Coca-Cola	6.20	5.82	0.38
Diet Coke	6.32	6.05	0.27
Orange Juice	5.61	5.00	0.61

Table 5.6
Mean salivary pH values after first sip and at lowest pH reached for each drink. The difference in pH between these 2 points is also shown.

these two points, indicating the importance of repeated exposure of the oral cavity to the test drink. When subjected to statistical analysis using paired t tests, this further fall was significant for *Coca-Cola* (p=0.003), *Diet Coke* (p=0.006) and orange juice (p=0.04).

The differences between "pH after the first sip" and "lowest pH reached" for the normal and erosive groups are shown in Table 5.7. When comparisons were made within the normal and erosion groups, this difference was only significant for the erosion group while drinking *Coca-Cola*, when pH fell from 6.04 to 5.43 (p=0.007).

5.3.11 Time spent below pH 6

The mean length of time for which salivary pH remained below 6 was 15.11 min for *Coca-Cola*, 2.78 min for *Diet Coke* and 15.44 min for orange juice, as shown in Table 5.8. When compared using the paired t test, a significant difference was found between orange juice and *Diet Coke* ($p<0.05$) but no difference was found between *Coca-Cola* and *Diet Coke* (p=0.09).

When the normal and erosive groups were compared using the two-sample t test, a significant difference was found while drinking *Coca-Cola*, with the normal group spending a mean of 2.6 min below pH 6, compared with a mean of 30.75 min for the erosion group (p=0.03). When consuming orange juice, the normal group spend on average 7 min below pH 6, compared with 26 min for the erosion group, although this was not statistically significant (p=0.1). These findings are summarised in Table 5.9.

Drink	First sip Mean salivary pH	Lowest pH Mean salivary pH	Fall in pH
	Normal group		
Coca-Cola	6.32	6.13	0.19
Diet Coke	6.50	6.21	0.29
Orange Juice	5.50	5.27	0.23
	Erosion group		
Coca-Cola	6.04	5.43	0.61
Diet Coke	6.11	5.85	0.26
Orange Juice	5.75	4.65	1.10

Table 5.7
Mean salivary pH values after first sip and at lowest pH reached for normal and erosive groups. The difference in pH between these 2 points is also shown.

Drink	Normal subjects					Erosive subjects				Mean	SD
	1	2	5	6	7	3	4	8	9		
Coca-Cola	0	0	1	12	0	8	52	15	48	15.11	20.58
Diet Coke	0	0	0	8	0	6	7	3	1	2.78	3.35
Orange Juice	0	4	14	13	4	3	55	30	16	15.44	17.47

Table 5.8
Length of time in minutes for which salivary pH remained below pH 6, with means and 1 SD (standard deviation) being shown for each drink.

Drink	Normal subjects					Mean	SD
	1	2	5	6	7		
Coca-Cola	0	0	1	12	0	2.6	5.27
Diet Coke	0	0	0	8	0	1.6	3.58
Orange Juice	0	4	14	13	4	7	6.16
	Erosive subjects						
	3	4	8	9			
Coca-Cola	8	52	15	48		30.75	22.47
Diet Coke	6	7	3	1		4.25	2.75
Orange Juice	3	55	30	16		26	22.26

Table 5.9
Length of time in minutes for which salivary pH remained below pH 6, with means and 1 SD (standard deviation) being shown for normal and erosion groups.

5.3.12 Time taken for recovery to baseline pH

Table 5.10 details the time taken for salivary pH to return to its baseline value. In most cases, pH rose back towards baseline, but as pH rose during drinking for some subjects, the time taken for salivary pH to fall back to baseline is also shown. It is interesting to note that, although salivary pH recovery usually commenced immediately after cessation of drinking for the normal volunteers, a return to baseline pH was not always achieved by the erosive subjects even after the 1 hour experimental period, which is indicated by "+" in the table. This phenomenon was particularly evident while drinking *Coca-Cola*. It can be seen in Figures 5.4 and 5.5 how prolonged the recovery was for the erosion subjects.

5.4 Discussion

The findings of this study were two-fold. These results show once again that not only are there differences between various soft drinks as seen in Chapter 3, but that there are also differences between normal and erosive individuals as shown in Chapter 4. The differences between various soft drinks are possibly greater than any differences between normal and erosive groups, although there is wide variation within each group.

The aims of this study were firstly to develop a new reproducible methodology by which the salivary pH could be monitored following repeated sips of a drink. The second aim was to use this methodology as a possible means of assessing whether the salivary pH profiles between erosive and non-erosive individuals might be different, while the third aim wished to assess the relationship between total titratable acidity and salivary pH. .

Drink	Time for salivary pH to:	Normal subjects				
		1	2	5	6	7
Coca-Cola	Rise back to baseline	40		15	17	
	Fall back to baseline		40			25
Diet Coke	Rise back to baseline			15	15	
	Fall back to baseline	25	40			30
Orange Juice	Rise back to baseline			17	17	20
	Fall back to baseline	20	20			
		Erosion subjects				
		3	4	8	9	
Coca-Cola	Rise back to baseline	60+	60+	60+	60+	
Diet Coke	Rise back to baseline		50	15	14	
	Fall back to baseline	20				
Orange Juice	Rise back to baseline	40	60+	60	60	

Table 5.10
Time taken in minutes for salivary pH to return to baseline value. Where salivary pH levels failed to return to normal within the 1 hour experimental time, this is indicated by "+".

With regard to the first aim, the results of this study have indicated that in most subjects tested the salivary pH continued to fall on repeated sips, albeit to a lesser extent, until the whole drink had been consumed. The pattern of change fell into three types. In the first group, the pH dropped to some extent, but the fall was not large; in the second group, there was a clear rise in pH; in the third group, there was a marked drop in pH with an accompanying slow recovery to normal pH. Three of the four subjects with dental erosion fell into this last category, with the salivary pH falling to a level where dissolution of tooth mineral may occur. This study indicates that the saliva of those with erosion behaves in a different fashion and, thus, may predispose individuals to erosion.

Although the aetiology of erosion is believed to be multifactorial, it is clear that for dental erosion (as well as caries) to occur, the oral fluids must be undersaturated with respect to tooth mineral. It is interesting, therefore, to speculate as to which factors may allow erosion to occur in these subjects. For example, perhaps the salivary buffering system in subjects with erosion simply cannot cope with the sustained low pH and high intrinsic acidity of soft drinks. This contrasted with the group whose salivary pH dropped, but to a lesser degree. Clearly, acidic drinks have the potential to cause erosion, but if the salivary defence systems and tooth structure are able to resist any pathological changes, erosion may not occur. This was highlighted by the salivary profiles of two "atypical" volunteers. One normal subject (PF6) consistently achieved a lower pH during drinking than an erosive volunteer (JM3), yet showed no clinical signs of erosion. This may be due to the rapid recovery period experienced by the normal volunteer.

The pattern of pH fall on subsequent sips was interesting in most cases and worthy of further comment. For example, it was particularly interesting to note a secondary rise and fall in the salivary pH at around 10 min into the experiment. The reason for this primary recovery and secondary fall in salivary pH is not clear, but may be due possibly to another salivary buffering system coming into effect. It has been reported that salivary proteins may play a role if the pH falls to a low value (Tenovuo, 1997), and this "secondary buffering system" may account for the pH changes seen in the earlier part of this experiment. It was also of interest to note that another fall in salivary pH occurring in erosive subjects once consumption of *Coca-Cola* had ceased. These are clearly areas worthy of further study. The differences between cola drinks and fruit juices have been shown clearly in vitro (Chapter 3). It was, therefore, of extreme interest to see that orange juice, with its higher titratable acidity, caused a greater fall in salivary pH than the cola drinks. However, *Coca-Cola* and *Diet Coke*, which showed similar titration curves *in vitro*, behaved quite differently in this study, with *Diet Coke* appearing to have less erosive potential. This may be attributable to the carbohydrate content of regular *Coca-Cola* being metabolised by oral micro-organisms and so increasing the acidogenicity of the drink. An *in vivo* study, such as the one described here, indicates that, although they may give some indication, *in vitro* results should be extrapolated with caution to the situation in the oral cavity.

This study agrees largely with other studies carried out to determine the effect of soft drinks on salivary pH. Tenovuo and Rekola (1977) found that after a 70 mL rinse for 2 min, a drop in salivary pH was detected. However, in that study it was reported that a cola-type drink caused a greater drop in pH than either orange juice or a carbonated orange drink. They hypothesised that a cola drink would stimulate

salivary flow less than an orange drink, thus resulting in a lower salivary buffering response to a cola-type drink. These results are in contrast to those reported here, where salivary pH fell lower while consuming orange juice. However, the recovery period was more prolonged following *Coca-Cola* consumption, indicating perhaps that, once the stimulus of drinking is removed, "erosive" saliva is less efficient at buffering acids remaining in the mouth. These finding agree with those of Frostell (1970), who showed that although fruit juices caused a sharper fall in plaque pH than other drinks, recovery was quicker.

The average pH values for the drinks used in this study are in keeping with the pH values of other soft drinks, which generally fall in the pH range of 2.5 – 3.5 (Chapter 3). In addition, soft drinks are buffered, which keeps the pH low. It is remarkable that, in two cases reported here, the pH of saliva actually rose. This highlights the exceptional ability of the saliva to buffer and neutralise drinks with a low pH and high intrinsic acidity. A study by Imfeld (1983) using telemetry showed that acids had the immediate and marked effect of lowering the pH of saliva. Drinking caused a less pronounced fall in pH than rinsing and allowed a quicker recovery. However, drinks with a high titratable acidity slowed pH recovery, so Imfeld concluded that drinks with a high acid content reflected this by causing greater pH changes in the mouth. This was in agreement with Jenkins (1981) who stated that the pH values of food and drink influence, not only the rapidity of pH drop, but also the lowest value reached. Further investigation of the relationship between titratable acidity and pH changes in the oral cavity may enable clinicians to give appropriate preventive advice to those individuals prone to erosion.

Several studies have monitored the effects of rinsing with acidic substances. Meurman *et al.* (1987) measured changes in the pH of the tongue after rinsing with 100 mL of an acidic drink for 1 min. The tongue pH dropped significantly but returned to baseline within several minutes. Bashir and Lagerlöf (1996) used a 5 sec rinse to investigate the effect of citric acid on salivary pH. A significant drop in pH was noted after 1 min but generally readings were back to baseline after 5 min, although there were large individual variations. The investigators noted the influence of other factors, including degrees of saturation and clearance patterns. These studies which use a single rinse may not be representative of normal drinking, as few people seldom have a single mouthful of a drink. The monitoring of pH following several, repeated exposures would seem like a more realistic and clinically relevant procedure. The study reported here addresses this issue by monitoring salivary pH immediately after consumption of the drink and throughout the drinking period. Not only did the pH drop following the initial sip, but also continued to fall with subsequent sips. Thus, measuring pH changes after only a single mouthful may fail to reveal the full extent of the pH fall. In fact, following the first sip of orange juice, the mean pH achieved by the normal group was lower than that of the erosive group. Despite this, the erosive individuals went on to achieve lower pH values during further consumption. This highlights the fact that prolonged and multiple exposure to acidic drinks may increase the risk of salivary pH falling to even lower levels.

There are variations in the methods of drinking and it has become apparent that many people hold or rinse drinks around their mouth before swallowing. There has been a case report in the dental literature concerning this (High, 1977), while a study by

Edwards *et al.* (1998) using videofluoroscopy confirmed that drinks are often held in or rinsed around the mouth prior to swallowing. There is obviously the potential then for significant damage to be caused to teeth, as rinsing produces higher concentrations of drinks at more locations around the oral cavity (Birkhed, 1984). Despite such differences in drinking pattern, studies investigating rinsing can still provide important information about salivary changes in relation to intake of acidic substances.

The present study measured the pH of whole saliva in an attempt to reflect changes in the pH of the oral cavity, but various techniques have been employed by other investigators. The pH at the tooth surface was measured by Millward *et al.* (1997), who used an electrode embedded in a vinyl splint. 100 mL of citric acid was drunk by glass, straw and feeder cup, all of which caused a sharp fall in pH, which recovered rapidly. This led them to advocate continuous drinking rather than sipping. It could be argued that the present study, by measuring whole saliva, presents a better case scenario as pH values measured at the tooth surface by Millward *et al.* fell to a lower level than those reported here. The methodology of the current study was relatively simple and may, therefore, have potential as a diagnostic tool, enabling screening of patients and identification of those with potential erosion to be given appropriate preventive advice regarding consumption of carbonated and other soft drinks. The *Dentobuff* kit has already been shown to be a poor predictor for susceptibility to dental erosion and so a simple diagnostic test would be useful to screen for those at risk of erosion.

There were some problems encountered in the analysis of the results of this study. As this was a novel methodology, there were no previous protocols or studies upon

which to base any analysis, thus making statistical interpretation difficult. In addition, the numbers in the normal and erosive groups were small and when comparisons were made between the groups, many were not significant. However, these non-significant results may be due to the small numbers involved and real differences could well exist between the groups. Subsequent studies using larger numbers may show further significant differences between normal and erosive groups.

Various points were chosen for data analysis and further studies may highlight which of these may be predictive parameters for susceptibility to erosion. Only a few specific points could then be used in the analysis of future studies. This would also reduce the problem of multiple significance testing that exists in the current study. The testing of so many parameters may well generate some spurious results, simply due to the number of tests being undertaken. Results of borderline significance in the present study should, therefore, be treated with caution. Formal methods, such as Bonferroni corrections, could be applied to adjust for multiple significance testing, although results which are currently highly significant are likely to remain so. In summary, this study is more likely to generate hypotheses rather than test them, but nevertheless is an important advance in the understanding of salivary interactions with acidic soft drinks.

5.5 Conclusions

These results indicate that repeated sipping of soft drinks causes a continued fall in intraoral pH, which only recovers slowly once consumption of the drink has stopped. Also, in subjects with diagnosed dental erosion, the pH fell to a significantly lower

level than in the non-erosive subjects and took longer to recover. The intrinsic acidity of the drink is reflected in this fall in salivary pH.

6 THE POTENTIAL ROLE OF SALIVARY PROTEINS IN DENTAL EROSION

6.1 Introduction

Saliva has several roles in the oral cavity, including buffering of acidic substances and modulation of calcium and phosphate levels (Section 1.4.1).

6.1.1 Salivary buffering

Salivary proteins are thought to be involved in buffering when pH levels fall to under pH 5, which is below the normal physiological range (Cole & Eastoe, 1988). However, it has been seen in the previous study (Chapter 5) that the pH of whole saliva can fall to this value and below while consuming acidic drinks. It is, therefore, conceivable that proteins exert some buffering action at the pH levels achieved while drinking low pH beverages. The differences observed between the salivary buffering capacity of normal and erosive individuals may be attributable to qualitative and quantitative variations in salivary proteins.

6.1.2 Pellicle

The organic elements in saliva are also involved in the formation of pellicle. Acquired pellicle is a layer of selectively adsorbed macromolecules formed on the tooth surface (Hay & Bowen, 1996). It acts to protect against acid attack and subsequent mineral loss (Zahradnik, Moreno & Burke, 1976) while still permitting remineralisation and repair (Hay & Moreno, 1993). The hydroxyapatite-binding proteins found in pellicle are believed to be mainly acidic proline-rich proteins or

PRPs (Kousvelari *et al.*, 1980; Bennick *et al.*, 1983) but also include glycoproteins or mucins (Cohen & Levine, 1989), statherin (Hay & Moreno, 1993), histatins (Oppenheim, 1989) and cystatins (Hay & Bowen, 1996).

The proteins that are concerned with inhibiting the precipitation of calcium and phosphate from saliva are also involved in pellicle formation and these different functions are now thought to be dependent on variations in the conformation of the molecule (Hay & Moreno, 1993). The concentrations of salivary proteins vary with glandular source and flow and may vary also with other physiological factors (Kousvelari *et al.*, 1980). Genetically-determined polymorphisms of salivary proteins have been discovered (Azen, 1989) and, as pellicle is believed to be protective against erosive insults (Meurman & Frank, 1991a), variation between individuals could cause a predisposition towards dental erosion.

6.1.3 Preliminary investigations

A pilot study by Bell, Creanor & Foye (1998) indicated that there were qualitative and quantitative differences between the saliva from normal and erosion individuals. Normal saliva showed greater amounts of proteins believed to be PRPs, although "erosive" saliva had overall a greater amount of protein. The study also looked at rate of pellicle formation, as well as the protein content of pellicle. The control pellicle had greater amounts of protein initially, suggesting that pellicle may be laid down more quickly by normal individuals than erosive individuals. Pellicle formed by "erosive" saliva contained greater amounts of proteins believed to be histatins and glycosylated PRPs respectively, while pellicle from normal saliva contained greater amounts of proteins, in the weight range of PRPs. The study concluded by

recommending that analysis of the saliva from a range of erosive and non-erosive individuals should be carried out, perhaps attempting to identify the proteins involved. The aim of the current study was, therefore, to collect saliva from a larger number of subjects for salivary protein analysis.

6.2 Materials and Methods

Twenty-two subjects, 11 males and 11 females from the age range 8-17 years, with diagnosed dental erosion were asked to give unstimulated and stimulated salivary samples, as described in Sections 2.2.1 and 2.2.2 respectively. Erosion was graded as I (mild), II (moderate) or III (severe) as summarised in Section 2.3.1. A clinical examination recording DMFT, DMFS and a brief orthodontic assessment was undertaken, as well as questions regarding dietary habits. In addition, 22 age-matched and sex-matched controls were also recruited for the study, bringing the final total to 44 subjects.

6.2.1 Electrophoresis

Electrophoresis is a technique used to separate and analyse protein mixtures. Proteins carry a charge at any pH other than their isoelectric point and will, therefore, migrate in an electric field. The most common support medium in which zone electrophoresis is carried out is polyacrylamide gel. These transparent gels can be produced with a wide range of pore sizes, acting as a sieve to separate proteins of different size ranges, and so polyacrylamide gel electrophoresis (PAGE) plays an important role in the experimental analysis of proteins (Hames, 1990).

6.2.2 One-dimensional discontinuous SDS-PAGE

The addition of anionic detergent such as sodium dodecyl sulphate (SDS) to the sample dissociates and denatures proteins into their constituent polypeptide units. SDS binds to proteins in a constant ratio resulting in a rod-like shape and masking the intrinsic charge of the protein. Consequently, electrophoretic separation depends on molecular weight (MW), which varies with the length of the rod. By using a set of known MW markers, the weight of the sample proteins can be calculated. To allow small volumes of sample to be analysed in sharp zones with good resolution, a discontinuous buffer system is employed. Samples are loaded into a large pore stacking gel, which has been polymerised on top of the small pore resolving gel. There is a discontinuity in buffer composition and pH between the two gels. The proteins are concentrated into narrow zones in the stacking gel before being separated during electrophoresis in the resolving gel. The most commonly used buffer system for SDS-PAGE is that devised by Laemmli (Dunn, 1993).

6.2.3 Sample preparation for SDS-PAGE

After collection, the salivary samples were centrifuged for 10 min at 7000 rpm. The supernatant was transferred to a clean Eppendorf tube and frozen at –20° C until use. On the day of SDS-PAGE analysis, salivary samples were allowed to come to room temperature. Once defrosted, saliva was treated with Laemmli sample buffer. Sample buffer consists of:

62.5 mM Tris HCl pH 6.8 (trishydroxymethylmethylamine with pH adjusted by hydrochloric acid)
2% SDS
2% mercaptoethanol (to cleave disulphide bonds)
12% glycerol (to increase the density of the sample)
0.5% bromophenol blue (to mark buffer front during electrophoresis).

The sample buffer was made up at twice the normal concentration and mixed in a ratio of 1 to 1 with the salivary samples, giving the final concentrations noted above. To ensure optimal reaction with SDS the samples were heated with buffer to 100° C for at least 3 min.

6.2.4 Gel preparation

A 15% resolving gel solution was used, consisting of:

2.45 mL deionised water
2.5 mL 1.5 M Tris HCl pH 8.8
5 mL acrylamide/bis solution 30% stock
50 µL ammonium persulphate (APS)
5 µL tetramethylethylenediamine (TEMED).

Polyacrylamide gels are formed by the polymerisation of acrylamide monomers into long chains which are crosslinked with bis (methylene-bis-acrylamide) to form a lattice. APS acts as a catalyst, while TEMED accelerates the polymerisation process. As acrylamide is a known neurotoxin and is also possibly carcinogenic, gloves are worn at all times when preparing gels.

SDS-PAGE was carried out using a Mini-Protean II Dual Slab Cell (Bio-Rad, Hemel Hempstead, UK). The resolving gel was cast between 2 glass plates, separated by a 0.75 mm spacer. The solution was overlaid with butanol saturated water, to ensure an even gel surface, and allowed to polymerise for 45 min to 1 hour. The butanol was then washed off and the gel allowed to dry.

A 4% stacking gel was then prepared. This consisted of:

6.15 mL deionised water
2.5 mL 0.5 M Tris HCl pH 6.8
1.33 mL acrylamide/bis solution 30% stock
50 µL ammonium persulphate (APS)
10 µL tetramethylethylenediamine (TEMED).

The stacking gel was poured on top of the resolving gel. In order to form sample wells, a plastic comb was inserted immediately into the stacking gel and the solution allowed to polymerise around the comb. Once the stacking gel had polymerised the comb was removed and the sample wells washed out with running buffer (0.25 M Tris, 1.92 M glycine, 1% SDS, pH 8.9). Gloves were worn when handling all equipment used for SDS-PAGE to minimise any contamination from skin proteins.

6.2.5 Sample loading and electrophoresis

The prepared salivary samples were loaded into the sample wells. The volume of sample added depended on the eventual staining technique, and was either 2-3 µL for silver staining or 12-15 µL for Coomassie Blue staining. The samples were applied using a graduated glass syringe and were added slowly to the sample wells to prevent overspilling into the next lanes. Molecular weight markers consisting of proteins

with MW 12 kD, 17 kD, 30 kD, 43 kD, 66 kD and 78 kD (BDH, Poole, UK) were added to the left hand lane of each run. The gel apparatus was assembled and running buffer added to the upper and lower reservoirs. Electrophoresis was carried out at 200 V constant voltage until the bromophenol blue marker reached the end of the resolving gel, normally 45 min.

6.2.6 Protein staining

Following electrophoresis, the gels were recovered prior to fixing and staining. The two staining techniques used in this study were silver and Coomassie Blue R-250. Silver staining is more sensitive and is able to detect small amounts of protein (Hames, 1990). The gels were first fixed for 15 min in either a 40% methanol, 10% acetic acid solution or 12.5% trichloracetic acid (TCA). Fixation precipitates and immobilises the separated proteins within the gel and removes any non-protein substances within the gel that may interfere with subsequent staining. There are many variations in the methodology of silver staining, but all rely on the reduction of ionic silver to metallic silver (Dunn, 1993). The protocol for this study involved immersing the gels in 0.05% dithiothreitol (DTT) for 15 min, which acted as a reducing agent. The gels were then placed in a 0.2% silver nitrate solution for a further 15 min. The silver nitrate was washed away by rinsing the gels in distilled water for 15 sec. The gels were next rinsed briefly in developer (3% sodium carbonate, 0.5% formaldehyde) to remove an initial silver precipitate and the bands visualised by further development. The staining reaction was stopped by placing the gel in 10% acetic acid.

Coomassie Brilliant Blue (CBB) R-250 is the most common stain used in PAGE. The gels were fixed and stained simultaneously in a solution of 1% CBB, 40% ethanol and 10% acetic acid. Following overnight immersion, the excess stain was removed from the gel, to allow the protein bands to be seen clearly, by using several changes of destaining solution (40% methanol, 10% acetic acid). Samples were subjected to staining by both staining methods since it is not unusual that proteins sensitive to one stain will be undetected by the other.

6.3 Results

6.3.1 Subject data

The twenty-two erosion subjects were graded on the severity of their disease. There were 6 with mild, 6 with moderate and 10 with severe erosion, with equal numbers of males and females in each category. The age range was from 8 to 17 years with a mean age of 14 years (SD 2.3). All had erosion of permanent teeth, except 1 male and 1 female in the severe group who showed marked wear of primary teeth. When these two subjects were excluded, the mean age of the mild group was 12.8 years (SD=1.5), the moderate group 14.5 (SD=1.0) and the severe group 15.8 years (SD=1.4). This data is summarised in Table 6.1.

6.3.2 DMF data

DMFT and DMFS scores were taken for each erosion and control subject. The mean DMFT for the erosion group was 2.95 (SD=3.23) with a range from 0-11. The mean DMFT for the control group was 5.5 (SD=5.7) with a range of 0-20. However, this

	Mild erosion	Moderate erosion	Severe erosion
	Age in years		
Male	15 13 11	16 15 13	17 15 14 14 *11*
n=	3	3	5
Female	14 12 12	15 14 14	17 17 17 15 *8*
n=	3	3	5
Mean	12.8	14.5	15.8
SD	1.5	1.0	1.4

Table 6.1
Age of 22 erosion subjects, 11 male and 11 female, graded by severity of disease. Ages in italics represents those with erosion in primary dentition rather than permanent dentition. The mean ages for each of the groups are also shown, with 1 SD.

Key: SD Standard deviation
n number

difference was not significant when compared using the two-sample t test (p=0.08). The DMFS scores also showed a wide variability, with the erosion group mean of 6.09, covering the range 0-30, while the mean control group DMFS was 13.64, range 0-74. However, once again, this difference was not statistically significant (p=0.16). The data was split into subgroups of male/female and erosion/control and the mean DMF scores displayed in Table 6.2. The female control group had a higher mean DMFT (7.27) than the male control group (3.73) and the female erosion group (3.36) but these differences were not statistically significant.

6.3.3 Salivary flow rates

There were no significant differences seen between the mean unstimulated salivary flow rate of normal and erosion groups, which were 0.30 and 0.22 mL/min respectively, when compared using the two-sample t test. However, the female erosion subgroup had a significantly lower flow rate (0.13 mL/min) than both the female control subgroup (p=0.02) and the male erosion subgroup (p=0.03), who both had mean unstimulated flow rates of 0.31 mL min. The stimulated salivary flow rates of the control and erosion groups were 1.2 and 1.35 mL/min respectively. No significant differences were seen between any of the groups or subgroups when the stimulated salivary flow rates were compared.

6.3.4 *Dentobuff* analysis

The stimulated saliva from 20 of the erosion subjects and their matching pairs was tested using the *Dentobuff* (*Vivacult BC*) system (Ivoclar Vivadent, Meridian, Leicester, UK), to give a grading for salivary buffering capacity. Fifteen of the

	Mean DMFT	SD	Mean DMFS	SD
AE	2.95	3.23	6.09	8.59
AC	5.50	5.7	13.64	20.28
ME	2.55	3.24	5.09	8.90
MC	3.73	4.17	9.18	13.83
FE	3.36	3.32	7.09	8.56
FC	7.27	6.62	18.09	25.08

Table 6.2
Mean DMFT and DMFS scores for erosion and control groups, and male and female subgroups.

Key:		
	AE	All erosion subjects
	AC	All control subjects
	ME	Male erosion subjects
	MC	Male control subjects
	FE	Female erosion subjects
	FC	Female control subjects
	DMFT	Decayed, missing and filled teeth
	DMFS	Decayed, missing and filled surfaces
	SD	Standard deviation

erosion and 10 of the control subjects showed high buffering capacity, with 2 erosion and 9 controls having medium buffering capacity. Low buffering capacity was found in 3 of the erosion and one of the control subjects. These results are shown in the histogram in Figure 6.1.

6.3.5 Orthodontic data

A brief skeletal class assessment was undertaken as part of the clinical examination. Fourteen of the 22 erosion patients had a Class I skeletal base (63.6%), 1 had a Class II (4.5%) and 7 a Class III (31.8%). The control group had 10 subjects with a Class I (45.5%), 3 with a Class II (13.6%) and 9 with a Class III skeletal base (40.9%). Figure 6.2 summarises these findings.

6.3.6 Dietary investigations

Although the dietary questions were brief, a wide range of answers was obtained and, therefore, only the main observations will be reported here. All erosion subjects admitted to consuming acidic soft drinks, with many admitting to "frothing" drinks around the mouth prior to swallowing. There was also a high incidence of consumption between meals and last thing at night. Many of the control group also consumed acidic beverages, but these were mainly drunk quickly at mealtimes.

6.3.7 Protein analysis

SDS-PAGE gels of saliva from erosive and control subjects stained with Coomassie Blue are displayed in Figures 6.3 and 6.4 respectively. These figures show examples

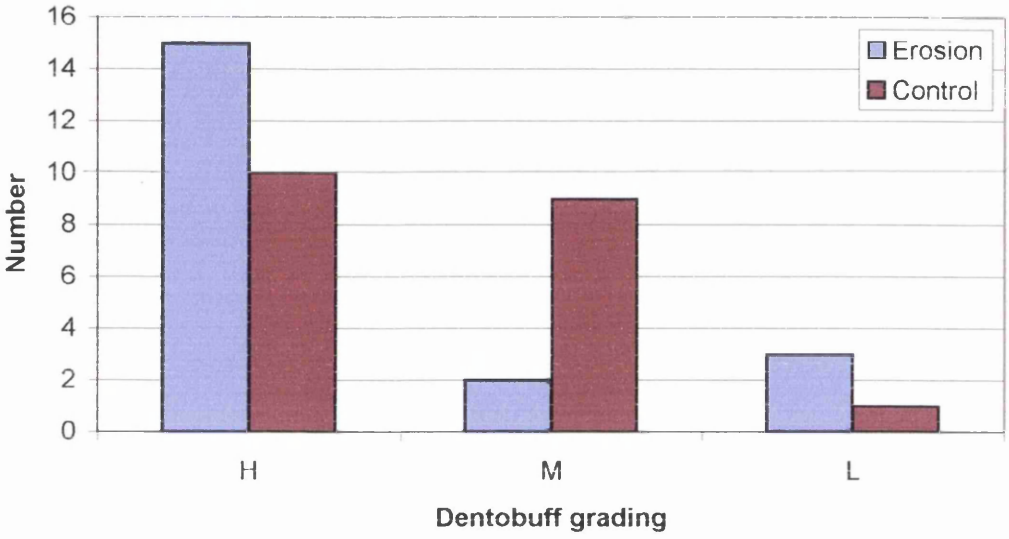

Figure 6.1
Histogram showing distribution of *Dentobuff* grading in erosion and control groups. Salivary buffering is categorised as high (H), medium (M) or low (L).

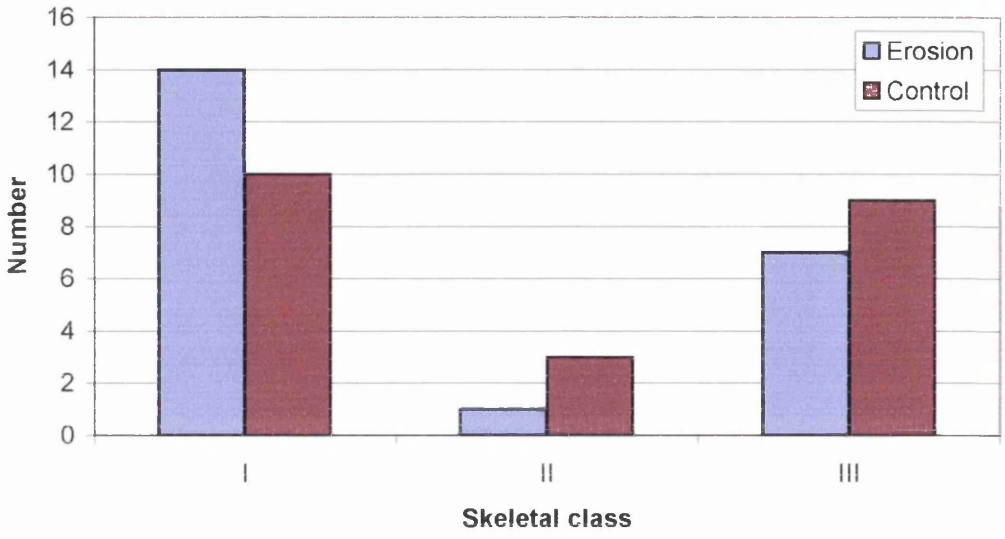

Figure 6.2
Histogram showing distribution by skeletal class of erosion and control groups.

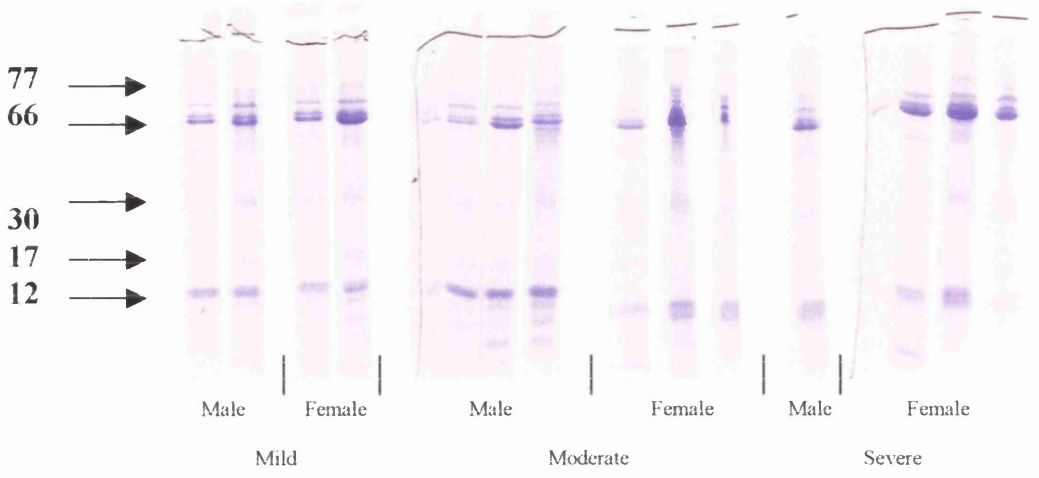

Figure 6.3
SDS-PAGE gels showing examples of subjects with mild, moderate and severe erosion. Molecular weight markers in kD on left hand side.

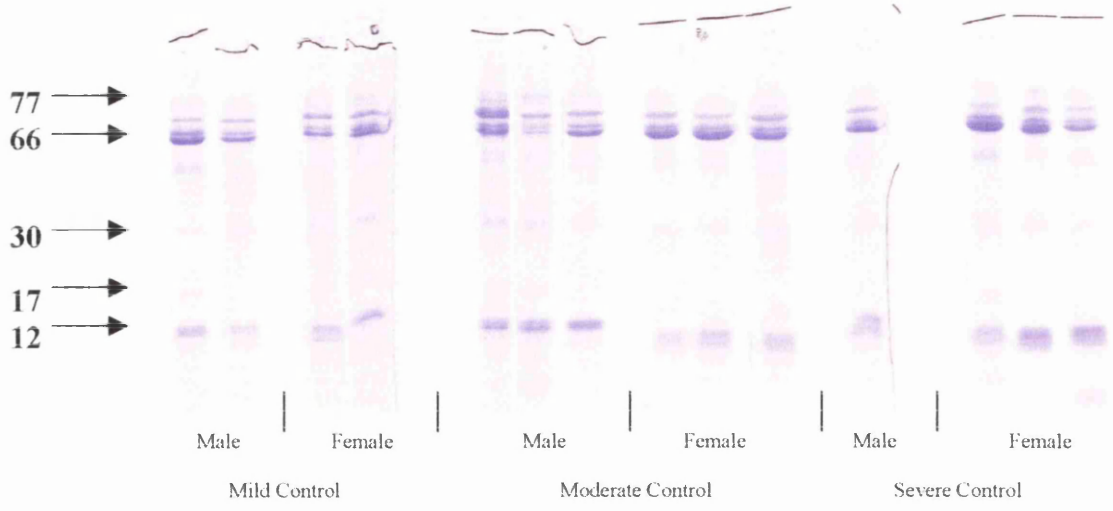

Figure 6.4
SDS-PAGE gels showing corresponding controls for subjects with mild, moderate and severe erosion. Molecular weight markers in kD on left hand side.

of subjects with mild, moderate and severe erosion, with molecular weight markers noted on the left. Results in the 66-77 kD region were variable. The mild erosion subjects showed an increase in this band compared with controls that was not as obvious in the moderate and severe subjects. However, the female moderate subjects could not be compared with their controls due to distortion of the lanes in the erosion gel. In addition, this observation of increased protein content in the 66-77 kD band of "erosive" saliva may be due to more overall protein in the erosion samples.

The male mild erosion subjects showed an increase in the 12 kD band, which was not as obvious in the female mild erosion subjects. The male moderate subjects also showed an increase in the 12 kD band, but again this was not as obvious in the female moderate subjects. No differences were observed in the 12 kD region between severe erosion subjects and their controls.

No large differences in band patterns were observed on the silver stained gels, although there was a suggestion that the total protein content in the saliva of the controls was higher than in erosion saliva.

6.4 Discussion

The results of the clinical part of this study gave some interesting insights into other possible factors involved in the aetiology of dental erosion. The severity of erosion increased with age, in that older teenagers had more marked erosion than younger teenagers. This finding could have been predicted, as the permanent incisors have been present in the oral cavity for longer. However, the incisors have only been erupted for, on average, 8 years and, as permanent teeth, are expected to last until old

age. It is distressing to note severe erosion in these young adults, which has occurred in the relatively short time since the teeth erupted.

Dental caries is a major public health problem in the West of Scotland, and so it was not surprising to see high DMF values in teenagers, which resulted in a large standard deviation. It was interesting to note a higher DMFT in the control groups, as many clinicians would agree that erosion patients tend either to be caries-free or to have a low caries rate. Other studies have not attempted to address the relationship between erosion and caries and although this result failed to reach statistical significance, it substantiates the "clinical hunch" that erosion patients tend to have lower caries experience. Caries and erosion are clearly different disease processes (Section 1.2.3) and it is not unexpected that some patients are predisposed to one entity and not the other. Further studies of larger numbers of subjects may show significant differences between the caries experience of normal and erosive individuals. An additional point of interest concerns the unexpected finding of a higher caries rate in the female control group than the male control group. This is unusual, as it is widely accepted that males have a higher incidence of caries than females.

The influence of salivary flow rates in the aetiology of dental erosion has already been studied extensively (Section 1.4.2). The finding that erosion patients had lower unstimulated flow rates than normal patients agrees with, amongst others, the studies by Jarvinen, Rytomaa & Heinonen (1991) and Woltgens *et al.* (1985). No differences were found between the stimulated flow rates of normal and erosion groups, which agrees with the results of Woltgens *et al.* (1985) but is in contrast to the results of Jarvinen *et al.* (1988). There is ongoing debate about whether low

unstimulated or low stimulated flow rates are more important as risk factors for dental erosion. On the basis of this study, unstimulated flow rates have been found to be significantly lower in erosion subjects.

This study showed no correlation between normal subjects having high salivary buffering capacity and erosive subjects having lower buffering ability. This is in contrast to other studies (O'Sullivan & Curzon 1995; Gudmundsson *et al.*, 1995) which demonstrated a clear relationship, with erosive subjects having significantly lower buffering capacity. However, the accuracy of *Dentobuff* in predicting susceptibility to erosion has already been brought under scrutiny (Chapter 4) and the results from the current study seem to underline these earlier findings.

The orthodontic examination was included as, once again, previous investigations have not sought to determine a relationship between occlusion and erosion. However, the sample size in the current study is relatively small, so no firm conclusions can be drawn. The erosion patients were taken mainly from the Paediatric Dentistry unit, to which patients had been referred for specialist treatment. In contrast, many of the control subjects were patients referred to the Orthodontic department for treatment, and so a higher incidence of skeletal Class II and III might be expected in this group. A larger epidemiological study would be required to investigate the potential influence of occlusion on erosion.

Some interesting trends were shown by the dietary questionnaire, which agree with previous studies investigating dietary factors pertinent to erosion (Millward *et al.*, 1994; Jarvinen, Rytomaa & Heinonen, 1991). Full analysis of a more detailed questionnaire would be more meaningful and would minimise the risk of leading

questions. Despite this, there was a high reported incidence in the erosion group of soft drink consumption between meals and last thing at night, and many erosion patients also admitted to frothing drinks around the mouth prior to swallowing. All of these are known to be risk factors in the development of dental erosion (Johansson *et al.*, 1997; Millward *et al.*, 1994; High, 1977).

The analysis of salivary proteins from a range of normal and erosive individuals gave some interesting insights. The preliminary suggestion that saliva from erosive subjects may contain more proteins in the 66-77 kD band and 12 kD band agreed broadly with the findings of the pilot study, indicating that the protein content of saliva varies between normal and erosive individuals. The major proteins in saliva have been characterised by Beeley *et al.* (1991). From this information, salivary proteins in the weight range 66-77 kD might be either glycosylated PRPs or amylase. Low molecular weight proteins of 12 kD are possibly histatins or basic PRPs. It is widely accepted that both PRPs and histatins play roles in modulating calcium phosphate precipitation and crystal growth, as well as being found in acquired pellicle (Kousvelari et al., 1980; Oppenheim, 1989). It is possible, therefore, that these proteins may play a role in the progress of erosion.

Although differences may exist between normal and erosion groups, it is important to note that there was also significant variation within the control group, probably due to variability in salivary protein expression, secretion and possibly post-secretion modification. Further research such as amino acid sequencing would be required to identify accurately the proteins involved. There was an indication from the silver stain gels that the total protein content in the saliva of the controls was higher than in the saliva from erosive subjects. However, this observation should be treated with

caution, as silver stain is non-quantitative and further tests such as protein assays would determine protein concentration more accurately.

Although this research did not identify stark variation between normal and "erosive" salivary protein content, other methods may be used to look further at protein differences. Mineral-binding proteins may be protective against erosion and specific tests to identify these may reveal more marked variation in salivary protein content between normal and erosion individuals. The current research looked at whole saliva, the overwhelming protein content of which might mask subtle differences in mineral-binding proteins. There is certainly potential to continue to address the issue of differences in organic content between the saliva of normal and erosive individuals, both for use as a diagnostic indicator and a predictive test for erosion susceptibility.

6.5 Conclusions

The results from this landmark study indicate that there are differences between normal and erosive subjects, in terms of unstimulated salivary flow rates and potential differences in caries experience. There may also be organic differences in the saliva of normal and erosive individuals that might influence susceptibility to dental erosion.

7 DISCUSSION

Dental erosion has a multifactorial aetiology, with some individuals being more susceptible than others. As stated in the Introduction, the purpose of this research was to further knowledge on the role of various soft drinks and the influence of salivary factors on dental erosion. Specifically, the aims of this research were:

- To rank various soft drinks in order of their erosive potential.
- To challenge normal and "erosive" saliva *in vitro* to assess its ability to buffer soft drinks.
- To monitor salivary pH changes in normal and erosive individuals *in vivo* during drinking.
- To examine salivary proteins present in the saliva of normal and erosive individuals.

These aims have been fulfilled. The results of this research indicate that not only do soft drinks vary in their buffering capacity and, therefore, their erosive potential, but also that individuals vary in their ability to cope with the acidic challenge of soft drinks.

The four phases of the research project followed on from one another in a natural progression. The initial stage of investigating some of the chemical properties of soft drinks was a prerequisite in order to give baseline information. The introduction of saliva to the applied *in vitro* study was a unique concept, which took some account of variations that might occur in the oral cavity. The ranking, with regard to erosive potential of soft drinks, was maintained when saliva was used as a biological base.

The use of saliva also gave an early indication that normal and erosive individuals might vary in their ability to buffer acidic substances, which was an original finding not been shown by previous research. At the third *in vivo* stage, decisive information was gained both about distinctions in salivary pH profiles when consuming various soft drinks and about differences in salivary buffering ability of normal and erosion subjects. Once again, it was exciting to see differences never before shown between normal and erosive individuals. Finally, the fourth, ground breaking phase attempted to identify the organic factors in saliva that might account for the variations evident between normal and erosive groups at the other stages of the research.

The initial *in vitro* study employed a tested scientific method to characterise a range of drinks available on the market and showed distinct differences between various groups of drinks. Many quote the pH values of soft drinks in an attempt to implicate beverages in the aetiology of dental erosion. While it is true that most soft drinks have low pH values, the current study showed that total titratable acidity gives more information about the relative erosive potential of drinks. The pH values of cola-based drinks are, on average, 1 pH unit lower than fruit juices. However, the total acid content of fruit juices is much higher than that of non-fruit flavoured carbonated beverages. In addition, comparing the carbonated drinks with their fruit-based counterparts and the sparkling mineral waters with the flavoured ones showed the impact of fruit flavouring on the acid content of drinks. This is an important distinction to make, as the drinks are packaged and consumed in a similar manner and tend to be classified generally as "fizzy drinks". Indeed, the contribution of carbonic acid from the carbonation process may be relatively unimportant compared

with the addition of fruit flavouring, as the buffering capacity of carbonated mineral water was significantly less than that of all other soft drinks tested.

The simple protocol of an acid-base titration has been expanded to give a major methodological advance in describing the total titratable acidity of a given drink or group of drinks and enables beverages to be ranked in order of erosive potential. By disseminating this information, it is hoped that the concept of total acidity rather than actual pH value of drinks will now be adopted by dentists, when referring to soft drinks and when giving advice to patients.

In vitro acid-base titrations are useful in the initial characterisation of beverages, but results may change once the drink is exposed to the oral cavity. Saliva is a powerful buffering agent that is produced constantly and so the situation in the oral cavity is not static. In addition, the many interacting factors present during drinking can complicate the investigation of any single agent. An original study designed to bridge the gap between purely *in vitro* studies and *in vivo* investigations has been presented here and shows distinct advantages. The use of saliva as a biological base in acid-base titrations is a novel concept, which gives for the first time information on the buffering ability of saliva when challenged by acidic soft drinks.

The fact that the ranking of drinks in order of erosive potential was maintained showed that saliva is effective at neutralising acidic drinks. There were clear differences found between drinks, depending on their total titratable acidity. The preservation of the ranking also added weight to the results of the purely *in vitro* study, which may have been treated with circumspection by clinicians. In addition, for any given drink, differences were also seen between the saliva of normal and

erosive groups with regard to initial pH, fall in pH (actual pH) and rate of fall (delta pH). The saliva of individuals with erosion was shown to cope less well with the acidic challenge of soft drinks, an important finding in understanding the aetiology of erosion. Further baseline information on the salivary characteristics of the subjects in this study would have been useful. Future studies should consider assessing unstimulated and stimulated pH, flow rates and buffering capacity, in order to build up background data on potential differences between normal and erosive subjects.

This was a static model, as no further saliva was added to the saliva/drink mixture during the titration. Although this is obviously unrepresentative of the situation in the oral cavity, it does allow the role of bicarbonate to be assessed more closely. It was particularly interesting to note that, when the protective paraffin seal was omitted from the protocol, the pH took much longer to stabilise after each increment of drink had been added. This may have been due to fewer ions moving across the electrode following the loss of bicarbonate. The pH also appeared to fall less after the addition of the first few increments of the chosen drink. The fact that there were few overall differences between the titration curves with and without the paraffin seal suggests that bicarbonate may not be the principal buffering agent when saliva is exposed to acidic substances. The low pH values achieved during the drinking of acidic beverages may call for buffering from other salivary agents, as yet unknown, but possibly including salivary proteins. This concept clearly requires further investigation, as it calls into question the role of the supposed main buffering agent in saliva, namely bicarbonate. However, it certainly appears that many elements are involved in the buffering of acidic food substances.

The monitoring of saliva during drinking employed a novel protocol to assess the ability of saliva to cope with multiple exposure to an acidic agent. This was more representative of the real life situation, when an entire can or glass of drink will be consumed. Therefore, this study superseded others reported in the literature that examined the affect of a single acidic challenge and allowed differences between subjects to be teased out. At the outset, it was expected that salivary pH would fall, even just slightly, during drinking and so it was surprising to see normal individuals who experienced a rise in salivary pH. However, this rise in pH was observed for various individuals on several occasions with different drinks and must, therefore, be assumed to be an accurate, if unexpected, finding. It shows indeed the remarkable buffering capability of saliva, which is obviously lacking in some individuals, hence predisposing them towards dental erosion. The consistent finding of three distinct salivary profiles with each drink was extremely interesting and indicated further that the saliva of individuals varies greatly in its buffering ability. This *in vivo* test and the applied *in vitro* study have both been crucial in identifying discrepancies in the capability of saliva when challenged by extremes of acidity. Significant potential exists to develop these investigations further for diagnostic purposes.

The monitoring of pH after the drink was finished provided valuable information about patterns of recovery. Additional measurements taken at this stage may have given further insights and future studies should, therefore, consider taking extra samples in the period immediately following cessation of drinking. The recovery pattern experienced by the erosive subjects after drinking *Coca-Cola* is worthy of further note. Salivary pH fell again during recovery, suggesting that the salivary stimulus provided by cola drinks is shorter lived than that following consumption of

fruit juices. The acid content of the drink being consumed is obviously a major factor in determining salivary pH responses during both drinking and recovery.

As seen in the applied *in vitro* study, *Diet Coke* and *Coca-Cola* behaved quite differently, despite their apparently similar total acid content. Salivary pH fell to a lower value with regular *Coca-Cola* than with *Diet Coke*, perhaps reflecting the higher pH value of the diet drink. In addition, *Diet Coke* has no fermentable carbohydrate, the metabolism of which might lead to acid production by oral microorganisms, although the amount would be small in comparison with the intrinsic acidity of the drink. It seems that cola drinks may be the "safest" drinks for those prone to dental erosion, preferable to fruit-based beverages, and it might be speculated that a diet cola is the best drink for those with erosion. Another interesting observation came when one subject was tested following physical exercise. Salivary pH fell while drinking in this instance much lower than on the other occasions when the drink was tested. Many people consume soft drinks after exercise to combat dehydration. It may be that in theses circumstances, fruit drinks may have more erosive potential and, therefore, individuals prone to erosion should be given appropriate preventative advice. In addition, this observation agrees with others that xerostomia predisposes to dental erosion.

The final experimental stage sought to investigate why there were differences in the preceding two salivary studies. A pilot study had already indicated that the salivary protein content might vary between erosive and normal individuals. The current research screened larger numbers of erosion subjects to compare with carefully matched controls. The main observation of this important landmark investigation agreed with the pilot study, in that the saliva from some erosion subjects appeared to

have more proteins at the extremes of low and high molecular weights. Proteins are thought to be involved in buffering at extremes of pH, as might be found while consuming acidic soft drinks. In addition, salivary proteins are adsorbed onto the tooth surface to form pellicle. There has been significant interest recently concerning the protective properties of pellicle against acid attack of enamel. This is interesting, as pellicle was once considered a major factor in the development of caries and periodontal disease because it acted as a substrate for plaque deposition. However, it now seems that pellicle might play a protective role in the erosion process. Therefore, protein content will be important, as this will determine which proteins are available to adsorb onto the enamel surface. Low molecular weight proteins might be less efficient at protecting against acid erosion. There is scope for further work in this area to sequence the amino acids of the proteins involved in these processes. Identification of such key proteins might also be used to indicate susceptibility to erosion.

In summary, these experiments show that many buffers are involved in the salivary defence system, some of them as yet unidentified. In addition, although saliva is derived from blood, it appears to adopt individual characteristics that vary between subjects. This might explain why the results of proprietary tests for salivary buffering capacity showed poor correlation with the outcomes described here. These tests were designed to indicate susceptibility to caries, which is clearly a different disease process with its own predisposing factors. Future research should aim, therefore, to develop tests which are specific for erosion susceptibility.

Some volunteers were common to both the applied *in vitro* and the *in vivo* studies. When an overall view is taken of the various stages of the research, it becomes

apparent that saliva from the same person behaves differently *in vitro* and *in vivo*. One of the erosion subjects behaved poorly *in vivo* but better *in vitro*, in contrast to another subject whose saliva coped better *in vivo* than *in vitro*. Only the saliva of one subject who took part in the two experiments performed poorly in both. An additional interesting finding concerned the differences between erosion and normal groups in baseline unstimulated and stimulated salivary pH measurements. Stimulated saliva was used for the applied *in vitro* experiment. The pH of this was used as a baseline reading and the erosion group was found to have a significantly lower stimulated salivary pH than the normal group. In the *in vivo* study, unstimulated saliva was taken as a baseline measurement before drinking commenced. In this case, no significant differences were found between the unstimulated salivary pH of normal and erosion groups. Although this discrepancy may be attributable to differences between unstimulated and stimulated saliva, it also shows the importance of repeated sampling within large study groups to show reproducibility in a given study. These observations also highlight that any one set of test circumstances should not be taken alone to indicate erosion susceptibility.

Although this research has addressed the dual issues of buffering capacity of drinks and buffering ability of saliva, no account has been taken of the chemical structure of the erosive agents. Citrate is a powerful chelator and may play a role in erosive tooth tissue loss. Other organic acids in drinks may also have chelating properties. By binding calcium in saliva, chelators reduce the degree of supersaturation with respect to tooth mineral, while strong chelating agents may enhance direct dissolution of enamel. There is considerable debate as to whether chelation is operative at the low

pH values found in the mouth. However, as erosion in known to be multifactorial, all possible factors should be taken into account.

Clearly, dental erosion is a complex process, which is presenting a significant problem to the dental profession. The aetiology is multifactorial, with the exact disease mechanism as yet unknown, although this research has contributed significantly to our understanding of the role of salivary buffering. It has also identified the importance of individual susceptibility and the need to find predictors for this. Further work in the area will focus on developing a predictive test on an epidemiological basis. It is planned that the labour intensive protocol for repeatedly sipping a soft drink will be adapted to make it less time consuming, with readings taken only between eight and ten minutes into the experiments as well as baseline salivary pH measurements. It is expected that some individuals will experience a fall in pH while others will show a rise in pH, thus forming the basis for a predictive test. In addition to this, salivary protein profiles may also indicate which individuals might be prone to erosion.

In conclusion, this study has used a logical sequence of experiments to identify differences between drinks and between individuals. It has clearly shown significant variations between the saliva of normal and erosive individuals, which should be identified further to form predictive tests for erosion susceptibility.

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