Mechanistic Profiling of Novel Wafer Technology Developed for Rate-Modulated Oramucosal Drug Delivery

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Master of Pharmacy

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DECLARATION

I, Rupal Patel declare that this dissertation is my own work. It is being submitted for the degree of Master of Pharmacy in the Faculty of Health Sciences in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any other examination at this or any other University.

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This 12th Day of April 2005.

Research Presentations

Design of a Novel Rapidly Dissolving Wafer System for Oramucosal Drug Delivery (poster), Rupal Patel, Viness Pillay and Michael P. Danckwerts, Academy of Pharmaceutical Sciences Conference, Durban, South Africa, September 2003.

Porosity-Controlled Lyophilized Crosslinked Polymer Wafers for Prolonged Oramucosal Drug Delivery (poster), Rupal Patel, Viness Pillay and Michael P. Danckwerts, Annual Meeting of American Association of Pharmaceutical Scientists, Salt Lake City, Utah, United States of America, 26-30 October, 2003.

Mechanistic Evaluation of Novel Wafer Technology Developed For Oramucosal Drug Delivery: *In vitro* and *in situ* Design Strategies (podium), Academy of Pharmaceutical Sciences Conference, Grahamstown, South Africa, September 2004.

A Textural Profile Study of Lyophilized Wafers Designed for Oramucosal Drug Delivery (poster), Academy of Pharmaceutical Sciences Conference, Grahamstown, South Africa, September 2004.

Experimental Design to Probe the Physicochemical and Physicomechanical Transitions Within Lyophilized Polymeric Wafer Systems (poster), Rupal Patel, Viness Pillay, and Michael P. Danckwerts, South African Pharmacology Society Congress, Free State, 12-15 September, 2004.

Assessment of the Disintegration and Hydration Dynamics of a Lyophilized Polymeric Wafer System (poster), Rupal Patel, Viness Pillay, and Michael P. Danckwerts, Annual Meeting of American Association of Pharmaceutical Scientists, Baltimore, United States of America 7-11 November, 2004.

ABSTRACT

A lyophilized polymeric wafer system was formulated for the provision of rapid drug release in the oramucosal region. Lyophilization produced a porous sponge-like matrix which allowed simulated saliva to be rapidly imbibed into the hydrophilic structure. This surge of simulated saliva resulted in rapid disintegration of the wafer.

Hydroxypropyl cellulose (HPC) was selected as the polymeric platform based on its low gelation potential. Other excipients incorporated into the system were lactose and mannitol as diluents, and glycine as a collapse protectant. A Face Centred Central Composite Design was chosen to establish the significant effects of the independent formulation variables on the physicochemical and physicomechanical properties of the wafer. The formulation variables investigated were, HPC concentration, type of diluent (lactose, mannitol or mixture), concentration of diluent, quantity of glycine and fill volume. An analysis of these variables elucidated the influential factors that may be controlled to form an 'ideal' wafer. The concentration of HPC significantly affected the disintegration rate ($p=0.003$), influx of simulated saliva ($p=0.011$) and friability ($p=0.023$). The quantity of diluent present in the system also had significant effect on matrix tolerance ($p=0.029$) and friability ($p=0.032$).

Statistical optimization was undertaken using stepwise forward and backward regression, and Artificial Neural Networks to predict the ideal combination of the independent variables that would produce an ideal formulation. This wafer was required to produce a matrix disintegration of 3.33%/s, friability of 0.1% loss and maximum matrix resilience. Formulations manufactured with and without model drug, diphenhydramine hydrochloride, reflected no significant differences in their physicomechanical and physicochemical properties.

In an attempt to expand the scope of this technology, a preliminary investigation was undertaken to develop a prolonged release wafer system. This was successfully achieved trough the application of crosslinking technology. It was possible to achieve drug released over a period of 6 hours.

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List of Abbreviations

ANN	- Artificial Neural Networks
ANOVA	- Analysis of Variance
AUC	- Area Under the Curve
BHN	- Brinell Hardness Number
CCC	- Circumscribed Central Composite Design
CCD	- Central Composite Design
CCF	- Face Centered Central Composite Design
CCI	- Inscribed Central Composite Design
DOE	- Design of Experiments
DSC	- Differential Scanning Calorimetry
GFF	- General Feed Forward
GIT	- Gastrointestinal Tract
GRG2	- Generalized Reduced Gradient Algorithm
HEC	- Hydroxyethyl cellulose
HPC	- Hydroxypropyl cellulose
HPMC	- Hydroxypropylmethyl cellulose
IVIVC	- *In Vitro-In Vivo* Correlation
MCG	- Membrane Coating Granules
MLP	- Multi Layer Perceptron
MSE	- Mean Square Error
OLCS	- Opthalmic Lyophilisate Carrier System
PCG	- 2%w/v Pectin, 2%w/v Carmellose Sodium, 2%w/v Gelatine
PEO	- Polyethylene oxide
PVA	- Polyvinyl alcohol

RSM	- Response Surface Method
SD	- Standard Deviation
Tg	- Glass Transition Temperature

Chapter 1 Introduction

1.1 Background

To achieve optimal drug therapy, one must ensure that patients receive the correct medication, at the right dosage, and at the most convenient dosing interval (Danckwerts, 2003). One of the major factors resulting in suboptimal therapeutic outcome is a lack of patient compliance, often due to inconvenient dosing systems or regimens (Goldberg et al., 1998; Playle and Keeley, 1998). Among the various routes of drug delivery, the oral route continues to be the most preferred mainly due to ease, convenience, safety and lower costs associated with drug administration. Furthermore, the pharmaceutical industry favours the formulation of orally administered tablets, due to the relative ease of production (Bredenberg et al., 2003).

Peroral application of drugs also has various limitations such as slow onset of action, and in many cases, incomplete and erratic absorption. This may be the result of hepatic first-pass metabolism and degradation by the gastrointestinal enzymes, acidic pH and/or microbial flora. Such events often lead to a significant reduction in the oral drug bioavailability. Patient acceptance may be a problem, particularly with children and geriatrics experiencing problems with swallowing tablets.

This study proposes to design an oral wafer system using the process of lyophilisation, for the rapid delivery of drugs on application to the oramucosa. The proposed wafer will dissolve in the sublingual region of the oral cavity within 30

seconds. This system aims to omit the rate limiting disintegration step of conventional tablets. The rapid disintegration of the system would also overcome problems experienced by patients who have difficulty swallowing tablets.

Commercially available lyophilised products that currently dominate the market include:

- Zydis® (R.P. Scherer; Basking Ridge, New Jersey, USA). The drug is physically trapped in a matrix consisting of a water soluble mixture of saccharide and gelatine. This is freeze-dried, leading to a product that dissolves rapidly when placed in the mouth. Drug candidates for this system would be chemically stable and water insoluble, having a small particle size, with a dose limited to 60mg. Large drug particles may sediment during the lyophilisation process (Seager, 1998);
- Lyoc® (Farmalyoc; Laboratoire L. Lefon. Maisons-Alfort, France). An oil-in-water emulsion is placed directly into the blister alveolus and subjected to lyophilisation. The emulsion is thick and paste-like containing the active as bulk or coated microparticles. A porous product is formed. This system can accommodate a high quantity of drug (Dobetti, 2001); and
- Quicksolv® (Janssen Pharmaceutica, Beerse, Belgium). This tablet is in a porous solid form obtained by freeze-drying an aqueous dispersion or solution of the active-containing matrix. The matrix is then dried by removing the water using an excess of alcohol. Drugs compatible with this system are limited to those with a low dose and that are insoluble in the extraction solvent (Gole et al., 1990).

1.2 Technology Applied In This Study

To achieve a rapidly dissolving wafer system, solubilised hydrophilic polymer combinations containing excipients either with or without drug will be moulded into small slabs through a lyophilisation process. Based on selection of appropriate polymer and/or excipient combinations the lyophilised system will be produced. Inherent in the lyophilisation process is the sublimation of water from the polymeric matrix which results in a highly porous, sponge-like polymeric network having a cylindrically planar geometry. On exposure of this system to the mucosa, saliva and mucus is spontaneously imbibed, the occurrence of which promotes rapid hydration and dissolution. Freeze-dried products, which are porous, lead to a high hydration capacity, and also tends to release their contents faster than products dried by other methods (Shojaei, 1998a).

The preliminary studies investigating the modification of the wafer technology to provide prolonged release wafer systems will utilise the crosslinking of a polymer network in order to reduce drug diffusion.

1.3 Advantages of Present Study

The main advantages of the wafer system over conventional buccal/sublingual delivery systems such as tablets and liquid-filled softgels include:

- Absence of matrix compression, thereby bypassing any deleterious phenomena associated with such manufacturing techniques, namely polymorphism, melting, degradation and crystalline/amorphous phase transitions. Peptides and proteins in particular are susceptible to destruction during consolidation processes and thus application of the proposed wafer system may avoid such denaturation.

- Inherent viscosity and release potential of selected hydrophilic polymer matrices may be easily modified through control of the rate and degree of sublimation. This would allow for the modification of matrix porosity which in turn would modulate fluid influx rate and hence drug diffusion rate. Thus, changes in the drug release profile may be induced without the need for specialised excipients. The availability of such choice in formulation development has a major impact on the provision of a cost-effective and thereby widely accessible delivery system.

1.4 Motivation for Study

Currently, the most popular commercially available rapidly dissolving lyophilised drug delivery system is Zydis®. The basic structure of the system is composed of gelatine. When gelatine is used in the production of rapid disintegrating dosage forms, it is necessary to heat the solution in order to effect solution. This heating step increases processing times and incurs heating costs. Conventional processing can require holding times of up to 48 hours. It has been observed that over this time the viscosity of the gelatine-based mixture can increase, leading to processing difficulties. Another known problem associated with gelatine-based fast dissolving dosage forms is the lack of homogeneity and sedimentation of the liquid mix during holding periods, as some mixtures incorporate the active substance as suspended particles.

A novel feature of this study is that it provides the possibility of eliminating gelatine from fast dispersing dosage forms. Hydrophilic polymers may be used as the primary structure-forming agent to form a physically robust matrix while maintaining the desired rapid dispersion characteristics of the product. By the

careful selection of an appropriate hydrophilic polymer it is possible to obtain particularly desirable properties of cold water solubility, no change in solution viscosity with time and improved stability and physical strength of the delivery system.

1.5 Objectives of Present Study

With the above in mind, the following objectives were outlined for this study to:

1. Conduct extensive preformulation studies to identify suitable excipients, establish appropriate lower and upper levels of concentration for a range of polymers and excipients and design preliminary native wafer platforms;
2. Optimise of the lyophilisation cycle for the development of these wafer platforms that demonstrate rapid polymeric dissolution;
3. Configure an Experimental Design strategy, namely the quadratic Face Centred Central Composite Design to systematically combine and test the candidate polymer and excipients for the production of wafer matrices;
4. Assess the physicochemical properties of the wafers, specifically the disintegration rate, friability and chemical interactions as a result of lyophilisation;
5. Gauge the physicomechanical (stress-strain) properties of the wafer matrix namely the yield value, tolerance, energy, resilience and Brinell Hardness Number;
6. Mathematically optimise the derived physicochemical and physicomechanical properties using the data generated from the Face Centred Central Composite Design using solver technology® and Artificial Neural algorithms; and

7. Undertake a preliminary investigation into the possible modification of wafer technology to produce a prolong release system.

1.6 Overview of This Study

Chapter One

The first chapter of this dissertation provides the introduction and rationale for the study. The introduction briefly describes the oral route as a site for drug delivery and provides a debate over advantages and limitations. The properties and manufacturing techniques of currently available lyophilised oral tablets are briefly outlined. This chapter finally outlines the approach, methodology and advantages of this study.

Chapter Two

The second chapter provides an insight into the background of the principles employed in this study. The utilisation of polymers for biomedical and drug delivery applications are outlined. This is followed by a description of the oramucosa as a site for drug delivery encompassing its anatomy, physiology and pros and cons of using this region. A concise description of commercially-available intraoral dosage systems is undertaken. The key principles involved in the fundamental technique of lyophilisation utilised in the preparation of the intraoral system, are discussed. In anticipation of the problems associated with poor physical strength of lyophilised products, the concepts governing stress-strain analysis conducted in this study are examined.

Chapter Three

To allow for an efficient and structured approach to experimentation, a statistical design was employed. Chapter three outlines the theory of the Design of

Experiments and highlights the various quadratic Response Surface Methods available. A motivation is provided for the use of the Face Centred Central Composite Design (CCF) in this study.

Chapter Four

The fourth chapter of this study describes the development of a lyophilised wafer system. Since it is critical that the wafer disintegrates rapidly on application to the sublingual region, it was essential that an appropriate polymer was selected. Once a suitable polymer was identified, other excipients and formulation variables were subsequently chosen. The upper and lower limits of the variables determined were used to generate the CCF used in this study.

Chapter Five

Chapter five includes the evaluation of the CCF with regard to the physicochemical and physicomechanical responses of the lyophilised wafer matrices. This includes the investigation of disintegration profiles; rate of influx of simulated saliva into the matrix; friability; matrix yield value; matrix tolerance; matrix absorption energy; matrix resilience; and Brinell Hardness Number.

Chapter Six

In addition to the responses measured for the CCF, the qualitative study, Differential Scanning Calorimetry (DSC) was conducted on the wafers and native excipients to analyse the effect of lyophilisation on the wafer constituents. This is elaborated in detail in chapter six.

Chapter Seven

The pinnacle of this study is reached in chapter seven which involves optimisation of the wafer system. The fundamental properties of the matrix (disintegration rate, friability and resilience), were optimised using multiple regression and Artificial Neural Networks (ANN). The model drug diphenhydramine hydrochloride was incorporated into the optimised formulation to establish the effect of active ingredient on the matrix.

Chapter Eight

Diphenhydramine hydrochloride was maintained as the model drug in the preliminary studies aimed at modifying the wafer technology to produce a prolonged release wafer system. The modification outlined in chapter eight involved incorporating cross linking technology to the existing system to ensure the release of active ingredient over a sustained period.

Chapter Nine

The final chapter of this study discusses the overall suitability of the wafer developed for oramucosal application. Recommendations are made for the improvement of such a delivery system for future studies.

Chapter 2 Polymers and Oramucosal Delivery Systems

2.1 Introduction

Polymers form an integral component of products manufactured for medical and pharmaceutical applications. Polymeric materials are amenable to various uses due to their mechanical characteristics, chemical stability, light weight and uncomplicated design possibilities. The first polymers to be utilised in biomedical applications were the widely used commodity polymers (e.g. polyethylene, polypropylene and polystyrene) (Streubel et al., 2003, Iconomopoulou et al., 2005, Palakurthi et al., 2005). These polymers were not developed at the onset with biocompatibility as a concern. During recent years many specialty polymers have been developed to meet the complicated demands for medical development, the optimisation of structure-property correlations and ultimately clinical use (Brocchini, 2001, Kholodovych et al., 2004). Polymers are applied to a large number of medical applications such as medical supplies, support replacement of malfunctioning body parts, and as drug reservoirs to provide a local therapeutic effect. A few examples of these polymers are listed in Table 2.1.

In addition to the applications listed in Table 2.1, polymeric materials have found extensive use in the design of drug delivery systems.

Table 2.1 Polymers for specific biomedical applications

Non-Degradable Polymer	***Biomedical Application***
Polyamides	Sutures
Polycarbonates	Device housing
Polyesters	Vascular Grafts
Poly(vinyl chloride)	Tubing and Blood bags
Polyurethanes	Tubing and Coatings
Silicones	Tubing and Soft tissue reconstruction
Biodegradable Polymer	***Biomedical Application***
Polylactic/glycolic acid	Sutures
Polyorthoesters	Bone plates
Cyanoacrylates	Wound closure
Polylactic acid	Tendon repair

2.2 Application of Polymers in Drug Delivery

Polymer macromolecules are a highly versatile and diverse group, many of which have been selected for specific applications in the field of drug delivery. Many of them play a role in solubilisation, nanoparticle formulations, surface modification and as macromolecular drug carriers (Khomyakov et al., 1965, Moghimi and Hunter, 2001, Tosi et al., 2005). The use of polymers in drug delivery continues to increase as clinical results show therapeutic benefits, novel applications are discovered, and sources of polymers and their derivatives become more accessible. Technology improvement of tablets such as the ability to control drug release profiles has been demonstrated by scientists using polymers as coating systems (Tarvainen et al., 2004) or incorporating the polymers as tablet excipients (Mahaguna et al., 2003; Toti and Aminabhavi, 2004).

Research into ‘intelligent’ polymers has boomed during the twenty-first century, in response to the growing need for site-specific drug delivery systems. Targeted drug delivery offers specific advantages over conventional dosage systems, such as a reduction in the frequency and severity of side effects. Attention has been given to site-specific drug release in the gastrointestinal tract (GIT), ocular cavity,

and malignant cells, enabling disease conditions to be treated from the affected site (Pillay and Fassihi, 1999 a,b; Gharat et al., 2001; Rudolph et al., 2001; Vandamme, 2002). The mechanisms utilised to provide site-specific delivery varies. pH sensitive systems allow dissolution of the device to be in a specified area of the GIT (Pillay and Fassihi, 1999a). In a study by Kono (2001), liposomes were developed whereby the contents, release behaviour, surface properties and affinity to cell surface could be controlled in a temperature-dependent manner. In another study by Piskin (2004), the delivery system responded to environmental stimulus such as changes in pH, ionic strength, light, electrical and magnetic field.

Concern was expressed by Pardridge (2002) about the use of nanoparticles containing detergents for site-specific drug delivery. Detergents such as cholic acid or polysorbate-80 are added to prevent aggregation and stabilise nanoparticles. However these detergents may be toxic *in vivo.* Polysorbate-80 causes disruption of the blood-brain barrier at a concentration as low as 3mg/kg (Azmin et al., 1985). On the other hand, Olivier and co-workers (1999) suggested that nanoparticles may mediate drug delivery to the brain just by temporarily disrupting the blood-brain barrier.

From the studies above, the significant impact of polymer technology in drug delivery systems can be clearly seen. The present study is specifically aimed at formulating a polymeric-based mucosal delivery system and, hence, the associated principles will be discussed in detail.

2.3 Transmucosal Drug Delivery

Due to the limitations of conventional oral systems discussed in Chapter 1, transmucosal routes of drug delivery (i.e. the mucosal linings of the nasal, rectal, vaginal, ocular and oral cavity) may offer distinct advantages over peroral administration for systemic drug delivery. Some of the reasons include:

- The drug is not subjected to the destructive acidic environment of the stomach;
- Therapeutic serum concentrations of some drugs can be achieved more rapidly; and
- The drug enters the general circulation without first passing through the liver.

Combinations of the above factors lead to a higher bioavailability (Bredenberg, 2003). In general, these mucosal surfaces are rich in blood supply, providing a means for rapid drug transport to the systemic circulation. Despite the abundance of mucosal areas amenable to drug application and delivery, the oral cavity still provides the most appeal to patients based on its convenience.

2.3.1 Oramucosal Drug Delivery

In addition to greater patient compliance, the oramucosal route offers distinct advantages over other mucosal drug delivery sites. There are no known adverse physiological effects, and the oramucosa is less vulnerable to damage or irritation than the nasal mucosa (Danckwerts, 2003).

The mouth is lined with a mucous membrane which is capable of serving as a site for the absorption of drugs. The oral mucosa is robust and shows short recovery times after stress or damage (Rathbone and Hadgraft, 1991; de Vries et

al.,1991; Squier, 1991). Also the absence of Langerhans cells provides a high level of tolerance to potential allergens (Bodde et al., 1990). Furthermore, drug absorption is facilitated by the continual washing action of saliva (0.5-2 litres per day) over the mucosal surface. This route also allows for excellent accessibility and easy removal of the system in case of an adverse drug reaction (Lee, 2002). These factors consequently support the oramucosal cavity as a highly feasible and rational site for systemic drug delivery.

2.3.1.1 Anatomic and Physiological Considerations

Four sites within the buccal cavity have been used for drug administration. The four regions have varying permeability, which plays a role in the absorption of drugs across the oral mucosa. As seen in

Figure 2.1, the four key areas are the buccal cavity, the lingual area, the palate and gingival region. The most commonly used sites for drug administration of the four mentioned above is the sublingual and buccal route. Using the sublingual route, the medicament is placed under the tongue, usually in the form of a rapidly dissolving tablet. The anatomic site for drug administration between the cheek and gingival is known as the buccal mucosa.

The oral mucosa is composed of three layers (Figure 2.2). The first layer is the stratified squamous epithelium; underneath this layer lies the basement membrane. The basement membrane overlies the lamina propria and submucosa.

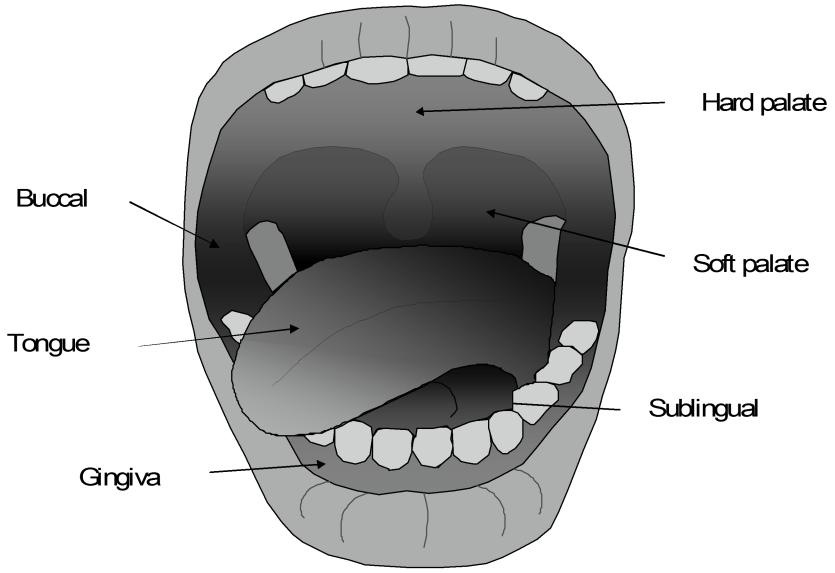


Figure 2.1 Mucosal regions of the mouth *(Danckwerts, 2003)*

The constitution of the epithelium within the different sites of the oral cavity shows dissimilarity. The gingival and hard palate are exposed to mechanical stress during eating, hence the epidermis is keratinised in a similar manner as the skin. The epithelium in the soft palate, buccal and sublingual area is not keratinised, therefore not containing ceramides and acylceramides which are associated with providing a barrier function (Squier, 1991; Wertz and Squier 1991; Harris and Robinson, 1992). The mucosa of the buccal and sublingual region have only small amounts of ceramide, and is thus more permeable when compared to other regions of the oral cavity (Shojaei, 1998b).

The presence of membrane coating granules (MCGs) accounts for the differences in permeability amongst the various regions of the oral mucosa. When cells go through differentiation from basal to flattened keratinous cells, MCGs are formed. At the apical cell surface, MCGs merge with the plasma membrane and their contents are discharged into the intercellular spaces. This

occurs mainly in the upper one-third of the epithelium. MCGs are present in both keratinised and nonkeratinised epithelia, however their composition is different. On the other hand, non-keratinised epithelium contains MCGs that are nonlamellar and include cholesterol, cholesterol esters and glycospingolipids (Wertz and Squier, 1991).

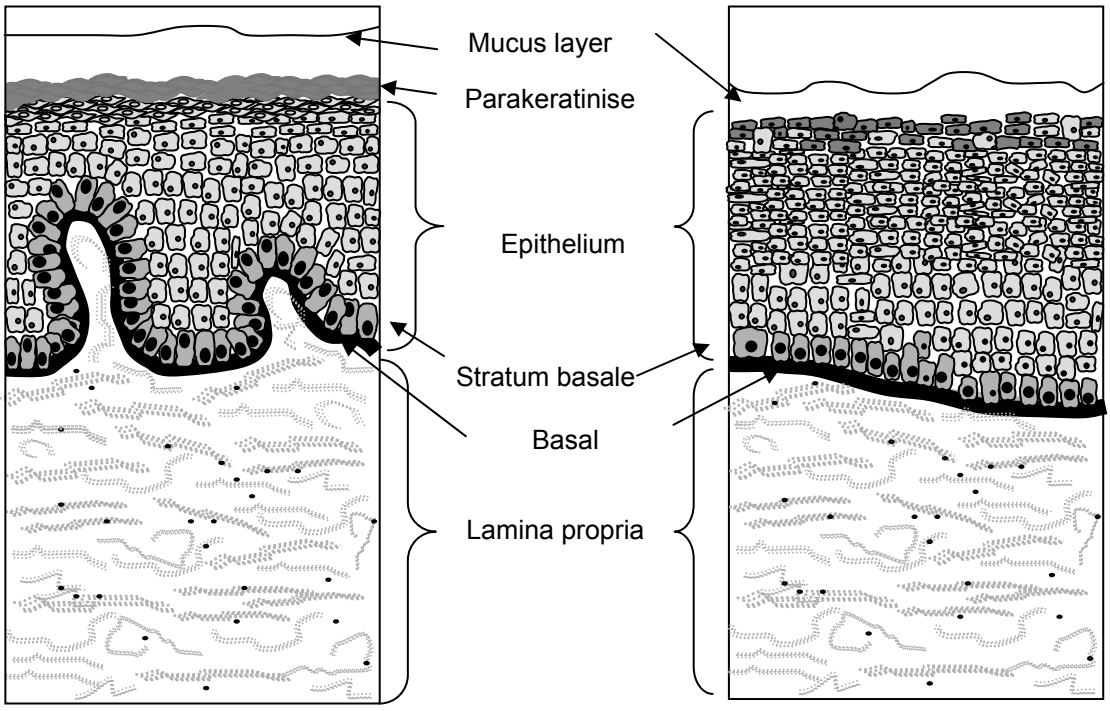


Figure 2.2 Composition of the layers of mucosal epithelium: (a) keratinised; and (b) nonkeratinised *(Danckwerts, 2003)*

A layer of mucus is present on the surface of the epithelial layer of cells. This plays a major role in cell-to-cell adhesion, oral lubrication, as well as mucoadhesion of mucoadhesive drug delivery systems (Peppas and Buri, 1985). A major feature in the environment of the oral cavity is the presence of saliva. The salivary glands produce saliva, responsible for protecting the soft tissues from abrasion during the mastication of food. Saliva plays an essential role in

facilitating the disintegration of quick-disintegrating drug delivery systems (Rathbone et al., 1994).

The buccal and sublingual regions are different from each other in terms of anatomy, permeability to drug, and their ability to retain a drug delivery system for a desired duration. Although the buccal mucosa is less permeable than the sublingual mucosa and does not yield a rapid onset of action as seen with sublingual delivery, mucosa of the buccal area has an expanse of smooth and relatively immobile surface, which is suitable for placement of a retentive system. For buccal drug delivery, adhesion to the oral mucosa permits not only the intimacy of contact and the possibility of improved drug absorption, but also the ability to achieve an optimum residence time at the site of administration (Martin et al., 2002). These characteristics make the buccal mucosa a more appropriate site for prolonged systemic delivery of drugs.

The sublingual route is however more suitable for delivery systems formulated either as rapidly disintegrating matrices or softgels. These systems create a highly significant drug concentration in the sublingual region prior to systemic absorption across the mucosa.

2.3.1.2 Absorption of Drugs

In general, drugs penetrate the mucous membrane by simple diffusion via paracellular and transcellular routes and are carried in the blood, which richly supplies the salivary glands and their ducts, into the systemic circulation via the jugular vein (Martin et al., 2002). Figure 2.3 depicts the pathway of drug absorption through the sublingual route.

Substances that can be administered by this route have limitations. The type of drugs absorbed via this route is dependent on the pH-partition hypothesis, pKa-partition theory and the lipid-water partition (Martin, 1993). The absorption of hydrophobic drug substances in the GIT is aided by the presence of bile acids. These are not present in the mouth and may compromise the availability of such drugs.

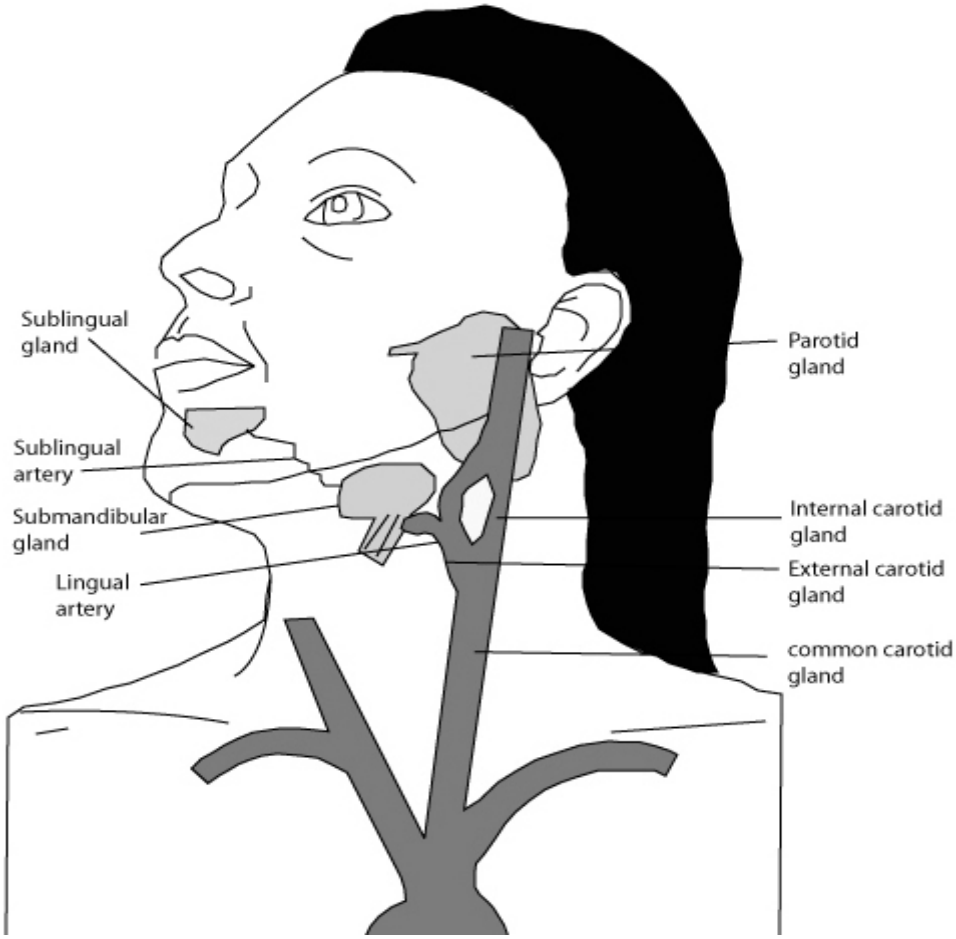


Figure 2.3 Absorption of drugs via the sublingual route *(Adapted from http:// abdellab.sunderland.ac.uk)*

2.3.1.3 Problems Associated with Buccal and Sublingual Systems

Similar to other mucosal membranes, the buccal mucosa as a site for drug delivery also has limitations. One of the major disadvantages associated with buccal drug delivery is the low flux that exists across the membrane, which may

result in incomplete transmucosal drug diffusion. In this respect, various compounds such as bile salt surfactants and chelators have been investigated as penetration enhancers in order to increase the flux of drugs through the mucosa (Aungst et al., 1988; Aungst and Rogers, 1988; Aungst and Rogers, 1989).

Other than the low flux associated with buccal mucosal delivery, a major limitation of the buccal route of administration is the lack of dosage form retention at the site of absorption. Consequently, hydrophilic polymers capable of gelation and swelling may be employed in the design of buccal drug delivery systems to enhance bioadhesiveness. Polymers may form hydrogen bonds with the mucosal surface and thus produce bioadhesive properties (Shojaei et al., 1998 a,b).

Although the oramucosal route is favoured due to the ease of administration, sublingual preparations may pose discomfort, due to the unpleasant feeling and may cause local irritation to the membranes.

Anatomical inconsistency in membranes such as the thickness and level of keratinisation may cause inter-patient variability in the level of bioavailability.

2.4 Drug Delivery Systems for Intraoral Application

Intraoral drug delivery systems are intended for the movement of drug through the oral mucosa. These systems generally fall into one of the four broad categories: mucoadhesive buccal patches and tablets, quick disintegrating solid dosage forms, solid intraoral delivery systems and aerosol intraoral drug delivery systems.

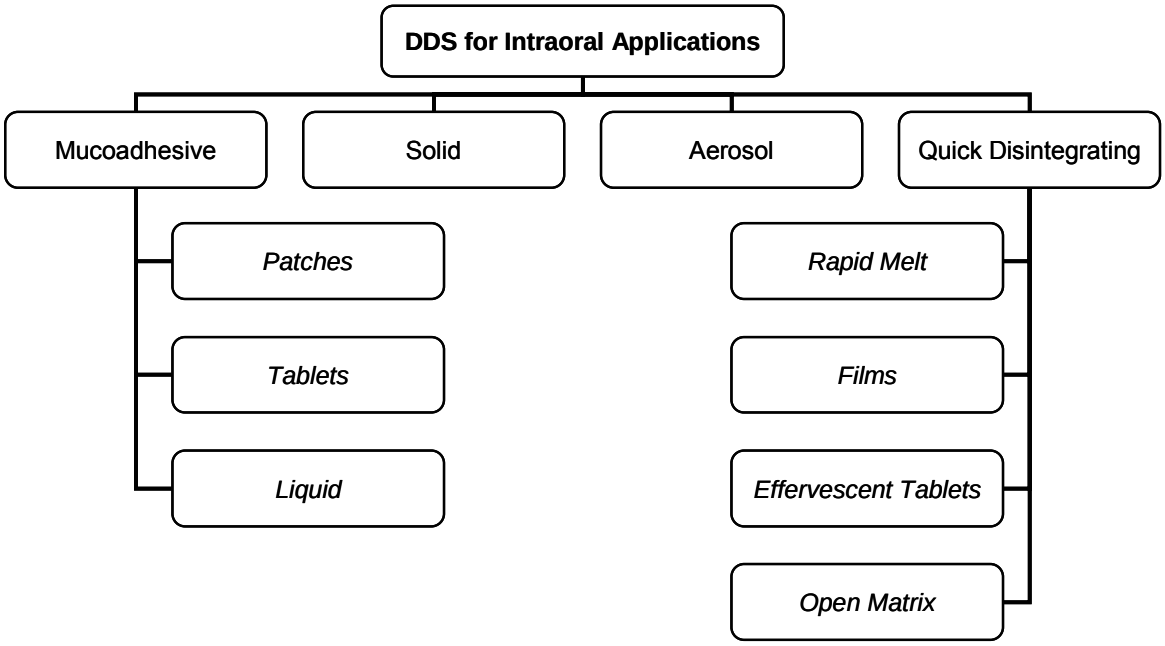


Figure 2.4 Classification of intraoral drug delivery systems

2.4.1 Intraoral Mucoadhesive Systems

2.4.1.1 Mucoadhesive Patches

The distinct advantage of mucoadhesive patches and tablets is that they provide a longer period over which to deliver the drug to and through the buccal mucosa. In contrast to the rapidly-disintegrating systems, they are not adversely affected by the risk of swallowing a large portion of the dose with saliva. In addition, mucoadhesive systems allow for controlled release of the drug to be delivered through the oral mucosa in the same fashion that transdermal systems do. Much research has been conducted on mucoadhesive polymers and therapeutic systems, however not many have reached commercial production. Effective bioadhesion and patient compliance still remain areas of concern (de Vries, 1991). The majority of mucoadhesive patches and tablets are formulated either

as solvent-cast mucoadhesive polymer discs or directly compressible flat-faced tablets of 1-3mm thickness.

2.4.1.2 Mucoadhesive Tablets

From an economic perspective, mucoadhesive tablets have the advantage that the technology used to produce them is usually the same process as compression technology used to produce conventional tablets. This makes them popular from a manufacturing point of view, reducing the number of equipment needed. A major limitation however is that a large portion of active drug is swallowed by the patient, which precludes drugs that are slowly absorbed through the oral mucosa. For this reason, drugs susceptible to instability or metabolism in the GIT are not compatible with this delivery system.

2.4.1.3 Mucoadhesive Liquids

A vast number of oral rinse formulations are available on the market. This may be suitable for oral hygiene applications, however for a drug delivery system, a longer contact time with the oral mucosa is desirable. Access Pharmaceuticals (Dallas, USA) has recently provided a solution to this problem. Their MucoAdhesive Liquid technology® provides an extended coating of the oral mucosa, due to the pseudoplastic and mucoadhesive nature of the liquid, allowing active ingredients to be present over extended periods of time.

2.4.2 Solid Intraoral Drug Delivery Systems

In the past, local mouth diseases and sore throats have been successfully treated using medicated oral lozenges. Most are manufactured as candy-type lozenges

or compressed tablets that are sucked by the patient. Previously, drugs with few systemic adverse effects were used with this type of system. More recently however, more potent drugs, with lower doses have been included into lozenges for the quicker intraoral absorption properties (Spijkervet et al., 1991; Okuno et al., 1997, Schachtel et al., 2002)

2.4.3 Aerosol Intraoral Drug Delivery Systems

In the search to develop alternative routes for the administration of insulin, an aerosol system was developed. Oralin® (Generex Biotechnology, Toronto, Ontario, Canada) delivers accurate doses into the mouth by use of a metered-dose aerosol. This aerosol formulation is rapidly absorbed through the buccal mucosa and oropharyngeal regions (Modi et al., 2002). Plasma insulin levels were sufficient to control postprandial glucose increases in diabetic patients.

The direct absorption of nitroglycerin in the form of an aerosol provides a faster onset of action as compared to the tablet (Reisin et al., 1988; Wight et al., 1992).

2.4.4 Quick-Disintegrating Intraoral Drug Delivery Systems

Some of the other terms used to describe this class of delivery system include, fast-dissolve, quick-dissolve, rapid melting and quick-disintegrating. Disintegration describes the system slightly more accurately than dissolving. Systems that undergo rapid disintegration in the oral cavity are predominantly intended for the patient to swallow the bioactive agents; absorption will occur in the GIT.

Quick-disintegrating intraoral drug delivery systems can be made by a number of processes including, direct compression, wet granulation and freeze-drying (lyophilisation).

2.4.4.1 Rapid-Melting Tablets

Quick-disintegrating intraoral tablets have also been created by using lipid waxy binders that melt at body temperature. Cherukuri (2000) patented a novel rapid-melt, semisolid, moulded composition including at least one melted wax binder, a salivating agent, diluent material, a slipping agent and an active ingredient. On the application of pressure, the composition becomes liquid. Hence once in the patient's mouth, application of pressure by the tongue, converts the semisolid into a liquid carrying the active substance. The final tablets may be coated to prevent melting on storage in warmer climates.

2.4.4.2 Disintegrating Films

Mucoadhesive and quick-disintegrating films have been patented by Zerbe and co-workers (1999). This delivery system has been used for pharmaceutical and cosmetic applications. Films are water-soluble with instant wettability, and immediate softening on application to the mucosal tissue. The dry film has adequate tensile strength to undergo cutting, slitting and packing operations.

2.4.4.3 Effervescent Tablets

Like conventional effervescent tablets that dissolve in water, OraSolv®, produced by Cima Labs Inc. (Eden Prairie, Minnesota, USA) is activated by saliva. On insertion into the patient's mouth, disintegration occurs rapidly without voluntary

action by the patient. The disintegrated tablet is swallowed and absorbed via the GIT. Due to the specialised packaging requirements, PakSolv®, Cima's packaging system and DuraSolv®, a more robust dosage form has been created. DuraSolv® can be packaged using conventional methods such as foil pouches or bottles. Cima Labs Inc. developed an effervescent tablet, OraVescent®, containing a pH-adjusting substance to facilitate the intraoral absorption of drugs.

2.4.4.4 Open Matrix-Type Wafers and Tablets

With the introduction of the Zydis® system (R.P. Scherer; Basking Ridge, New Jersey, USA) in the late 1970s, the concept of quick disintegrating drug delivery systems gained much attention. It was the first of this class of delivery systems to be manufactured on a large scale. It is a freeze-dried wafer made from various standard tablet adjuvants (Virley and Yarwood, 1990). The wafer essentially works on the principle of forming an open network containing the active ingredient. Figure 2.5 illustrates the Zydis® manufacturing process. The freeze-dried tablet disintegrates within 2-3 seconds, releasing the active ingredient. The drug either forms a dispersion or dissolves in the saliva, which is then swallowed and absorbed via the GIT.

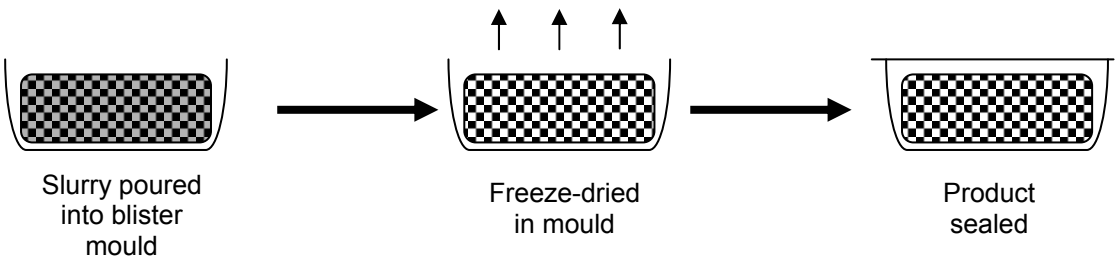


Figure 2.5 Production of the Zydis® lyophilised wafer *(Danckwerts, 2003)*

The WOWTab® (With-Out-Water tablet) has been produce by Yamanouchi Pharmaceutical Co. Ltd. (Tokyo, Japan). This tablet is manufactured using conventional granulating and compression. The rapid disintegration is attributed to the blending of a low and high moldability saccharide. The unique combination of saccharides provides sufficient mechanical strength as well as quick tablet disintegration.

Fuisz Technology Ltd. (Chantily, Virginia, USA) developed the Flash Dose® tablet, which can dissolve in the patient's mouth in less than 10 seconds. This has been achieved by the use of Shearform™ technology. The process involves a unique blend of sugars being placed in a fast spinning machine and subjected to flash heat. By this process, long cotton-like fibres called 'floss' are produced. The 'floss' is then cured by subjecting it to specific environmental conditions that induce crystallisation, at this stage crystallisation modifiers may also be added. The matrix is then blended with coated or uncoated microspheres containing the active drug. The floss is compressed using standard tabletting equipment (Misra et al., 1999). Figure 2.6 illustrates the manufacturing process.

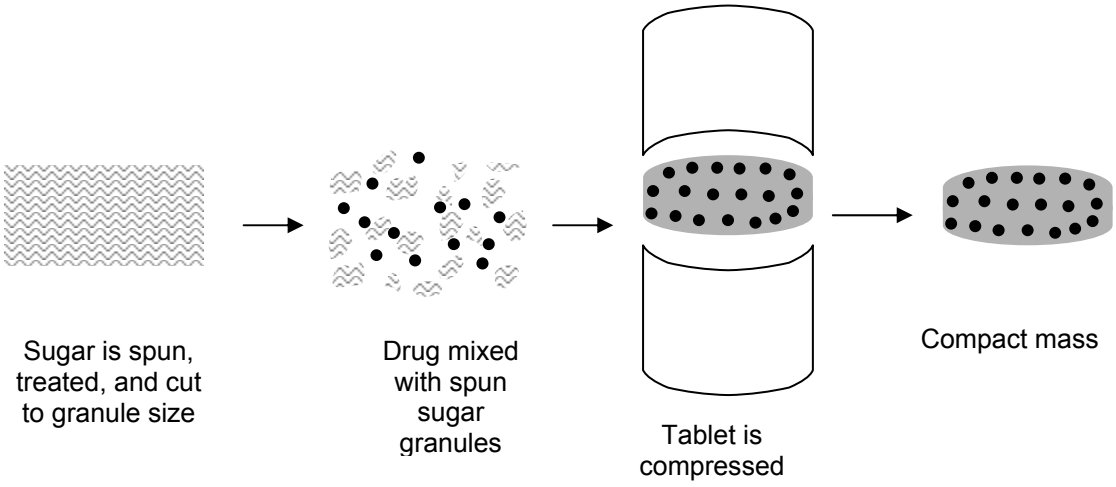


Figure 2.6 Manufacturing process of Flash Dose® *(Danckwerts, 2003)*

Of the various open matrix-type wafers on the market, the Zydis® system remains the most popular, as a result making lyophilisation the most frequently used process for the manufacture of these systems.

2.5 Lyophilisation

The principle of lyophilisation is to remove water or solvent material through the process of sublimation. Sublimation is a method whereby a substance changes from a solid directly into vapour. Water will sublime from a solid (ice) to a gas (vapour) when the molecules have sufficient energy to be liberated but the conditions are not conducive for a liquid to form.

There are two major factors that determine in which phase (solid, liquid or gas) a substance would exist, namely heat and atmospheric pressure. These parameters must be within a certain range for phase transitions to occur. Without these conditions, that phase of the substance cannot exist. The phase diagram below illustrates the necessary pressure and temperature conditions for different phases of water.

Each line (OA, OB and OC) Figure 2.7 provides the conditions when two phases coexist but a change in temperature or pressure may cause the phases to abruptly change from one to the other. The ‘triple point’ is the intersection on the phase diagram where three phases, consisting of ice, liquid and vapour, coexist in equilibrium but change into each other given a change in temperature or pressure.

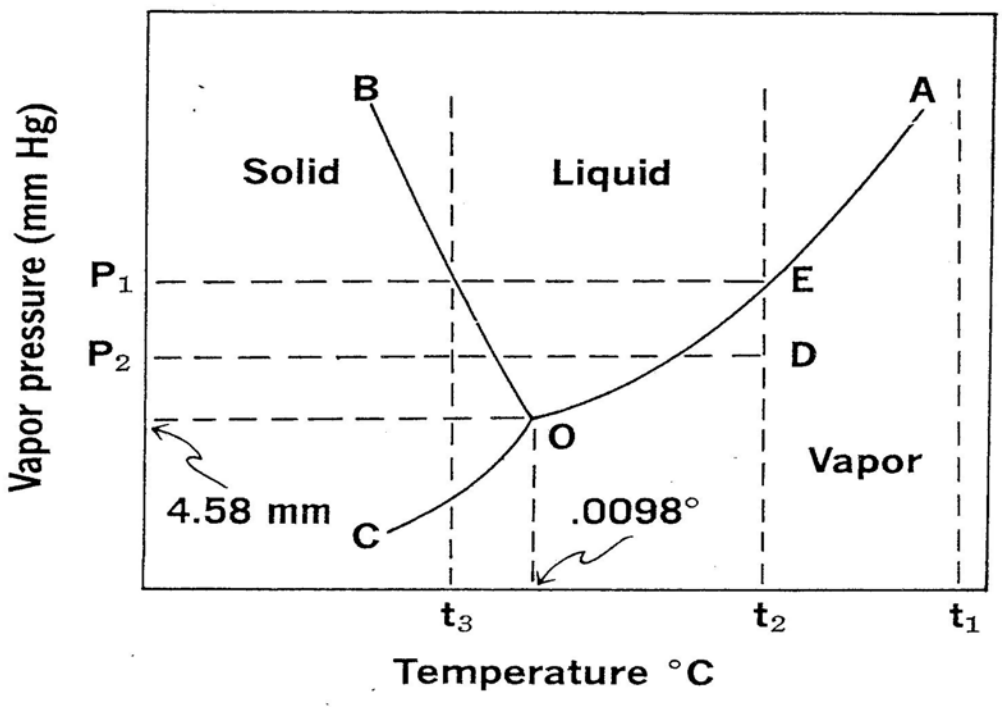


Figure 2.7 Phase diagram of water *(Martin, 1993)*

The ‘critical point‘ occurs when the properties of the two phases become indistinguishable from each other. The line between the gas and solid phase (OC) indicates the vapour pressure of the solid as it sublimes at different temperatures. The freeze-drying apparatus creates the conditions necessary for sublimation to occur.

2.5.1 The Lyophilisation Process

A typical instrument consists of a freeze-drying chamber with several shelves attached to heating units, a freezing coil connected to a refrigerator compressor, and a vacuum pump.

The lyophilisation process consists of three stages: freezing, primary drying and secondary drying. During the freezing phase, the material is placed onto the

shelves when it is still unfrozen. The chamber is sealed to begin the process; the compressors lower the temperature in the chamber. The material is frozen to a solid, which on a molecular level, separates the water from everything around it. The initial crystal size depends on the relative contributions of nucleation and crystal growth of ice. A rapid nucleation and growth rate resulting from a large degree of supercooling leads to a number of small ice crystals. Small ice crystals produce pores in the final product with lower volume-surface area.

Primary drying is accomplished by negative pressure, traditionally carried out at 40 – 400 Torr. The heating units apply a small amount of heat to the shelves (ranging from -30 to +10˚C), causing the ice to change phase. Throughout this stage, the product is maintained in the solid state below the collapse temperature of the product in order to dry the product with retention of the structure established in the freezing step. The product temperature remains relatively constant and drying follows a pseudo steady-state rate with heat removal by sublimation at the same rate as the heat input supplied by the shelves.

This is expressed thermodynamically in the form of an equation introduced by Pikal (1993) as:

$$\Delta H_S \times \frac{dm}{dt} = \frac{dQ}{dt} \qquad \text{(Equation 2.1)}$$

Where:

ΔH_S is the heat of sublimation;

$\frac{dm}{dt}$ is the sublimation rate; and

$\frac{dQ}{dt}$ is the rate of heat input.

The rate of sublimation can be expressed as:

$$\frac{dm}{dt} = \frac{(P_O - P_C)}{(R_P + R_S)} \qquad \text{(Equation 2.2)}$$

Where:

$(P_O - P_C)$ is the thermodynamic driving force, P_0 is the vapour pressure of ice in the frozen sample and P_c is the total pressure in the chamber; and

$(R_P + R_S)$ is the total resistance to sublimation, R_p is the product resistance and R_s is the resistance of the stopper of the vial.

The rate of heat input can be expressed as:

$$\frac{dQ}{dt} = A_V \times K_V \times (T_S - T_P) \qquad \text{(Equation 2.3)}$$

Where:

A_v is the cross-sectional area of the vial;

K_v is the heat transfer coefficient; and

$(T_S - T_P)$ is the heat difference between the shelf (T_s) and the product (T_p).

The vapour pressure (P_o) of the ice in the product increases exponentially with the temperature, so that an increase in product temperature will cause an increase in the rate of sublimation. The water vapour condenses onto the freezing coil in solid ice form. This continues for many hours, while the material gradually dries out. The gradual process is necessary as overheating the material can significantly change the composition and structure.

At the end of primary drying, secondary drying will begin spontaneously. This occurs when unfrozen water is removed from the matrix. This may include a small amount of bound moisture removed by desorption. Initially the rate of water

loss is large, followed by a plateau beyond which further water removal is very slow (below ≈ 2%). The rate of water removal is controlled by the rate of diffusion of the solute/vapour interface and the subsequent evaporation.

The resultant product is completely void of water, and may have a porous structure as a result of voids left where water was present (Craig et al., 1999; Bedu-Addo, 2004).

2.5.2 Pharmaceutical Application of Lyophilisation

Lyophilisation is used extensively and diversely in the pharmaceutical industry. It has been used to stabilise various pharmaceutical products, including vaccines, proteins and peptides, liposomes and small-chemical drug formulations (Mozhaev and Martinek, 1984; Colaco et al., 1992; Pikal et al., 1992 and Cleland et al., 2001).

Furthermore, freeze-drying has been used as the principle process in the development of numerous drug delivery systems. The desired characteristics of lyophilisation (Bedu-Addo, 2004) that make this process attractive to the pharmaceutical industry include:

- Long term stability;
- Short reconstitution time;
- Elegant cake appearance;
- Maintenance of the dosage form characteristics upon reconstitution, including solution properties, conformation of proteins, and particle size distribution of suspensions; and
- Isotonicity upon reconstitution.

Delivery systems using the process of freeze-drying include parenterals, suspensions, microspheres, eye applications and tablets (Mal et al., 1999; Ameye et al., 2002; Bouma et al., 2002; Donini et al., 2002; Kakish et al., 2002; Kim et al., 2004; and Suverkrup et al., 2004).

Particular attention has been given to the use of lyophilisation in the preparation of parenterals containing anti-cancer agents. In a study by Bouma and co-workers (2002) freeze-drying was used to increase the stability of NAMI-A, a novel antimetastatic ruthenium complex for at least one year. The shelf life of melphalan, an anti-neoplastic agent with poor aqueous solubility, was increased when lyophilised with specific excipients (Mal et al., 1999).

The drug loading capacity of polymeric micelles containing taxane was increased by applying the lyophilisation process to a solution containing drug and an amphiphilic copolymer in a water/butanol mixture (Fournier et al., 2004).

The freeze-drying of microspheres produces a product that is buoyant and thus has the ability to float on the gastric contents increasing the gastric retention time of the system (Whitehead et al., 2000; Kakish et al., 2002).

The ophthalmic lyophilisate carrier system (OLCS) is novel for the delivery of pharmacologically active ingredients or other substances improving the structure of the tear film to the eye. A drop of lyophilisate containing the drug and bulk forming water-soluble or swelling excipients is attached to a flexible hydrophobic carrier (Suverkrup et al., 2004).

In addition to employing freeze-drying in the preparation of bioadhesive tablets (Ameye et al., 2001), lyophilised tablets are increasingly popular as rapidly disintegrating systems (fast-melting tablets). Despite the rapid dissolution and disintegration times of these systems, high cost of production, the limitation to low dose of water-soluble drugs and poor physical resistance still remain a problem.

As a result of the poor physical strength of the rapidly disintegrating lyophilised systems, the physicomechanical properties of the wafers formulated in this study were extensively investigated.

2.6 Physicomechanical Analysis

In order to understand the impact of the mechanical properties of the wafer system on its physicochemical behaviour, textural profiling was undertaken. In general, mechanical characterisation of a material is an assessment to gain an understanding a of material's reaction to stress and strain in relation to its deformation. Stress is defined as the force per unit area acting on a material and tending to change its dimensions. It is the ratio of force to the area over which it is applied. This differs from strain which is the percentage deformation of a body when subjected to a load. Strain can be manifested as tensile, compressive, shear or volumetric changes (Martin, 1993).

The elastic theory describes deformation where the material rebounds to its original shape after the forces on it have been removed (Martin, 1993). Linear elasticity occurs when stress is directly proportional to strain in one dimension as described by Hooke's Law:

$\delta = E\varepsilon$ (Equation 2.4)

Where:

δ = Stress;

E = Young's modulus; and

ε = Strain.

Most materials deviate from Hooke's law (Equation 2.4), by exhibiting both elastic as well as viscous-like behaviour. These materials are termed viscoelastic substances. These materials responding to a deforming load, combine both viscous and elastic qualities. The relationship between stress and strain depends on time.

Some phenomena associated with viscoelastic materials are:

- If the stress is held constant, the strain increases with time;
- If the strain is held constant, the stress decreases with time (relaxation);
- The effective stiffness depends on the rate of application of the load;
- If cyclic loading is applied, hysteresis occurs, leading to the dissipation of mechanical energy;
- Acoustic waves experience attenuation;
- Rebound of an object following an impact may be less than 100%; and
- During rolling, frictional resistance occurs.

Figure 2.8 shows the reaction of polymeric substances in terms of strain on the application of stress.

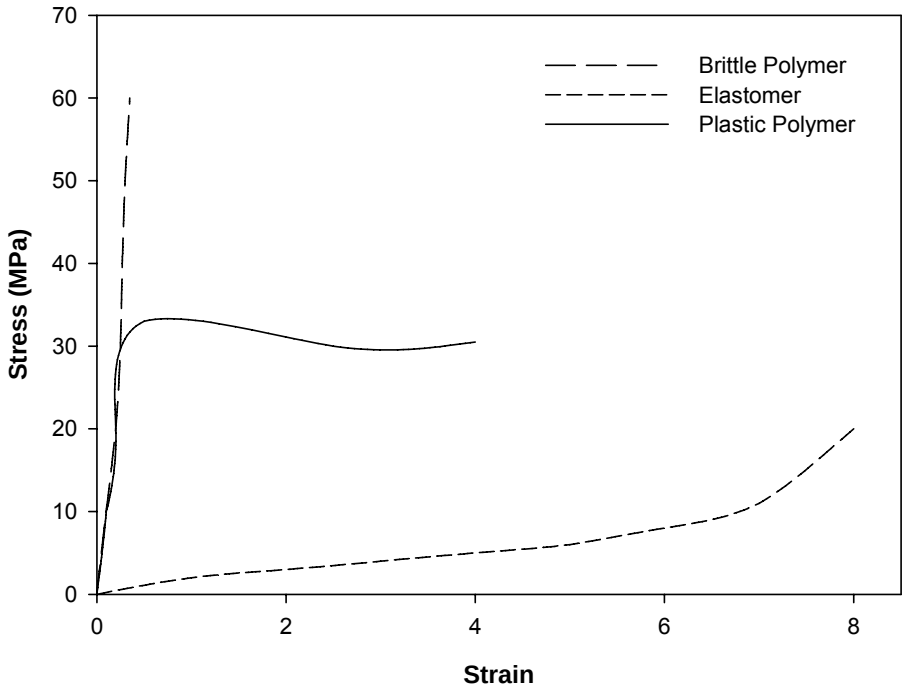


Figure 2.8 Stress-strain behavior of polymers

2.6.1 Stress – Strain Behaviour of Polymers

The moduli of elasticity for polymers range from 10MPa – 4GPa. The tensile strengths range from 10MPa – 100MPa, and elongation can be up to 100% in some cases. Mechanical properties of polymers are sensitive to the rate of deformation. Plastic deformation is defined by the interaction between crystalline and amorphous regions and is partially reversible.

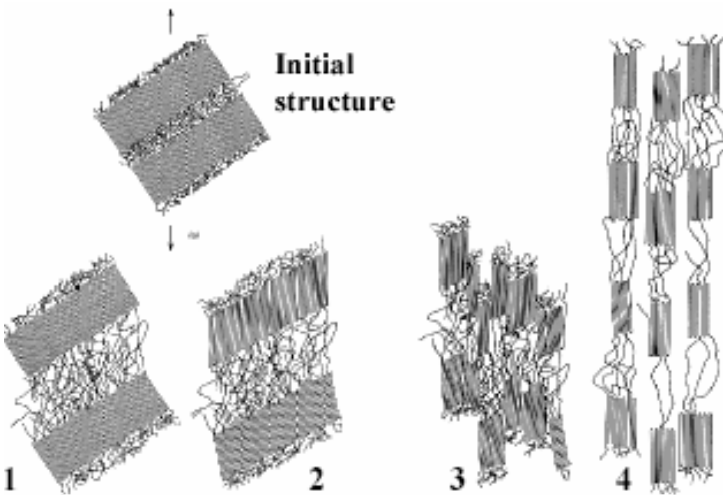


Figure 2.9 Stages of plastic deformation of semi-crystalline polymers

As seen in Figure 2.9, the stages of plastic deformation of semi-crystalline polymers include:

1. Elongation of amorphous tie chains;
2. Tilting of lamellar crystallites towards the tensile axis;
3. Separation of crystalline block segments; and
4. Stretching of crystallites and amorphous regions along tensile axis.

In polymers, energy elasticity represents the storage of energy resulting from the elastic straining of bond angles and lengths like springs from their equilibrium value. Entropy elasticity is caused by the decrease in entropy upon straining. In an unstressed state, the polymer molecules are free to adopt a number of random configurations, switching from one to another through rotation about bond angles. Under the application of tensile force, the molecules are stretched out, and fewer configurations are possible. It is therefore deduced that stretching decreases the entropy (Rosen, 1971).

2.6.2 Biomedical Applications of Mechanical Testing

Compression tests are commonly used to assess products with biomedical applications. Compressive stress and strain are calculated and plotted as a stress-strain profile which is used to determine the elastic limit, proportional limit, yield point, yield strength and for some materials compressive strength.

Practical applications include:

- Orthopedic testing of raw materials, impact loading of joint components and simulation for the evaluation of fatigue and wear properties *in vivo.* Biomechanic studies relate to the human body in motion. This is essential in the design and manufacture of prosthetic and mobility aids (Lee et al., 1999; Howard et al., 2002; Boylan et al., 2003 and Beingessnera et al., 2003);
- The relationship between the structure of a biomaterial (e.g. polymer) and its mechanical properties is assessed through mechanical testing and simulation. Commonly gel strength, elasticity and rupture force are measured (Jones et al., 1996; Park et al., 2001; Pillay and Danckwerts, 2002);
- Novel methods involving mechanical testing have also been developed to evaluate mucoadhesion and bioadhesion (Moss et al., 1999; Shojaei et al., 2000);
- In relation to the quality control of medical devices, the material strength of catheters, surgical tubing and fittings are often determined using mechanical testing. Furthermore the tensile strength of bandages, medical gloves, sutures, stents and adhesives are measured (Meyer et al., 2003); and

- Mechanical profiling has been used in the analysis of various dosage forms such as patches and films, tablet coating, capsules, gels, suspensions, parenterals and pellets (Campbell et al., 1999; Pillay and Fassihi, 1999b; Repka and McGinity, 2000; Park and Munday, 2003; Allahhama et al., 2004).

During this study, the energy of absorption, matrix yield value, matrix tolerance, matrix resilience and Brinell Hardness Number of the various formulations were assessed.

Chapter 3 Theoretical Framework for Design of Experiments

3.1 Introduction

To achieve the desired characteristics of a drug delivery system, it is necessary that we can identify factors that are influential to the properties of the formulation. During the preformulation stage, we deliberately change one or more process variables or factors, to determine the effect that the change may have on response variables. Design of Experiments (DOE) is an efficient statistical procedure for planning experiments so that the data obtained can be analysed to yield valid and objective conclusions.

DOE begins with determining the objectives and selecting process factors of an experiment. An experimental design is the laying out of a detailed experimental plan prior to conducting the experimentation. This serves to minimise the number of trial experiments that need to be conducted to determine optimal conditions of variables for a response. An appropriately selected experimental design can maximise the amount of information that can be obtained from a given amount of experimental data. The levels of each factor range from high to low.

Ensuring the successful choice and implementation of an experimental design lies primarily in clearly identifying the objectives of the experiment and determining the number of factors to be investigated.

3.1.1 Experimental Design Objectives

3.1.1.1 Comparative Objective

The primary goal of the experimentation is to draw a conclusion about one *a-priori* factor amongst several other factors under investigation. Of interest is whether or not there is a significant change in the response for different levels of that factor. This is classified as a comparative problem, and a comparative design solution is required.

3.1.1.2 Screening Objective

When the purpose of the experiment is to select the few important main effects from the many screening designs, main effects designs can be applied. This normally consists of trails run at the extreme lower and upper-bound level setting combinations of the variable study ranges. Screening designs enable researchers to select the best materials and equipment from the available alternatives.

3.1.1.3 Response Surface Method Objective

This type of design is used when the goal is shifted from product screening to product optimisation. Response Surface Method (RSM) designs contain trails in which one or more of the variables are set at the mid-point of the study range. This allows us to estimate interaction on direct effects, pair-wise interaction effects, curvilinear variable effects and quadratic effects, therefore giving us an idea of the local shape of the response surface that we are investigating.

RSM designs are used to:

- Find improved or optimal process settings;
- Solve process problems and identify weak points; and
- Make a product or process relatively insensitive to external and uncontrollable influences.

To satisfy the objective of our studies, a RSM was most appropriate, due to the allowance for curvature.

3.1.2 Response Surface Method Designs

Response surface models may involve just main effects and interactions, or in order to account for curvature, may have quadratic and cubic terms.

3.1.2.1 Advantages of the Response Surface Design

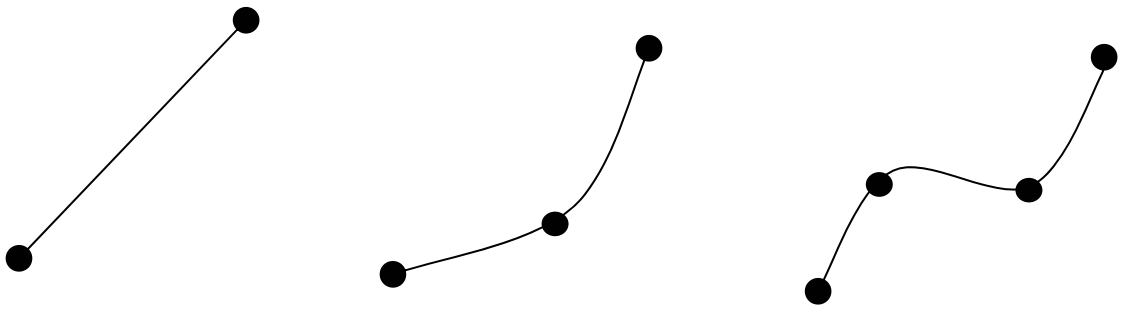
- Often, fewer simulation loops are required than a Monte Carlo Simulation method;
- Low probability levels can be evaluated;
- The goodness-of-fit parameters provide an approximation function as to how accurate the approximation function describes the true response parameter values. The goodness-of-fit can also provide a warning of when the approximation function is insufficient; and
- The individual simulation loops are inherently independent, hence making this design ideal for parallel processing.

3.1.2.2 Disadvantages of the Response Surface Design

- The total number of required simulation loops is dependant on the number of random input variables. This implies that if there is a large number of random input variables, then a probabilistic analysis would be impractical; and
- These methods are not suitable for cases where a random output parameter is a non-smooth function of the random input variables.

3.1.2.3 Response Surface Design Functions

RSM designs can have a linear, quadratic or cubic function. If the response behaves in a linear manner (Figure 3.1a), the factors need only to be taken on two-levels. A two-level design, even with centre points can only detect pure quadratic effects, but cannot estimate them. If a response behaves as in Figure 3.1b, then to quantify the pattern, a minimum number of three levels are utilised. A cubic function (Figure 3.1c) may be characterised by making use of a minimum of four levels of each factor. In general quadratic models are usually sufficient for industrial applications.



(a) (b) (c)

Figure 3.1 RSM functions: (a) Linear function, (b) Quadratic function and (c) Cubic function

3.1.3 Quadratic Designs

Classical quadratic designs were introduced during the 1950's. These designs can be classified according to two broad categories: Central Composite designs and Box-Behnken designs.

3.1.4 Central Composite Designs

The Central Composite designs (CCD) contain an imbedded factorial or fractional factorial design with centre points, in addition to axial points, which allow for curvature, cube points are also present. Axial points are created by a screening analysis. Cube points are determined from a Full Factorial Design, whereas centre points are created by a nominal design. If the distance from the centre of the design space to a factorial point is ±1 unit for each factor, the distance from the centre of the design space to an axial point is ±α (lαl >1). The precise value of α depends on certain properties desired for the design and on the number of factors involved.

The CCD is utilised to determine the coefficients of a second-order response surface model and is one of the most popular of the RSM designs due to the following three properties:

- A CCD can be run sequentially. It can be partitioned into two subsets of points. The first subset estimates linear and two-factor interaction effects while the second subset estimates curvature effects.
- CCDs are efficient, proving much information on experiment variable effects and overall experimental error in a minimum number of required runs.

- CCDs are very flexible. There are several varieties of CCDs that enables their use under different experimental regions of interest and operability.

Three main varieties of CCDs are available: circumscribed central composite design (CCC), inscribed central composite design (CCI) and face-centred central composite design (CCF).

3.1.4.1.1 Circumscribed Central Composite Design

CCC designs provide high quality predictions over the entire design space. However factor settings outside the range of the factors in the factorial part are required. The axial points establish new extremes for the low and high factor settings (Figure 3.2).

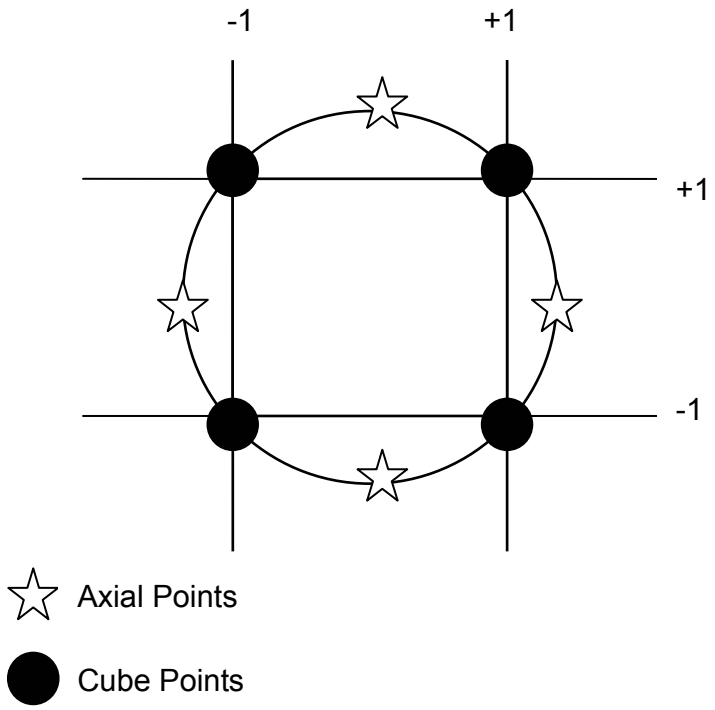


Figure 3.2 Arrangement of variable limits and axial points of the CCC

3.1.4.1.2 Inscribed Central Composite Design

In contrast to CCC designs, CCI designs use only points within the factor ranges originally specified, hence the CCI explores the smallest space. This design is used when the limits specified for factor settings are truly limits. As a result the CCI design uses the factor settings as axial points (Figure 3.3), the fractional factorial design is created within those limits. Although this design also requires 5 levels for each factor, it does not provide the same accuracy as the CCC.

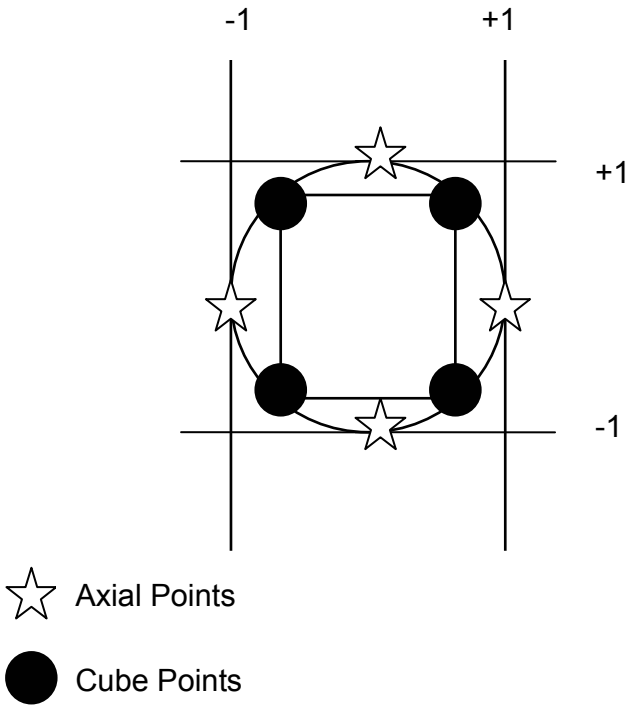


Figure 3.3 Arrangement of variable limits and axial points of the CCI

3.1.4.1.3 Face Centred Central Composite Design

The CCF design provides a relatively high quality of predictions over the entire design space. A distinct advantage that CCF designs have over CCC model, is that it does not require points to be set outside of the original factor range.

Making use of 3 levels of each factor, the axial points are at the centre of each face of the factorial space, so α =±1 as seen in Figure 3.4.

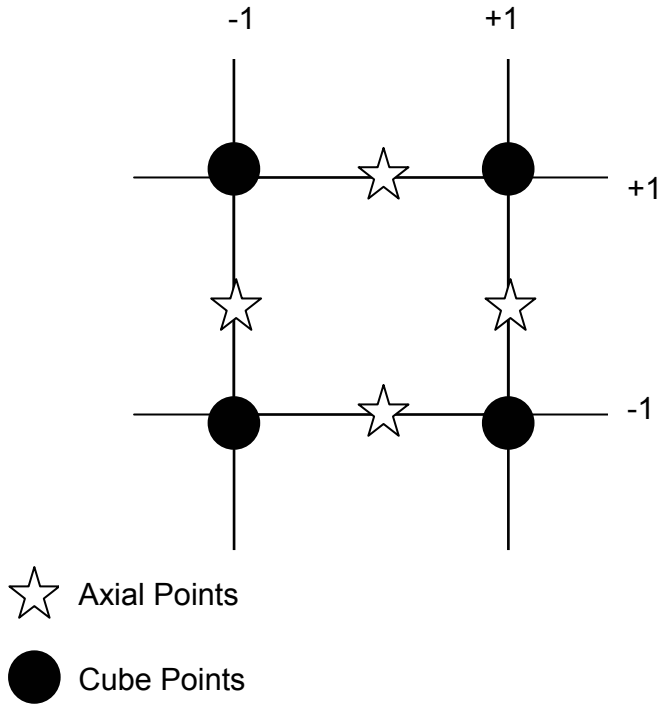


Figure 3.4 Arrangement of variable limits and axial points of the CCF

3.1.4.2 Box-Behnken Designs

The Box-Behnken design is a quadratic design that does not contain an embedded factorial or fractional factorial design. This design requires 3 levels of each factor. Like the CCI, the Box-Behnken design contains regions of poor prediction

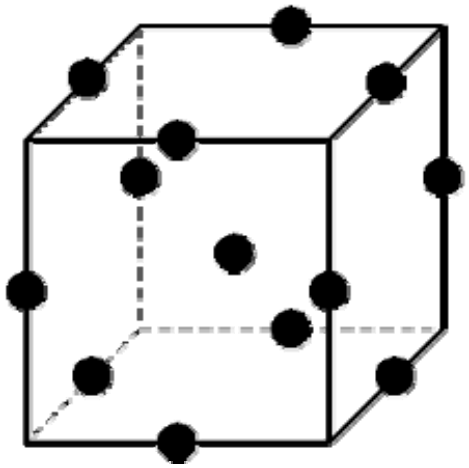


Figure 3.5 A Box-Behnken Design for three factors

Of the various quadratic designs described above, a CCF was used in this study. A number of responses were identified which would provide valuable information necessary for the optimisation of the wafer.

Chapter 4 Preformulation Studies

4.1 Introduction

For the formulation of a rapidly disintegrating wafer, a polymer with low gelation characteristics is desired. The gelation potential of polymers is highly dependent on it's solubility. General rules that govern the solubility of polymers (Rosen, 1971) are as follows:

- Like dissolves like; that is, polar solvents will tend to dissolve polar polymers and nonpolar solvents will tend to dissolve nonpolar polymers;
- In a given solvent at a particular temperature, the solubility of a polymer decreases with an increase in molecular weight; and
- The rate of polymer solubility
    - Increases with short branches, which loosen up the main-chain structure, allowing the solvent molecules to penetrate more easily;
    - Decreases with longer branches, because the entanglement of these branches makes it harder for the individual molecules to separate, and
    - Decreases with increasing molecular weight.

Other factors that may affect the gelation of polymers include polymer concentration and for certain polymers, degree of acetylation (Montembault et al., 2005).

We predicted that the polymer within the system would play a pivotal role in output factors such as solubility, disintegration, mechanical strength and

hardness. Hence, the aim of the series of experiments outlined in this chapter was to select the most suitable polymer to provide rapid disintegration of the system and identify the lower and upper limits for the other variables used to generate the CCF.

4.2 Materials and Methods

Polymers utilised in the study include: sodium alginate (E401,Kelco Int. Ltd, London, UK), hydroxypropylmethyl cellulose (HPMC) (Methocel K4m Prem CR, The DOW Chem. Corp., Midland, Michigan, USA), hydroxypropyl cellulose (HPC) (Klucel, EF Pharm, Hercules Inc., Wilmington, North Carolina, USA), hydroxyethyl cellulose (HEC) (Natrosol, 250 G-Pharm, Hercules Inc., Wilmington, North Carolina, USA), pectin (Pectin Classic CM701, Herbstreith and Fox KG, Neuenburg, Germany), polyethylene oxide (PEO) (MW 7,000,000, Union Carbide Corp., Danburg, Connecticut, USA), polyvinyl alcohol (PVA) (MW 124,000 - 186,000, Alrich, Milwaukee, Wisconsin, USA). Additionally lactose (Merck Lab Supplies Pty. Ltd., Midrand, Gauteng, South Africa) and. polystyrene cylindrical moulds of total volume 60.31mm^3 (diameter 16mm and depth of 2.4mm) were utilised.

Material used in the preparation of simulated saliva were: Potassium Phosphate Monobasic (KH_2PO_4) (Protea Lab Services Pty. Ltd., Gauteng, South Africa), Disodium Hydrogen Phosphate (Na_2HPO_4) (Saarchem Pty. Ltd., Krugersdorp, South Africa), Sodium Chloride (NaCl) (Labchem, Edenvale, South Africa).

4.2.1 Preparation of wafers

Polymers suitable for oramucosal preparations were identified based on information provided in literature (Guo, 1994; Shojaei, 1998a; Miyazaki et al., 2000; Yong et al., 2001; Ameye et al., 2002; Martin et al., 2002; Nafee et al., 2003).

A polymer (Table 4.1) (1%w/v) and lactose as a bulking agent (6%w/v) was added to deionised water and mixed for 45 minutes. 1.5mL of the various polymer solutions were pipetted into the cylindrical cavities pre-oiled with mineral oil. The formulation was subjected to a freeze-phase in a freeze-dryer (Bench Top 2K, Virtis, New York, USA) at -60°C for 2 hours. The drying-phase was executed at a pressure of 25 mtorr for 24 hours. Wafers were stored in glass jars with 2g of desiccant sachets.

4.2.2 Analysis of wafers

4.2.2.1 Weight Uniformity

Weight uniformity was used to assess the reproducibility of wafer production process. Individual wafers were weighed, and standard deviations calculated. All experimentation was conducted in triplicate.

4.2.2.2 Gelation of Matrices

The main objective of this study was to formulate a rapidly dissolving wafer system. Thus the matrix formation characteristics required assessment and formed the basis for the selection of a suitable polymer. Gelation of the dosage

form would delay the disintegration and ultimately the release of active substance.

A novel method was developed in order to assess the matrix forming profiles of the wafers. Wafers were weighed before being placed in a petri dish (diameter 85mm, depth 10mm) containing 20mL of simulated saliva (pH 7.1). The petri dish was agitated for a period of 30 seconds on a Vortex Genie2 (Scientific Industries Inc. Bohemia, New York, USA) on the slowest setting. The contents of the petri dish were sieved through a stainless steel mesh (pore size 1mm). The mass of the remaining residue was determined on a balance (AB104-s, Mettler Toledo, Greifensee, Switzerland) and used to calculate the rate of matrix formation.

The simulated saliva solution comprised 2.38g Na_2HPO_4, 0.19g KH_2PO_4 and 8g NaCl in 1000mL of deionised water (Tan et al., 2001).

4.2.3 Determination Limits for Formulation Variables

The lower and upper limits were determined using a trial and error method. Wafers of varying polymer and diluent concentrations (up to 30%w/v of each) were made and inspected visually.

4.2.4 Development of the Manufacturing Process

To establish the suitability of a mould in terms of ease of the system removal, well plates, blister packs and disposable polystyrene trays were assessed.

To overcome problems of wafers sticking to the mould, various lubricant systems were considered. Magnesium Stearate, Span 60, Maize oil and mineral oil were evaluated for their anti-adhesive properties.

It was also necessary to determine suitable timeframes for the lyophilisation process.

4.3 Results and Discussion

4.3.1 Weight Uniformity

The reproducibility of the production process was demonstrated by the low standard deviations (SD) calculated from the mass for each of the various polymer systems. Table 4.1 Shows the results obtained from the various polymer wafer systems.

Table 4.1 Mean weight of wafers manufactured (N=3)

Polymer	***Mean (g) ± SD***
HPC	0.126 **±** 0.0017
HPMC	0.122 **±** 0.0002
Pectin	0.134 **±** 0.0055
PEO	0.119 **±** 0.0045
PVA	0.118 **±** 0.0011
Sodium alginate	0.109 **±** 0.0007

Although the standard deviation of the samples is low, slightly higher values were observed for polymers such as pectin and PEO. This may be attributed to the high viscosity of the initial solution, and therefore greater variability in the production process.

4.3.2 Gelation of Matrices

Polymers such as sodium alginate, pectin and PEO tended to form a gel-like substance when hydrated and agitated rather than undergo disintegration. Sodium alginate produced the highest amount of residue, possibly due to its low water solubility. In sharp contrast, the highly hydrophilic polymers such as HPC were completely disintegrated within 30 seconds into small particles which were able to penetrate through the pores on the sieve. Figure 4.1 shows the mass of intact material after sieving of the various dissolved wafers tested.

Based on the results obtained, HPC was identified as the most suitable polymer for the wafer system, because no residue was produced after 30 seconds of hydration and agitation in simulated saliva. This may be attributed to the fact that HPC is highly soluble in polar solvents and therefore undergoes disintegration rapidly without forming a gel residue, ensuring rapid matrix disintegration.

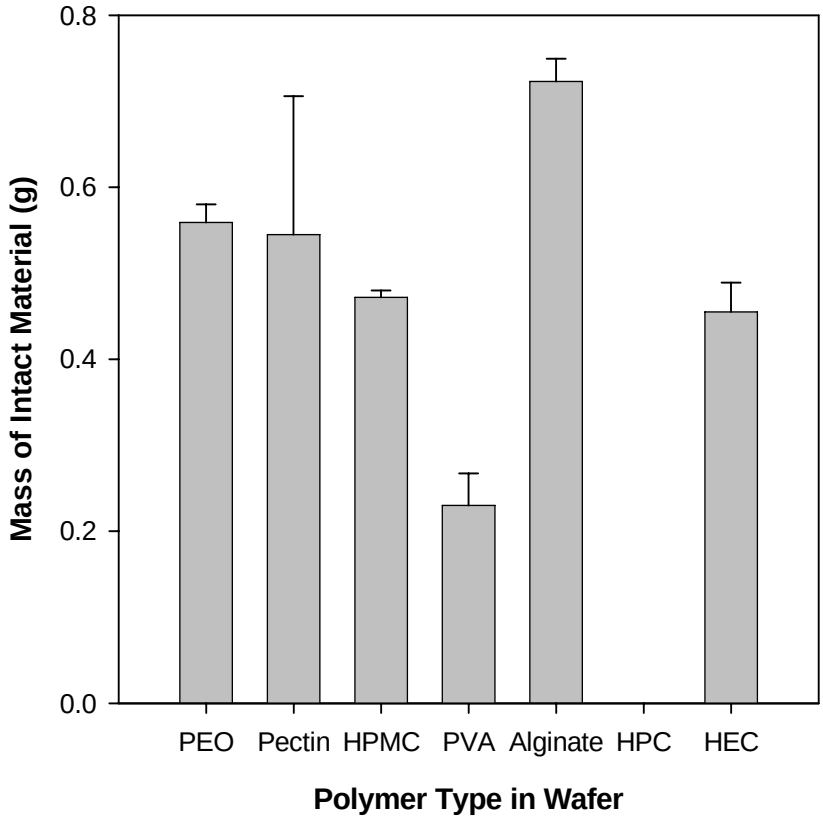


Figure 4.1 Mass of intact wafer after gelation studies using various polymers (N=3)

4.3.3 Established Parameters of Formulation Variables

4.3.3.1 Concentration of HPC

Lower and upper limits were determined to be 1%w/v and 10%w/v respectively. The upper limit of 10%w/v was set because wafers of higher polymer concentrations were difficult to remove from the mould. Some wafers produced with polymer concentrations below 5%w/v collapsed. Less than 1%w/v of HPC was not sufficient to form the wafer matrix.

4.3.3.2 Concentration of Diluent

The concentration of the diluent would affect both the solubility and textural properties of the matrices. Lower and upper limits were determined to be 1%w/v and 5%w/v respectively. Concentrations of lactose higher than 5%w/v caused the wafer to be powdery and extremely fragile.

4.3.3.3 Type of Mould

A major problem that was encountered was the removal of the wafers from the moulds without disrupting the delicate structure. Polystyrene trays proved to be the most successful, with minimal deformation of the final product as these moulds could be easily split down the middle to release the wafer.

4.3.3.4 Type of Lubricant

As mentioned above, removal of the wafers from the mould was problematic. Mineral oil produced the greatest ease of removal of the product as compared to the other lubricants analysed, imparting minimal hydrophobicity and having no effect on the taste of the final product as opposed to other substances such as maize oil.

4.3.3.5 Freeze-Drying Parameters

Although the wafers appeared to be dry after a period of 24 hours, ‘melting’ and discolouration of the matrices occurred on storage. This was attributed to moisture present within the products, indicating that the freeze drying process needed to be conducted for a longer period. In future processes, this was increased to 48 hours.

4.4 Concluding Remarks

It was necessary to gain a firm understanding of the key factors involved in the successful production of a lyophilised wafer system. HPC was selected as the most appropriate polymer of the seven that were assessed. It was expected that the type of diluent used in the wafer matrix would affect the disintegration rate of the wafers. Mannitol which is more quickly soluble than lactose will be included in the experimental design to assess its influence of this inclusion on the disintegration rate. The diluents will either be used on their own or in a 1:1 combination.

To solve the problem of wafers collapsing, Seager (1998) recommended that glycine be used as a collapse protectant. Therefore, concentrations of up to 0.6%w/v will be included in subsequent formulations.

The selection of a suitable polymer, determination of future formulation parameters and creation of problem-free manufacturing techniques formed the basis of this part of the study.

Chapter 5 Experimental Design Strategy to Formulate and Evaluate the Wafer Matrix

5.1 Introduction

Once a suitable polymer was selected, in order to conduct a scientifically sound and rational study, a factorial experimental design was used. It was necessary to identify and optimise factors that were influential on the properties of the wafers, as well as the manufacturing process.

The series of experiments discussed in this chapter aims to identify the effects of formulation variables on the physicochemical and physicomechanical properties of the wafers using a CCF to provide a systematic approach to the experimentation.

5.2 Materials and Methods

Ingredients used in the production of the wafers were hydroxypropyl cellulose (HPC) (Klucel, EF Pharm, Hercules Inc., Wilmington, North Carolina, USA), lactose (Merck Lab Supplies Pty. Ltd., Midrand, Gauteng, South Africa), mannitol (Merck Lab Supplies Pty. Ltd., Midrand, Gauteng, South Africa) and glycine (Aminoacetic Acid, Hopkin and Williams Ltd., Essex, England, UK) as a collapse protectant.

5.2.1 Statistical Approach to Wafer Formulation

A Face Centered Central Composite design was developed with 5 factors and 4 centre points (Table 5.1).The equation for the design was as follows:

$$\text{Response} = b_0 + b_1*s + b_2*t + b_3*u + b_4*v + b_5*w + b_6*s*s + b_7*t*t + b_8*u*u + b_9*v*v + b_{10}*w*w + b_{11}*s*t + b_{12}*s*u + b_{13}*s*v + b_{14}*s*w + b_{15}*t*u + b_{16}*t*v + b_{17}*t*w + b_{18}*u*v + b_{19}*u*w + b_{20}*v*w$$

(Equation 5.1)

Where:

s = Polymer Concentration;

t = Diluent Type;

u = Diluent Amount;

v = Glycine Concentration; and

w = Fill Volume.

The responses that were measured are:

- Disintegration profiles;
- Rate of influx of simulated saliva into the matrix;
- Friability;
- Matrix yield value;
- Matrix tolerance;
- Matrix absorption energy;
- Matrix resilience; and
- Brinell Hardness Number (BHN).

Table 5.1 Randomised experimental runs generated from the CCF

Formulation Number	***[Polymer] (%w/v)***	***Diluent Type***	***[Diluent] (%w/v)***	***[Glycine] (%w/v)***	***Fill Vol. (mL)***
1	10	1	5	0.6	2
2	5.5	0.5	3	0.6	1.5
3	1	1	1	0	1
4	5.5	0.5	3	0.3	1.5
5	5.5	0.5	1	0.3	1.5
6	10	1	1	0.6	1
7	5.5	0	3	0.3	1.5
8	5.5	1	3	0.3	1.5
9	10	0.5	3	0.3	1.5
10	10	1	1	0	2
11	5.5	0.5	3	0.3	2
12	10	0	5	0.6	1
13	1	0	5	0.6	2
14	5.5	0.5	3	0	1.5
15	1	0	1	0.6	1
16	10	1	5	0	1
17	10	0	5	0	2
18	5.5	0.5	5	0.3	1.5
19	1	1	1	0.6	2
20	1	0	5	0	1
21	10	0	1	0.6	2
22	1	0	1	0	2
23	10	0	1	0	1
24	1	0.5	3	0.3	1.5
25	5.5	0.5	3	0.3	1.5
26	5.5	0.5	3	0.3	1
27	1	1	5	0	2
28	1	1	5	0.6	1
29	5.5	0.5	3	0.3	1.5
30	5.5	0.5	3	0.3	1.5

**Parenthesis indicate concentration*
**Diluent type: 0=lactose, 1=mannitol, 0.5= 1:1 mixture of lactose and mannitol*
**Glycine = matrix consolidator to increase rigidity*

5.2.2 Preparation of Wafers According to the CCF

The composition of the wafers was specified by the CCF (Table 5.1). Ingredients were dissolved in deionised water and left to stir for 30 minutes. The specified volume of the solution was pipetted into polystyrene moulds pre-oiled with 2 drops of mineral oil. The formulation was subjected to a freeze-phase in a freeze-

dryer at -60°C for 2 hours. The drying-phase was executed at a pressure of 25 mtorr for 48 hours. Wafers were stored in glass jars with 2g of desiccant sachets.

5.2.3 Evaluation of CCF Responses

5.2.3.1 ANOVA Test

An Analysis of Variance (ANOVA) was conducted on the input variables of the wafers to determine which input variables had a significant effect on the recorded output properties of the wafers. The ANOVA was carried out using Essential Regression and Experimental Design V2.207 (Yeaton, Stepper and Werner, 1998). Only the linear terms were used to regress the data, since we were only interested in the effect that each input variable had on the measured output variables at a 95% confidence interval.

5.2.3.2 Disintegration Profiles

The definition of a fast melting (or disintegrating) tablet appeared in a compendial publication for the first time in 1998. However, neither the US Pharmacopeia nor the European Pharmacopeia have defined a specific disintegration test (Dobetti, 2001). As a result, a novel method was developed to assess and compare the disintegration profiles of the 30 samples manufactured according to the CCF.

Wafers were weighed before being placed in a petri dish containing 20mL of simulated saliva (as described in Chapter 4, section 4.3.2.2). The dish was allowed to slowly agitate on a vortex mixer for a period of 20 seconds. The contents of the dish were sieved through a stainless steel mesh (pore size 1mm). Particles that were able to pass through the pores of the sieve were considered

to be sufficiently disintegrated, while those captured by the sieve were termed the 'residue'. The residue represents the portion of the wafer that was not sufficiently disintegrated. The residue was measured in both the hydrated and dry state. For wafers that were eroded very rapidly, the agitation time was reduced to 10 seconds. Tests were conducted in triplicate. Based on the measurements documented, the following information was calculated, providing a comprehensive disintegration profile for each wafer formulation:

Normalised Percentage Matrix Disintegrated per second (%/s)

$$\frac{\left(\frac{Mass\ of\ Disintegrated\ Wafer}{Mass\ of\ Unhydrated\ Wafer\ at\ t_0}\right)\times 100}{Time} \qquad \text{(Equation 5.2)}$$

To account for the different times used for certain formulations, as well as the difference in wafer size caused as a result of the various fill volumes (1mL, 1.5mL and 2mL) used in the CCF, the Percentage Matrix Disintegration was calculated per second. This process expresses the results as a fraction such that differences in formulation are taken into account, and are comparable on a normalised level.

Influx Rate of Simulated Saliva within Wafer (%/s)

$$\frac{\left(\frac{Residual\ Mass\ of\ Hydrated\ Wafer - Mass\ of\ Dry\ Residual\ Wafer}{Residual\ Mass\ of\ Hydrated\ Wafer}\right)\times 100}{Time}$$

(Equation 5.3)

Similar to the disintegration profiles, the influx of simulated saliva is calculated as a rate, allowing the various formulations to be compared on the unit percentage per second.

5.2.3.3 Friability

Rapidly disintegrating systems prepared by the process of lyophilisation are known for having the characteristic disadvantage of poor physical resistance (Dobetti, 2001). Problems anticipated as a result of this include: breakage of tablet edges during handling and the inability of the tablet to be ejected and removed from a conventional blister alveolus. These features need to be taken into consideration when determining the packaging of the product.

Friabilty was measured using a Roche friabilator (Hoffman la Roche, Basel, Switzerland). The wafers (N=3) were accurately weighed before being placed into the friabilator. A rotation time of 4 minutes at 25 rpm was used. Tablets were removed and loose particles brushed off the surface. Wafers were re-weighed and the percentage weight loss was calculated.

5.2.3.4 Textural Analysis

This study focuses on the characterisation of matrix resilience, energy of absorption, matrix yield value and matrix tolerance, using the TA.*XTplus* Texture Analyser (Stable Micro Systems, Surrey, UK) fitted with a 5kg load cell.

5.2.3.4.1 Energy of Absorption

The energy of absorption is an indirect indication of the porosity of the wafers. A highly porous wafer will exhibit a greater value for the energy of absorption because energy is accommodated within the voids in the matrix. The energy of absorption is calculated by determining the area under the curve (AUC) of a profile illustrating force (N) and distance (m) (Figure 5.1). Note that for the AUC, the units of Newton metre (Nm) are equivalent to Joules.

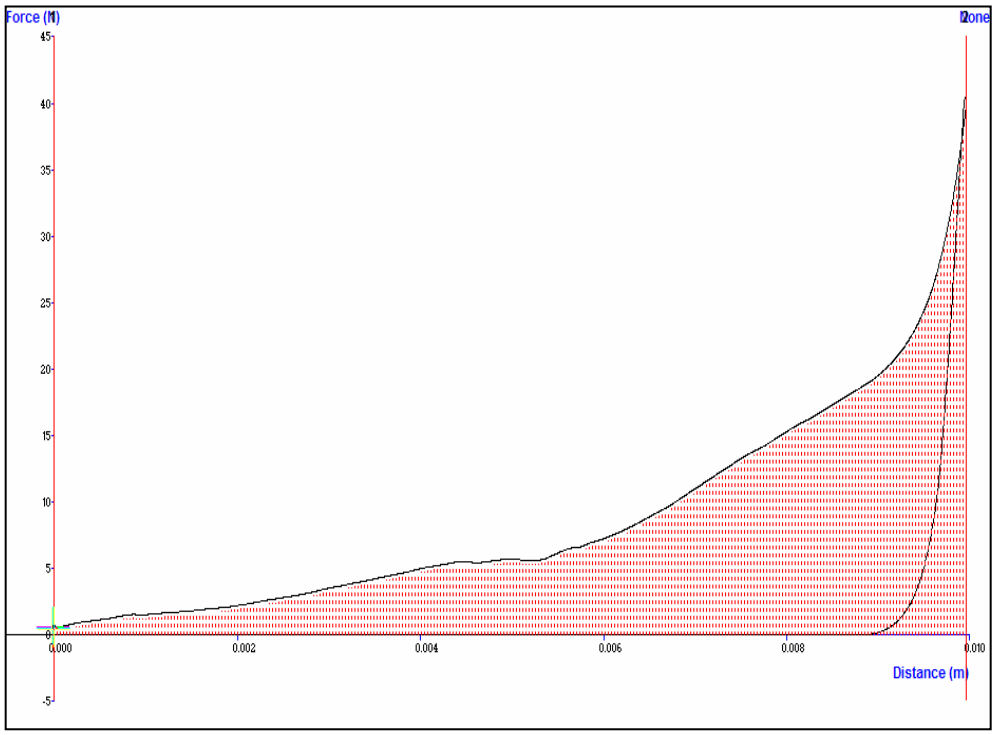


Figure 5.1 Calculation of energy of absorption (i.e. AUC)

5.2.3.4.2 Matrix Yield Value

This test is indicative of a surface phenomenon, providing information about the superficial, surface structure of the wafer. The matrix yield value is determined by creating a gradient between anchors 1 and 2 (Figure 5.2). Anchor 2 represents

the first point of major inflection on the force-distance profile. This is indicative of primary fracture of the wafer matrix which results in a reduction of force.

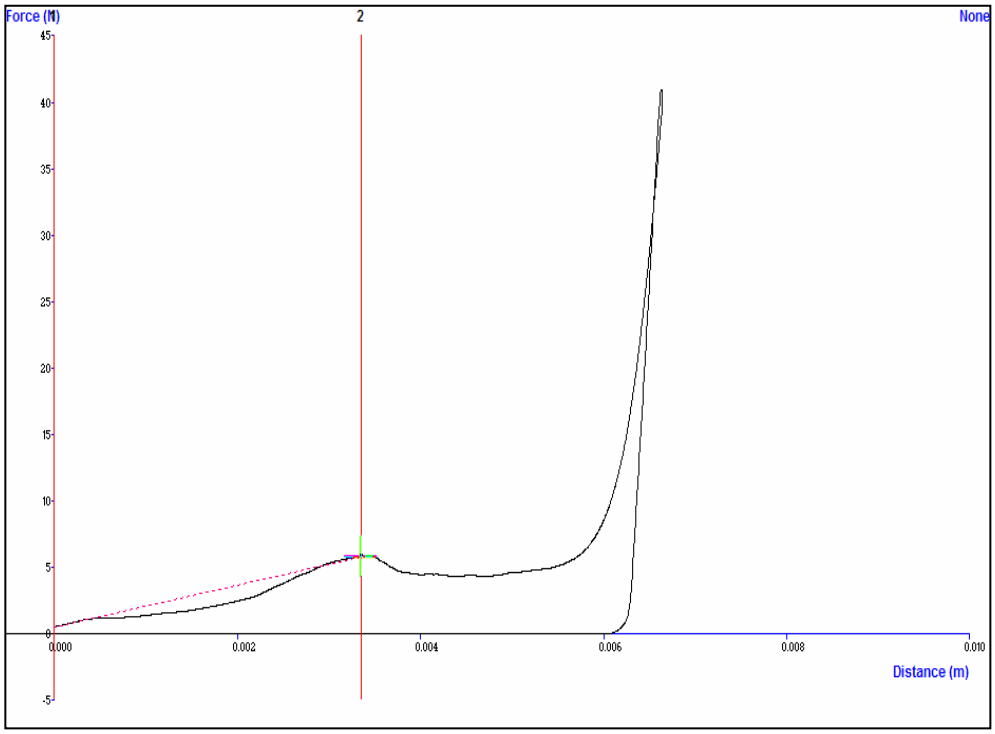


Figure 5.2 Determination of matrix yield value

5.2.3.4.3 Matrix Tolerance

On further application of force, the residual intact matrix undergoes complete fracture (Figure 5.3). The matrix tolerance value is indicative of the overall strength of the wafer. The second anchor indicates the point of maximum force. The gradient between anchors 1 and 2 in Figure 5.3 is the matrix tolerance value. This indicates the point at which total collapse of the matrix occurs.

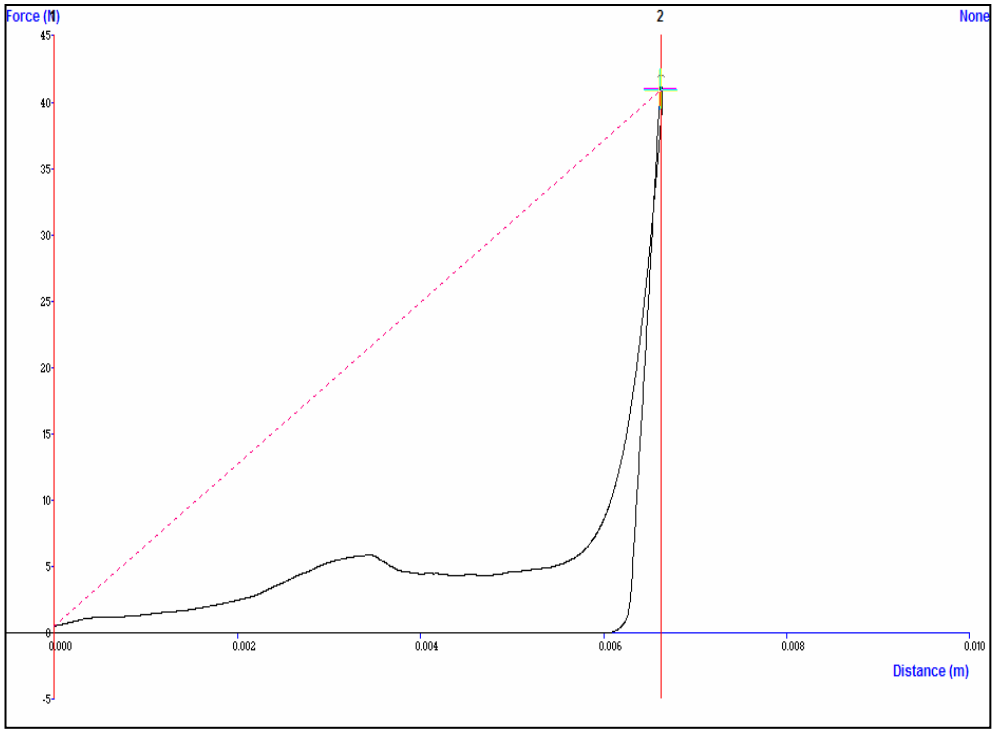


Figure 5.3 Determination of matrix tolerance

5.2.3.4.4 Matrix Resilience

Matrix resilience profiles provide us with an understanding of the deformation characteristics and the ability of the wafer to withstand pressure. The calculation of matrix resilience is provided by the ratio of the AUC between anchors 2 and 3 and between anchors 1 and 2 as shown in Figure 5.4.

Figure 5.4 Force-time profile for the calculation of matrix resilience.

5.2.3.4.5 BHN

The BHN is an indication of the force required to indent the surface of the wafer, and is thus a measure of the hardness of the surface of the wafer. BHN is calculated using the following equation:

$$BHN = \frac{\frac{2F}{\Pi}}{D\left(D - \sqrt{D^2 - d^2}\right)} \qquad \text{(Equation 5.4)}$$

Where:

D = Diameter of ball probe = 3.175mm

d = Depth of indentation = 0.25mm

F = Force, determined from Figure 5.5.

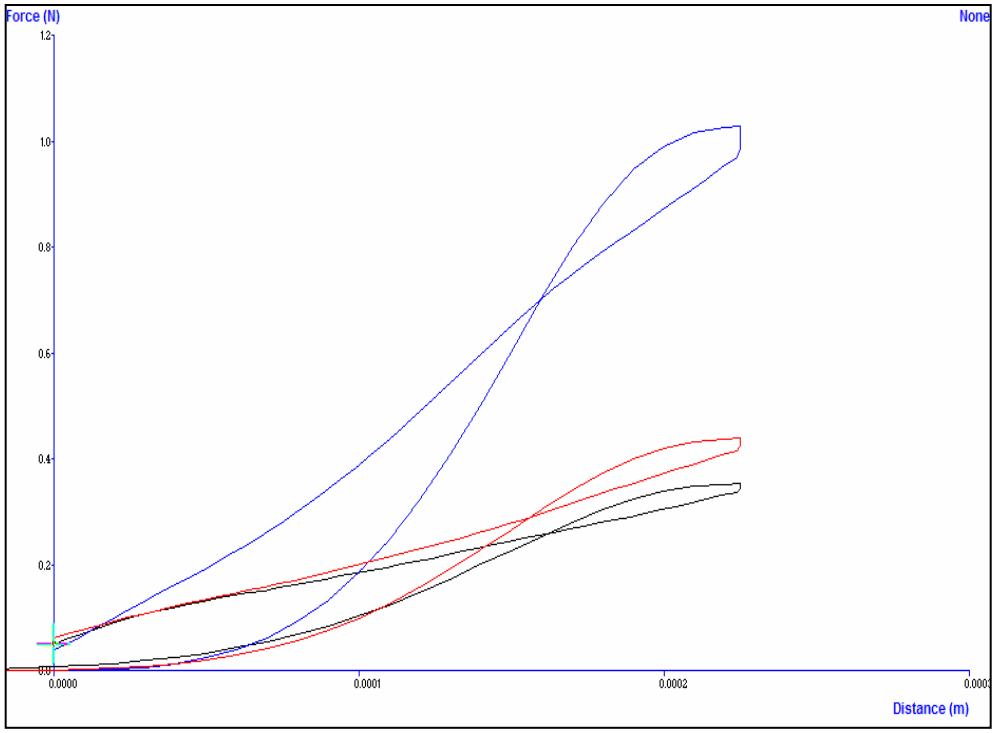


Figure 5.5 Force-distance profile for the computation of the BHN

5.3 Results and Discussion

The complete results for the ANOVA are shown in Table 5.2. The measured responses of the CCF are shown in Table 5.3 and Table 5.4.

Table 5.2 Results of ANOVA conducted on wafer systems

Output Variable	***Term***	***Coefficient***	***t - Statistic***	***Significance (p value)***
Disintegration Rate (%/s)	Constant	4.899	1.255	0.223
	[HPC] %w/v	-0.770	-3.369	0.00277
	Diluent Type	1.870	1.817	0.08287
	[Diluent] %w/v	1.100	2.138	0.04385
	[Glycine] %w/v	1.972	0.575	0.571
	Fill Vol. (mL)	-0.706	-0.343	0.735
Influx of Simulated Saliva (%/s)	Constant	8.729	2.346	0.02838
	[HPC] %w/v	-0.603	-2.767	0.01124
	Diluent Type	2.025	2.064	0.05098
	[Diluent] %w/v	0.738	1.505	0.147
	[Glycine] %w/v	0.762	0.233	0.818
	Fill Vol. (mL)	-1.783	-0.909	0.373

Table 5.2 continued

Output Variable	***Term***	***Coefficient***	***t - Statistic***	***Significance (p value)***
Friability (% loss)	Constant	3.034	0.465	0.647
	[HPC] %w/v	-0.932	-2.435	0.02344
	Diluent Type	1.637	0.951	0.352
	[Diluent] %w/v	1.967	2.285	0.03233
	[Glycine] %w/v	-8.109	-1.413	0.172
	Fill Vol. (mL)	1.176	0.341	0.736
Energy Absorbed (J)	Constant	-0.02816	-2.685	0.01354
	[HPC] %w/v	0.00374	6.088	3.975e-06
	Diluent Type	0.00117	0.422	0.677
	[Diluent] %w/v	0.00144	1.045	0.307
	[Glycine] %w/v	-0.00444	-0.482	0.634
	Fill Vol. (mL)	0.02189	3.958	0.000668
Matrix Yield Value (N/mm)	Constant	5.430	2.958	0.00728
	[HPC] %w/v	-0.211	-1.962	0.06255
	Diluent Type	0.609	1.259	0.221
	[Diluent] %w/v	-0.411	-1.697	0.104
	[Glycine] %w/v	0.261	0.162	0.873
	Fill Vol. (mL)	-1.070	-1.105	0.281
Matrix Tolerance (N/mm)	Constant	58.68	3.950	0.000682
	[HPC] %w/v	-2.686	-3.086	0.00540
	Diluent Type	2.799	0.715	0.482
	[Diluent] %w/v	-4.570	-2.334	0.02915
	[Glycine] %w/v	1.185	0.09074	0.929
	Fill Vol. (mL)	-11.37	-1.451	0.161
Matrix Resilience (%)	Constant	22.59	2.088	0.04863
	[HPC] %w/v	-0.603	-0.951	0.352
	Diluent Type	-3.832	-1.343	0.193
	[Diluent] %w/v	1.640	1.150	0.262
	[Glycine] %w/v	-12.28	-1.291	0.210
	Fill Vol. (mL)	-9.019	-1.581	0.128
BHN (N/mm^2)	Constant	-0.122	-0.09834	0.923
	[HPC] %w/v	0.454	6.237	2.816e-06
	Diluent Type	-0.114	-0.348	0.731
	[Diluent] %w/v	0.268	1.637	0.116
	[Glycine] %w/v	2.513	2.301	0.03124
	Fill Vol. (mL)	-0.937	-1.430	0.167

Table 5.3 Data generated from disintegration and friability analysis

Formulation Number	***Rate of Matrix Disintegration (%/sec)***	***Rate of Simulated Saliva Influx (%/sec)***	***Friability (% Loss)***
1	1.60	3.32	0.46
2	1.94	3.39	1.28
3	2.44	8.30	0.67
4	2.77	4.14	0.77
5	2.35	3.97	0.08
6	0.55	3.37	1.14
7	1.44	3.51	0.69
8	4.02	4.04	0.60
9	0.64	3.04	0.05
10	0.89	2.98	0.42
11	0.06	3.54	0.19
12	0.01	3.05	0.34
13	5.73	3.38	2.63
14	1.39	3.59	0.51
15	5.77	7.62	1.72
16	2.32	3.92	0.31
17	1.13	3.38	0.50
18	2.34	3.46	0.44
19	3.40	3.80	0.53
20	4.67	4.22	19.73
21	1.82	3.53	0.38
22	3.45	4.37	0.96
23	0.70	3.40	0.33
24	3.42	3.95	0.55
25	1.02	3.55	4.11
26	1.16	3.62	0.07
27	18.18	18.18	40.72
28	25.00	25.00	11.90
29	0.84	3.59	0.28
30	0.62	3.46	0.50
R^2	0.969	0.955	0.942

**R^2 – for validation of model according to predicted data*

The data in Table 5.3 is shown to two decimal places, however four decimal places were employed during calculations to ensure precision and accuracy.

Table 5.4 Textural profiling analysis conducted on wafers

Formulation Number	***Matrix Yield Value (N/mm)***	***Matrix Tolerance (N/mm)***	***Matrix Absorption Energy (J)***	***Matrix Resilience (%)***	***BHN (N/mm^2)***
1	1.25	4.47	0.06	4.66	6.19
2	1.10	5.69	0.03	4.36	2.35
3	10.03	80.22	0.01	3.97	1.50
4	2.09	4.98	0.03	3.71	1.90
5	0.14	5.44	0.02	4.27	1.92
6	1.19	6.14	0.03	6.09	6.67
7	0.77	4.64	0.04	3.00	6.11
8	0.21	4.70	0.02	5.24	2.88
9	0.88	5.79	0.04	5.25	4.53
10	0.98	4.02	0.08	5.49	2.41
11	1.12	4.47	0.06	4.40	0.46
12	1.73	7.65	0.04	6.90	8.14
13	0.09	4.40	0.02	2.65	1.01
14	0.27	5.14	0.02	5.38	0.52
15	3.12	54.64	0.01	7.13	0.05
16	0.57	7.10	0.03	8.64	3.59
17	0.70	3.84	0.07	3.10	5.20
18	0.74	4.58	0.03	3.13	1.69
19	3.94	41.12	0.01	3.18	0.08
20	0.17	8.61	0.01	7.18	0.65
21	1.36	7.17	0.04	6.50	3.58
22	0.20	8.67	0.01	4.78	0.20
23	0.75	7.65	0.02	5.71	1.81
24	7.24	63.84	0.01	2.39	0.44
25	0.79	4.93	0.03	3.15	1.76
26	0.40	8.10	0.02	4.59	3.77
27	0.20	4.77	0.02	2.10	0.01
28	1.50	5.11	0.01	3.20	1.39
29	0.32	5.27	0.02	3.75	0.01
30	0.86	4.75	0.03	3.14	2.71
R^2	0.855	0.916	0.923	0.956	0.897

R^2 – for validation of model according to predicted data

The data presented in Table 5.4 is to two decimal places, however four decimal places were employed during calculations to ensure precision and accuracy.

5.3.1 ANOVA

Table 5.2 lists the results of the ANOVA on the variables tested together with the regression coefficients of the linear terms.

The ANOVA for a total of 28 points for each output variable reveals that for friability, disintegration rate and matrix tolerance, the concentration of HPC and diluent are the only formulation factors that had any significant effect on them ($p < 0.05$). The type of diluent, concentration of glycine and fill volume had no significant effect on the friability, disintegration rate and matrix tolerance of the wafers tested. The concentration of HPC also had a significant effect on the influx of simulated saliva rate, BHN and energy absorbed. The only output variables that the concentration of HPC had no significant effect on were the matrix resilience and yield value. In fact no input variables had any significant effect on the matrix yield value and resilience of the wafers. Therefore, the matrix resilience and yield value were not considered to be reliable measures that would discern between the effects of different input variables on optimising the wafer formulation.

Glycine concentration did however have a significant effect on the BHN, probably due to the fact that as it increases in concentration, it decreases the plasticity of the wafers. The only factor that was influenced by the type of diluent used was the rate of saliva influx. The different diluents may contribute to varying the solubility of the wafers. Besides the matrix absorption energy, the fill volume did not influence any other variable measured in this study.

5.3.2 Disintegration Profiles

Based on the results (Table 5.3), it could be seen that the rate of disintegration of the wafers was primarily dependent on the concentration of HPC, and secondarily on the concentration of the diluents (Figure 5.6). It was generally noted that higher polymer concentrations were associated with lower rates of

disintegration. Due to the highly soluble nature of the diluents, an increase in the amount accounted for higher matrix solubility and thus faster rates of disintegration.

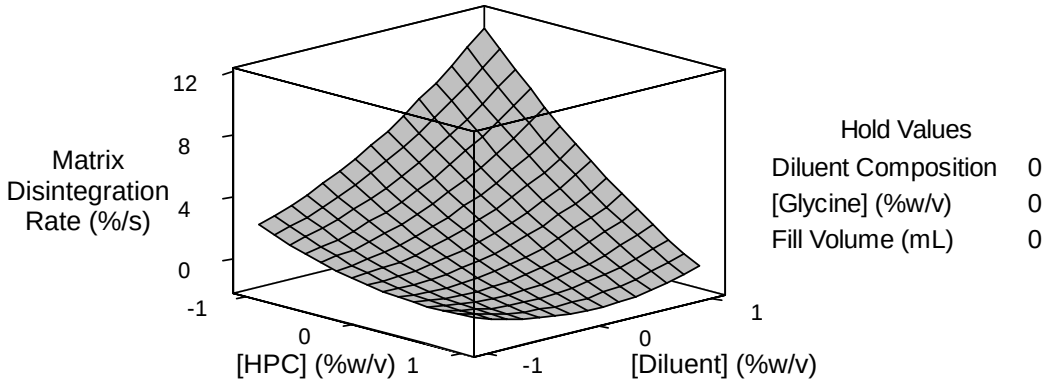


Figure 5.6 Surface plot illustrating the effect of diluent and HPC concentration on the rate of matrix disintegration

Formulations containing low polymer concentrations, accompanied by high concentrations of diluent, underwent significantly rapid disintegration. It was also noted that the presence of mannitol in the formulations promoted more rapid disintegration than those containing lactose. This phenomenon can be explained by comparing the solubility of the two sugars. Although solubility of mannitol and lactose are similar (1g in 5.5 and 5mL of cold water respectively, Windholz et al., 1976), it was noted that lactose dissolve at a slower rate than mannitol. The more rapid disintegration rates of formulations containing mannitol can be directly attributed to its better solubility than lactose.

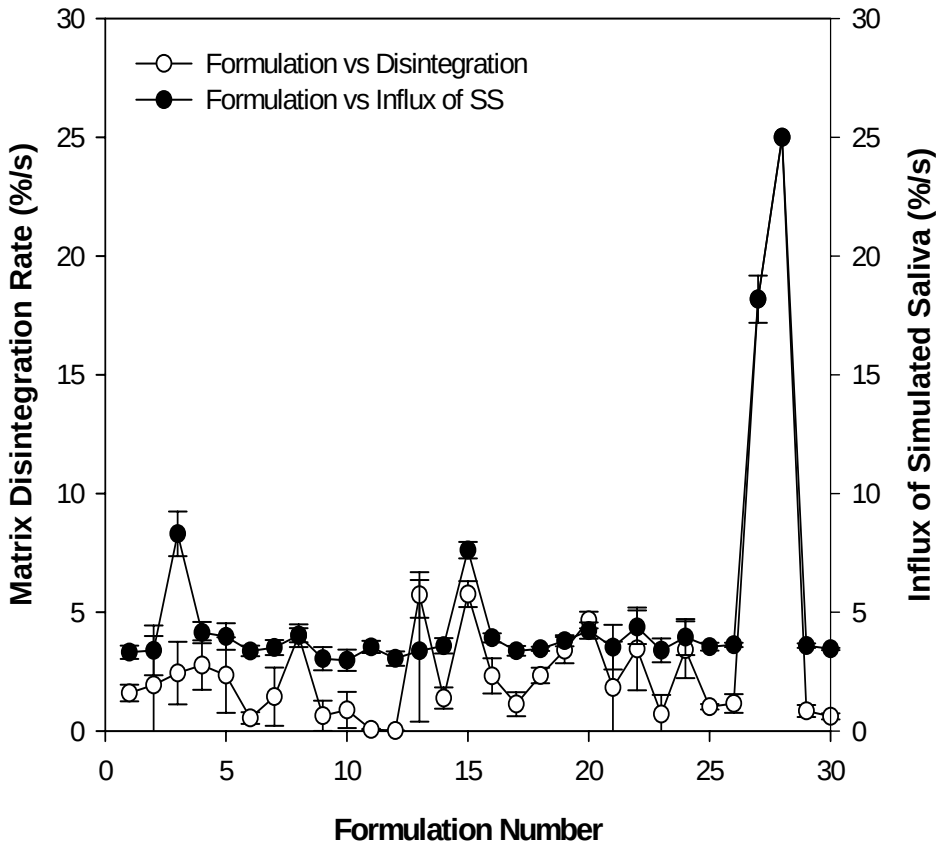


Figure 5.7 Relationship between the influx of simulated saliva and disintegration of the wafers (N=3)

Another factor that affected the rate of disintegration was the influx of simulated saliva. It was observed that as saliva was imbibed into the wafer, disintegration was promoted (Figure 5.7). The ability of saliva to be imbibed into the wafer was attributed to the porous structure created, as a result of the freeze drying process. The results of the ANOVA analysis (Table 5.2) indicated that the formulation variable to have the most significant effect on the influx of saliva was the concentration of HPC. It was therefore deduced that an increase in the concentration of HPC allows for the creation of pores within the wafer during the lyophilisation process. The concentration of diluent also plays an important role in the rate of saliva influx.

5.3.2.1 Predicted Disintegration Response

The close correlation between the experimental and predicted responses for the rate of matrix disintegration can be clearly seen in the plot below (Figure 5.8). The correlation coefficient (R^2) obtained is 0.97. The standard deviation for this comparison is 1.66.

The equation that describes the response (Figure 5.8) is as follows:

$$y = 1.2013 – 3.4672*s + 1.8698*t + 2.2004*u + 0.5916*v – 0.3528*w +0.8852*s*s + 1.5822*t*t + 1.1977*u*u + 0.5152*v*v - 0.5348\ w*w – 1.7330*s*t – 2.3389*s*u – 0.7629*s*v + 0.5606*s*w + 2.5017*t*u +0.2095*t*v – 0.4505*t*w + 0.1239*u*v -0.3421*u*w – 2.0179*v*w$$

(Equation 5.5)

Where s, t, u, v and w are as described in Equation 5.1.

Similarly, the high accuracy of the CCF predictions can be seen for the rate of influx of simulated saliva (Figure 5.9). The standard deviation and R^2 were 1.79 and 0.96 respectively.

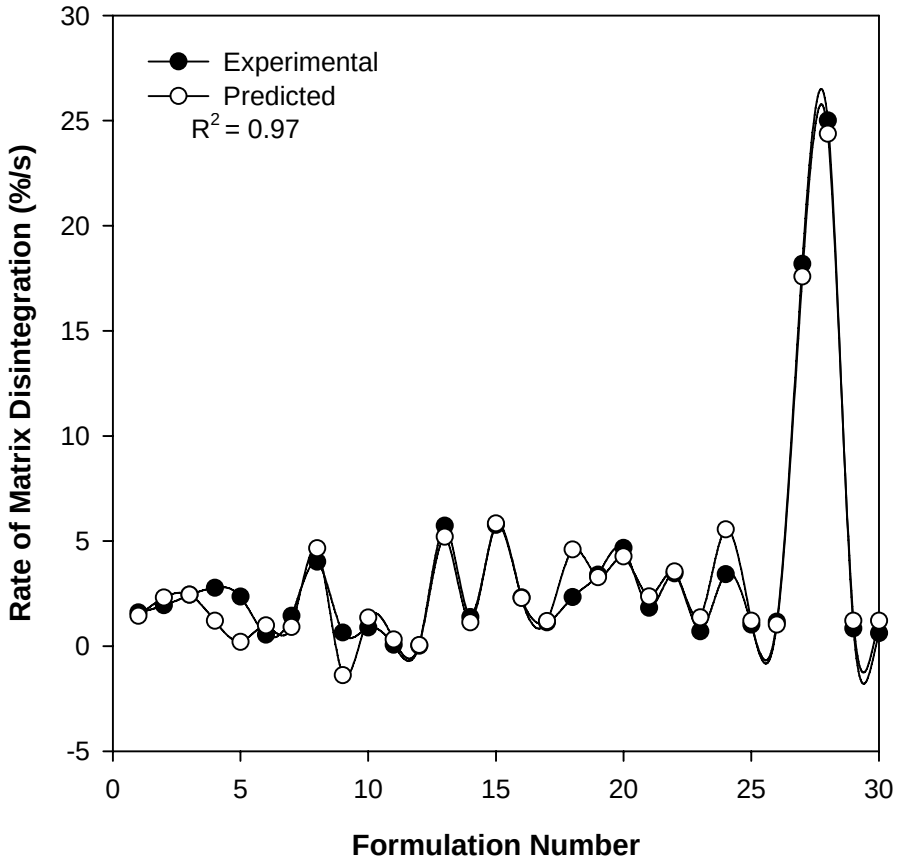


Figure 5.8 Comparison of the experimental and predicted responses for the matrix disintegration rate

The equation describing the response (Figure 5.9) is as follows.

$$y = 3.22095 - 2.71411*s + 2.02478*t + 1.47594*u + 0.22856*v - 0.89128*w + 0.50857*s*s + 0.78557*t*t + 0.72607*u*u + 0.50057*v*v - 0.59207\ w*w - 2.21656*s*t - 1.64331*s*u - 0.32056*s*v + 0.92981*s*w + 2.30381*t*u - 0.00669*t*v - 0.54206*t*w + 0.36031*u*v - 0.00369*u*w - 2.13006*v*w$$

(Equation 5.6)

Where s, t, u, v and w are as described in Equation 5.1

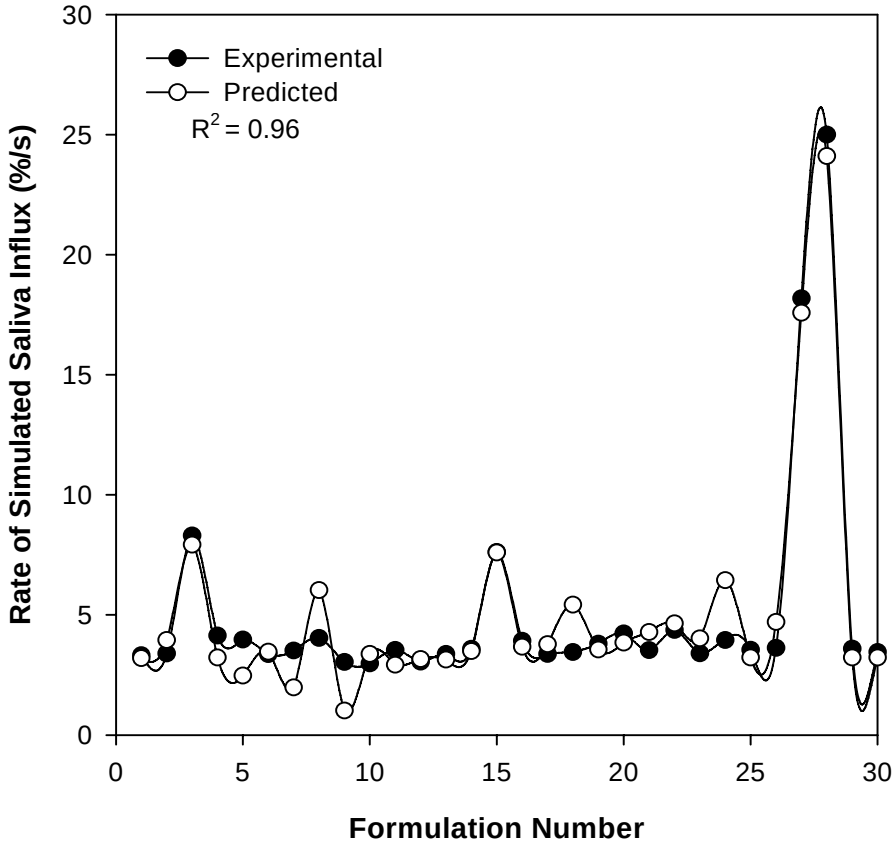


Figure 5.9 Comparison of the experimental and predicted responses for the rate of influx of simulated saliva

5.3.3 Friability

Preliminary investigations have shown that lactose may possess a superior binding ability to mannitol during wafer preparation. From the results in Table 5.2 it was observed that the friability of the wafers was dependant on the concentration of polymer (p= 0.063). Low friability was seen in wafers containing high concentrations of HPC. The most friable wafers were those containing low concentrations of polymer accompanied by high concentrations of diluent, as seen in the surface plot (Figure 5.10). From this it may be concluded that the polymer served as a binding agent, thus imparting robust qualities to the wafer. When determining optimal concentrations for the diluent, it should be kept in mind

that although high diluent concentrations promoted rapid dissolution, this also led to an increase in friability.

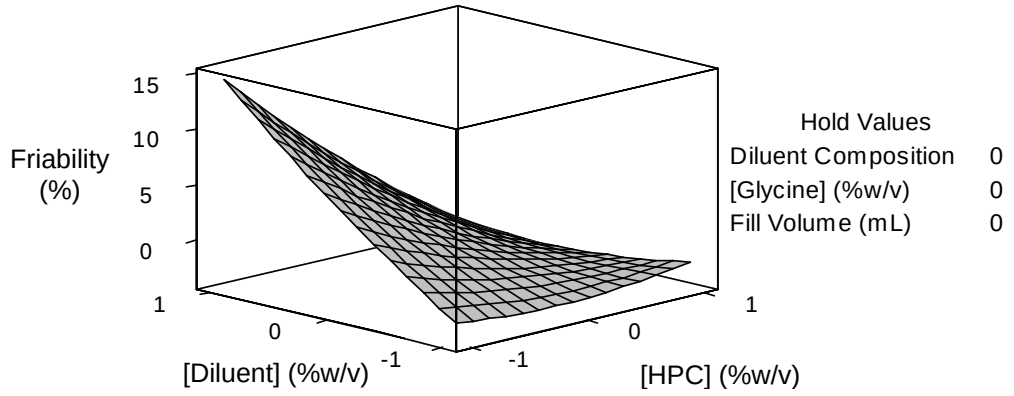


Figure 5.10 Surface plot of friability demonstrating the effects of diluent and HPC concentration

5.3.3.1 Predicted Friability Response

The precision of the design prediction for the friability of the wafers is highlighted by the R^2 value of 0.94.

The equation below describes the response (Figure 5.11)

y = 0.10301 – 4.1935*s + 1.6374*t + 3.9343*u – 2.4327*v + 0.5879*w + 0.8511*s*s + 1.1986*t*t + 0.8095*u*u + 1.4470*v*v + 0.6785 w*w – 1.7506*s*t – 4.4849*s*u + 2.8785*s*v – 0.6974*s*w + 1.9261*t*u – 0.7268*t*v + 2.8599*t*w – 2.9570*u*v + 0.8504*u*w – 2.0417*v*w

(Equation 5.7)

Where s, t, u, v and w are as described in Equation 5.1.

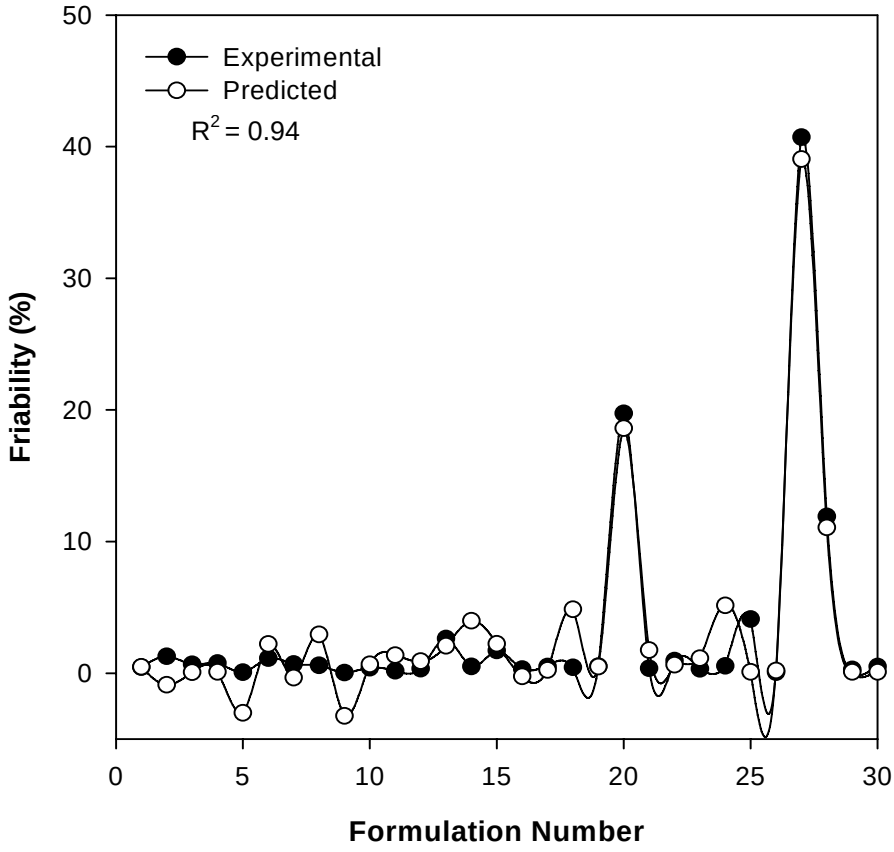


Figure 5.11 Comparison of the experimental and predicted responses for friability

5.3.4 Values Generated from Textural Profiling

Table 5.4 depicts the observed values of the textural responses for the 30 wafer formulations, as per CCF. It is apparent that all wafers possessed significantly different textural properties, based on the wide intra-response variation.

No input variables had a significant effect on the matrix yield value and matrix resilience (Table 5.2). The concentration of polymer and diluent were shown to cause a decrease in the matrix tolerance (Figure 5.12). It was postulated that an increase in the HPC concentration resulted in an increase in the porosity of the wafer. Resulting from an increase in porosity, a corresponding increase in

plasticity was also seen. The matrix was therefore unable to resist the force applied by the probe and was fractured by lower forces. On the other hand, an increase in the amount of diluent present in the system created a consolidated wafer resulting in greater compactness of the matrix. This compact matrix was brittle in nature and fractured by lower forces.

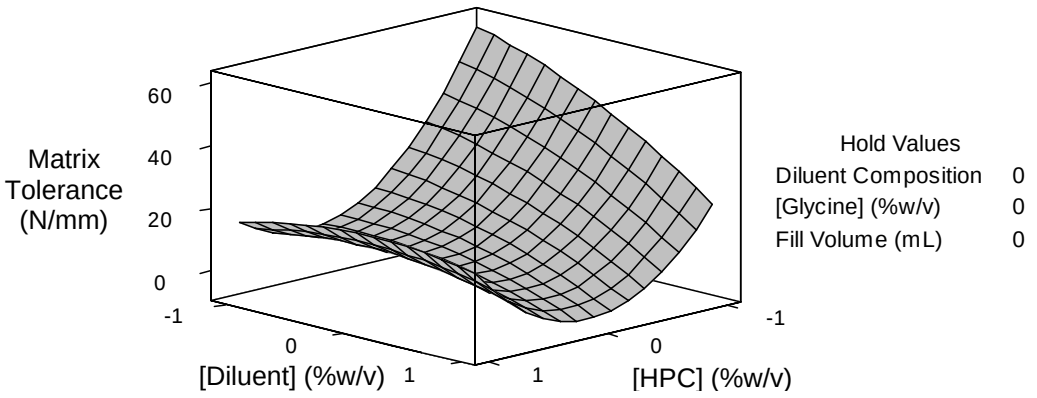


Figure 5.12 Surface plot illustrating the reduction in matrix tolerance as a result of increasing diluent and HPC concentration

The concentration of HPC also had a significant impact on the BHN. The HPC imparts rigidity and thus increases the surface hardness of the wafers. An increase in the concentration of glycine also resulted in an increase in the BHN (Figure 5.13). These results show that glycine was successful in acting as a consolidator.

The variables that significantly affected the matrix absorption energy were the fill volume and the HPC concentration (Figure 5.14). As the fill volume and hence the size of the wafer increased, the capacity to absorb energy increased as a direct result of greater area available for the propagation and dissipation of energy. As mentioned earlier, an increase in the concentration of HPC enabled the wafer with a greater ability to form pores. The spaces within the wafer allowed

for the entrapment of energy and therefore a greater ability for energy absorption with increasing concentrations of polymer.

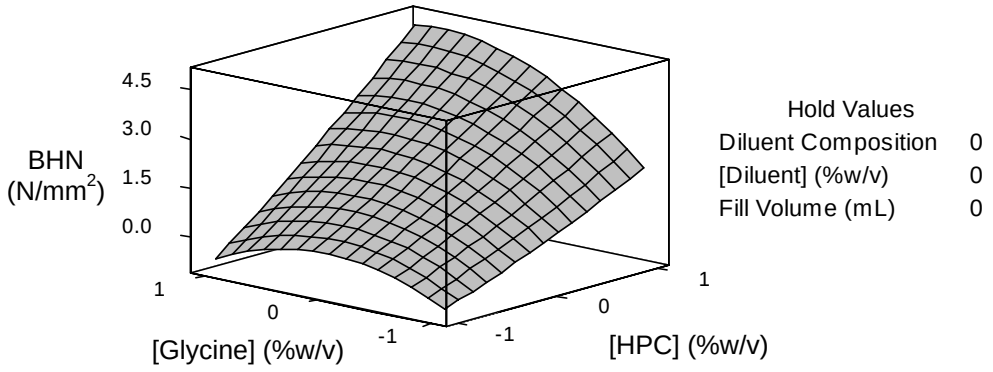


Figure 5.13 Surface plot illustrating the effect of diluent and HPC concentration on the BHN

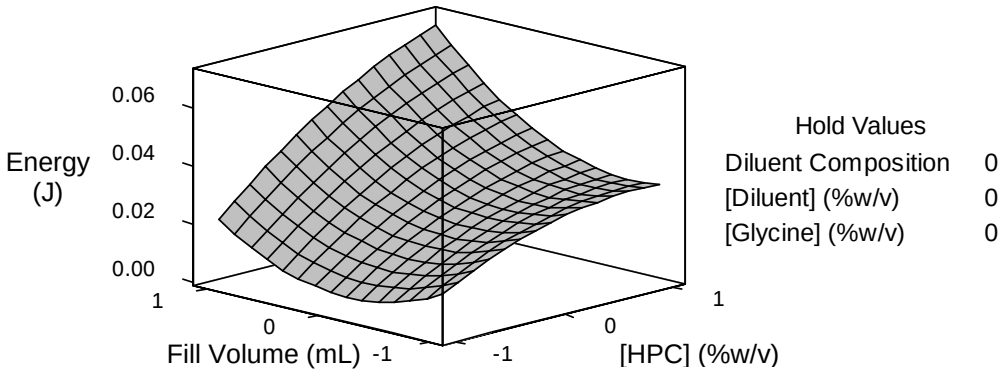


Figure 5.14 Surface plot illustrating the effect of fill volume and HPC concentration on the matrix absorption energy

5.3.4.1 Prediction of Textural Parameters by the CCF

A close relationship was observed between the experimental and predicted values (Figure 5.15) depicts the relationship between the observed and predicted values for each response.

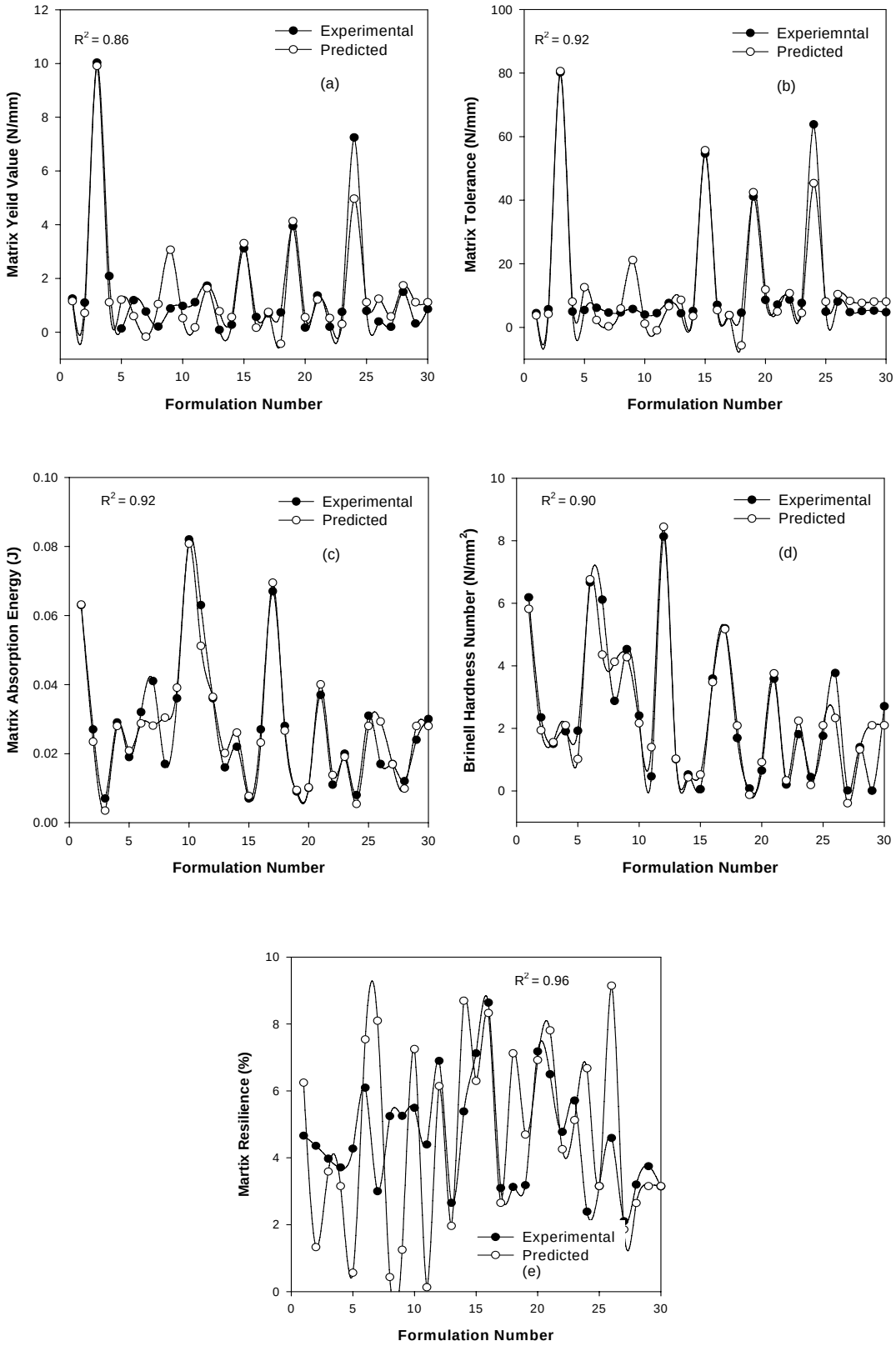


Figure 5.15 Profiles depicting the relationship between the experimental and predicted values for each dependent variable: (a) Matrix yield value; (b) Matrix tolerance; (c) Matrix absorption energy; (d) BHN and (e) Matrix resilience

A close correlation can be seen between these profiles. The similarity of the experimental and predicted data is highlighted by an average correlation coefficient of 0.91 for the above responses.

5.3.5 Porosity of the Wafers

Poor resilience may be attributed to large pores and voids within the spongy matrix. The irregular peaks seen between the anchors 2 and 3 in Figure 5.16 are indicative of the porosity of the wafers. The bounce on the curve was caused by the air pockets within the matrix. These were larger at the surface, as compression continued air was forced out of the spongy matrix.

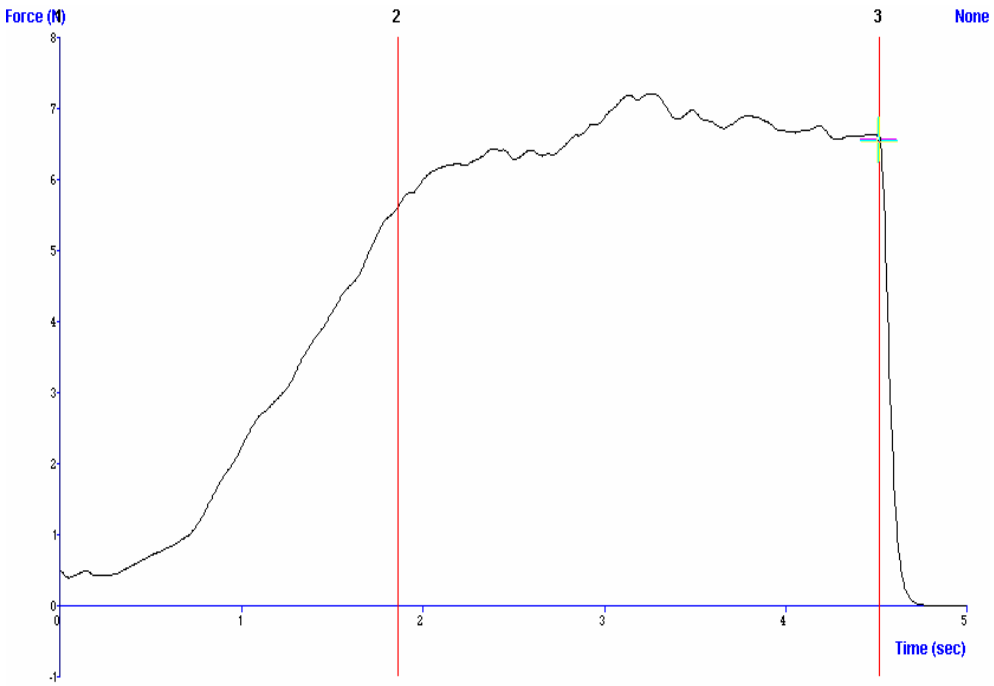


Figure 5.16 Typical force-time profile showing matrix resilience

5.3.6 Relationships between Textural Analysis Responses

5.3.6.1 Matrix Tolerance vs. Energy of Absorption

It was observed that an increase in matrix tolerance was accompanied by a decrease in the work performed during probe penetration (Figure 5.17). This indicated that as the matrix became more resistant to fracture, the energy generated by stress was not dissipated. The observed reduction in energy may be a result of its absorption within the wafer matrix. This absorption would also be facilitated by the large number of voids within the wafer.

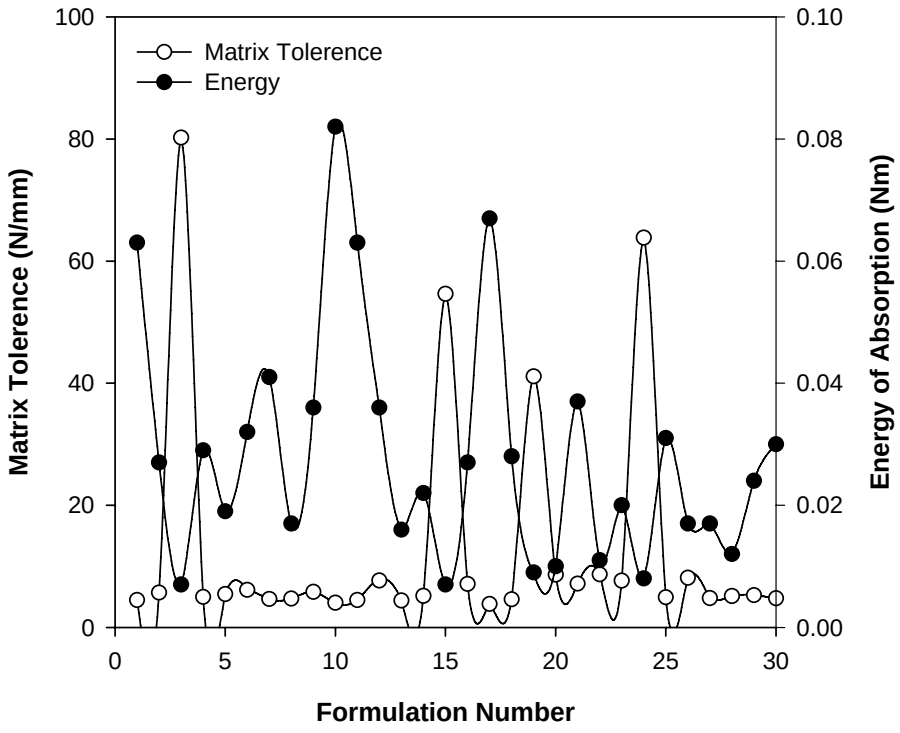


Figure 5.17 Inverse relationship between the wafer matrix tolerance and energy of absorption

5.3.6.2 Brinell Hardness vs. Energy of Absorption

The overall pattern of the profile (Figure 5.18) indicates that there was a directly proportional relationship between the BHN and the matrix absorptive energy. The absorptive energy is an indication of the wafer matrix ability to withstand distension prior to relaxation. The indentation hardness (depicted by the BHN) is a high pressure point measurement (force/unit area). Contact of the textural probe with the matrix resulted in the dissipation of high energy which was subsequently absorbed. The dissipation of energy appeared to be faster than the propagation and absorption, hence smaller energy values were apparent.

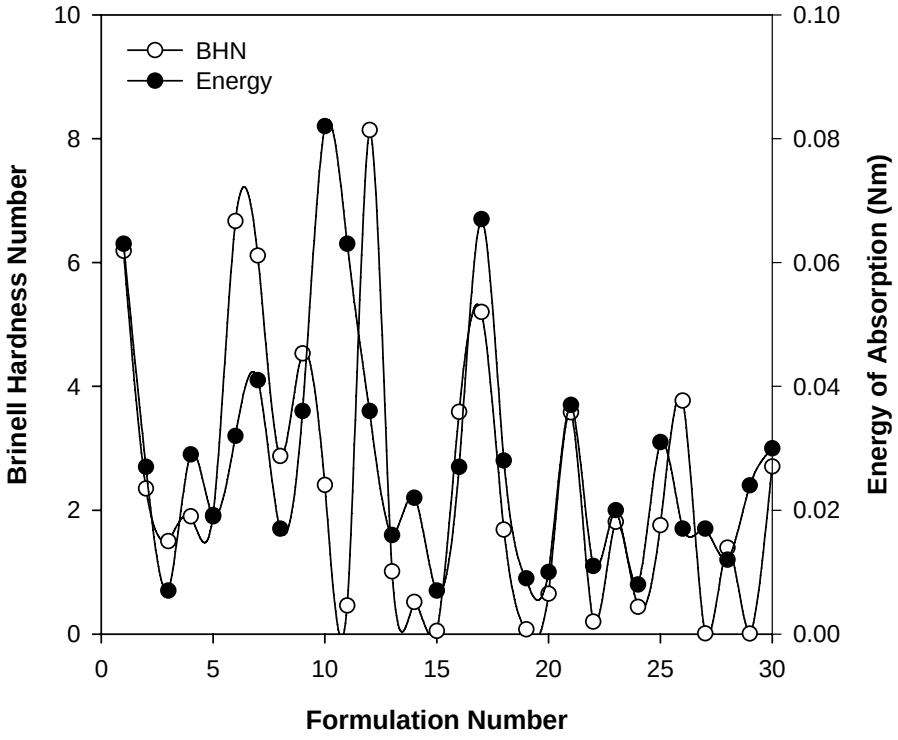


Figure 5.18 Comparison between Brinell Hardness Number and energy of absorption

5.3.6.3 Matrix Yield vs. Matrix Tolerance

A direct relationship between the matrix yield value and tolerance is illustrated in Figure 5.19. Significantly low yield values are due to the initial high energy levels within the wafer, therefore a low force was required to split the wafer. Once the wafer is fissured, the energy is dissipated throughout the matrix which eventually reaches a threshold called matrix tolerance, when the structure collapses.

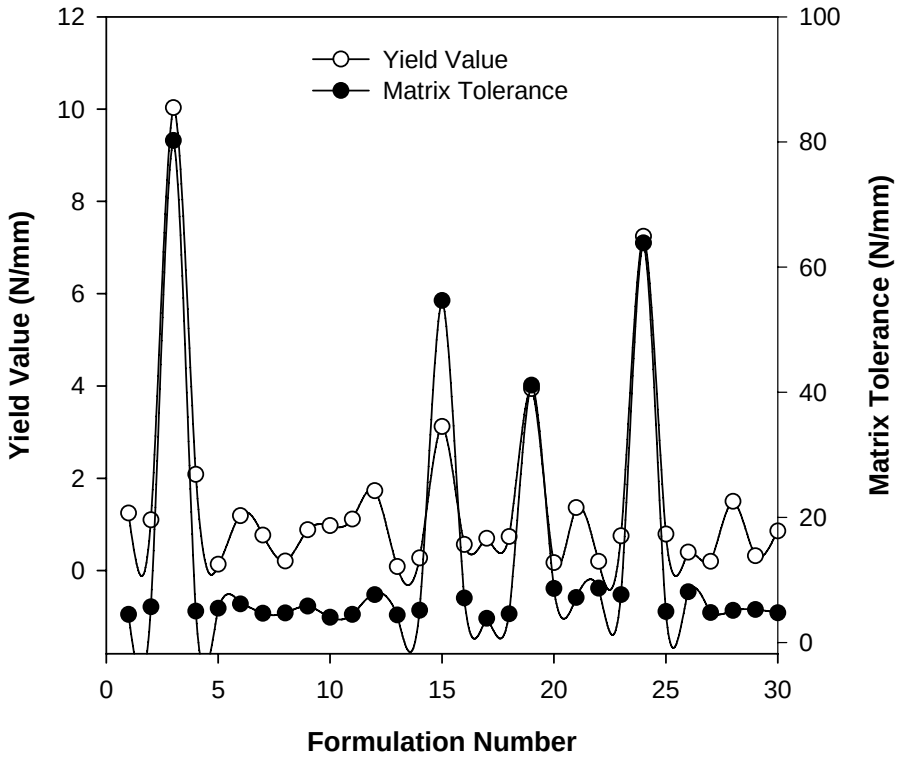


Figure 5.19 Correlation between matrix yield value and tolerance

5.4 Concluding Remarks

During the optimisation of the wafer system, matrix disintegration and friability are of utmost importance. The experiments prove that these responses are manipulated by varying formulation excipients such as HPC and diluent concentrations.

The elucidation of various textural parameters to ascertain the physicomechanical behaviour of the wafers proved to be sensitive based on subtle changes in the formulation as per statistical design. This can be beneficial in terms of being able to optimise these parameters to meet criteria acceptable to the pharmaceutical industry for manufacturing purposes.

Among the various parameters evaluated, the matrix resilience is key to understanding the deformation characteristics. This property will ultimately determine an appropriate packaging method.

The use of statistical design was successful in providing a structured approach to the formulation of the wafer system. The data generated through the measurement of responses was meaningful and allowed for the identification of the formulation factors which were influential in altering the response. This allows for further manipulation of these factors to gain an optimal wafer system.

Chapter 6 Assessment of Glass Transition Temperature

6.1 Introduction

Differential Scanning Calorimetry (DSC) is one method of assessing the glass transition temperature (Tg) of materials. Glass transition involves the transition from a “glassy” solid to a “rubbery” liquid-like state. This change occurs within a temperature range characteristic for each material. The mid-point temperature of such a change is taken as the Tg (Sobral et al., 2001). With respect to polymers, as the temperature of the material drops below the Tg, they behave in a brittle manner, and as the polymer temperature rises above the Tg, it becomes more rubber-like.

It was reported by Simon and co-workers (2003) that the process of lyophilisation affects the Tg of the material. Glass transition temperature is depressed by freeze-drying from dilute solution and by precipitation from dilute solution (Bernazzani et al., 2002, Simon et al., 2003). It is hypothesized that the Tg is an important parameter for storage, stability and quality of dried or frozen products (Sobral et al., 2001). Freeze-dried products are less likely to collapse if stored below Tg (Craig et al., 1999). It was therefore necessary to gain a complete understanding of the behavior of our wafers during the freeze drying-process.

In addition to the quantitative studies used as responses for the CCF, DSC was also carried out on the wafers. The qualitative nature of this experiment did not lend itself for inclusion into the CCF, however the effect of the lyophilisation process on wafer components was important to assess. The Tg of material also

provides an indication of stability, permitting information about storage conditions to be extrapolated.

6.2 Materials and Method

The 30 wafer formulations that were previously manufactured (Chapter 5, section 5.3.2) according to the CCF were analysed.

6.2.1 DSC

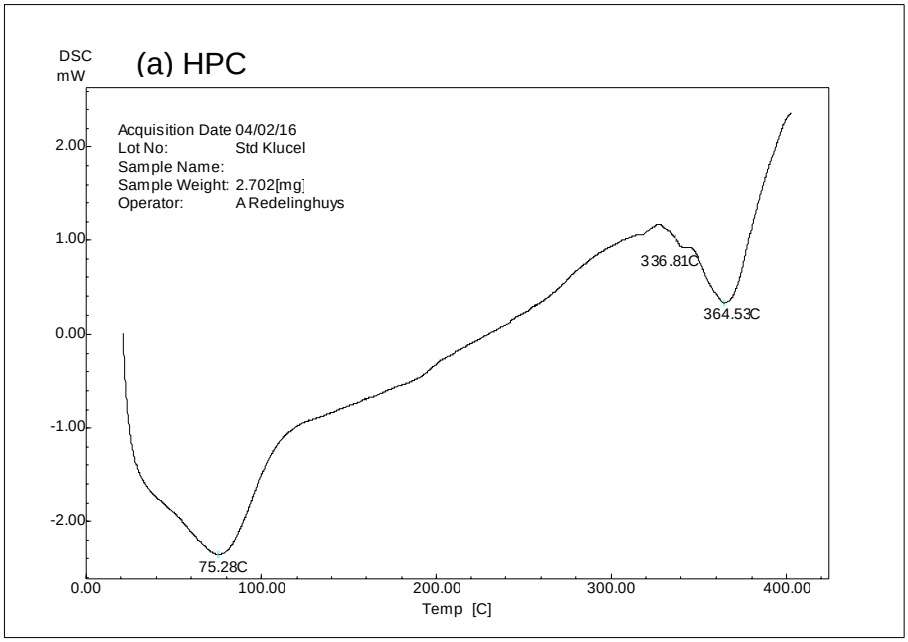
DSC studies were conducted on the 30 wafer formulations as well as individual wafer components. A linear temperature gradient at a rate of 5˚C per minute was performed from 25˚C to 400˚C (Perkin Elmer Pyris-1). Samples of 2-3mg were placed within crimped aluminium pans and subjected to the test.

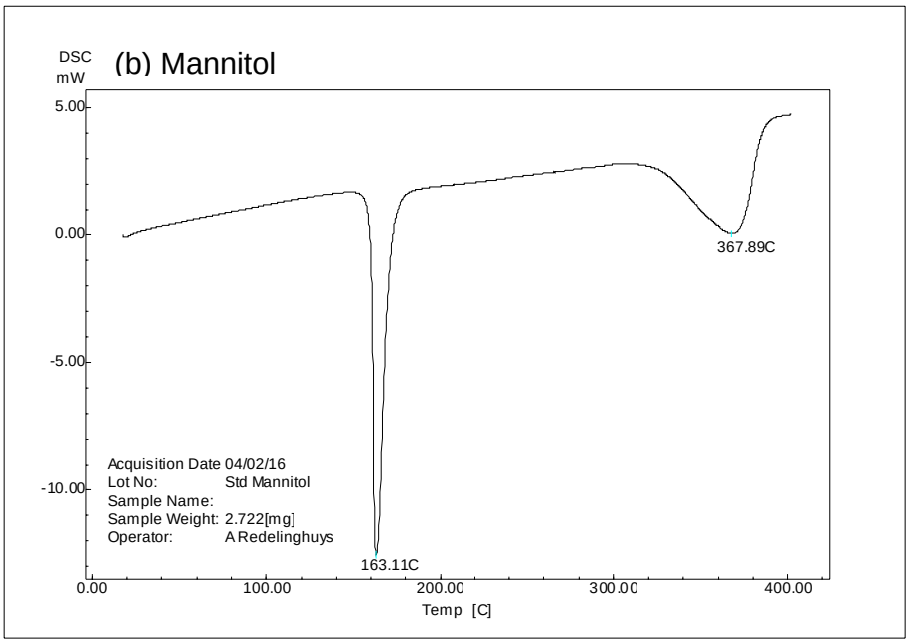
6.3 Results and Discussion

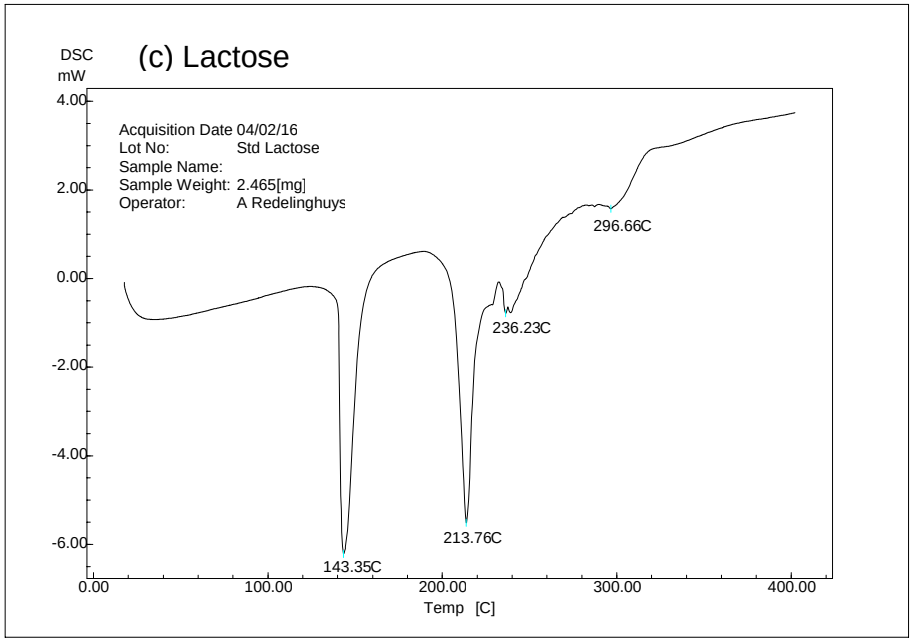
6.3.1 DSC

Typical thermal curves of the pure components of the various wafer systems are shown in Figure 6.1. The Tg of HPC occurred at 75.28˚C. The large endothermic peak at the Tg may be due to vaporisation of low molecular mass components of the polymer (Hatakeyama and Quinn, 1999). Degradation of the polymer structure was observed at 336.81˚C and 364.53˚C. Mannitol exhibited a Tg at 163.11˚C, while also undergoing degradation above 360˚C. The thermogram for lactose showed not only the Tg at 143.35˚C, but also an endothermic peak in the region of 210˚C indicative of the melting of the sugar. Peaks that were seen at 236˚C and 296˚C may be the result of impurities present in the lactose, or

degradation products of the sugar. The experimental Tg of glycine was found at 167.58˚C, and the highly endothermic peak at 262˚C is indicative of its degradation.







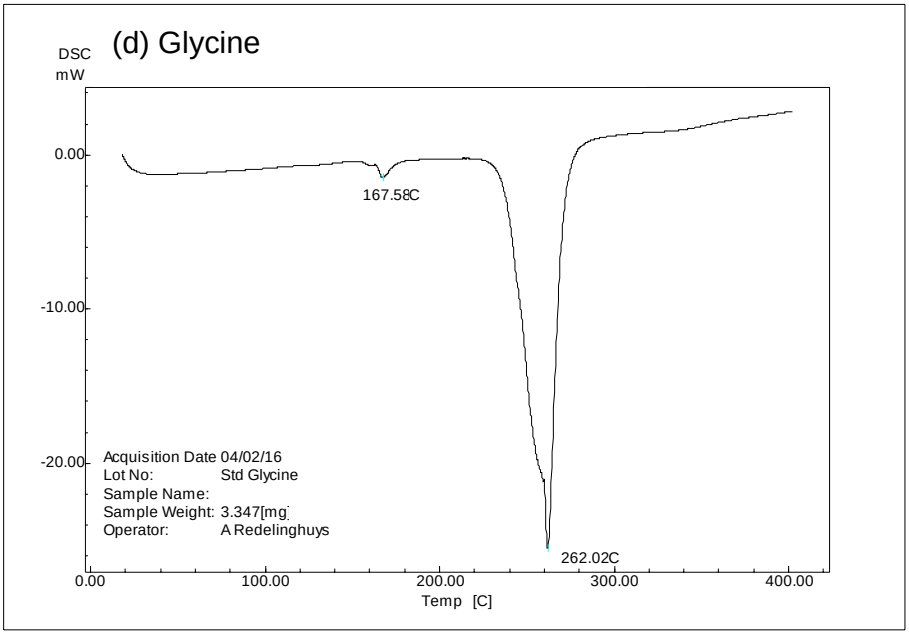


Figure 6.1Differential Scanning Calorimetry profiles for: (a) HPC; (b) Mannitol; (c) Lactose; and (d) Glycine

In general a reduction in the Tg of the HPC was observed for the 30 wafer formulations, with the transition temperature ranging from 59.99˚C to 73.43˚C. A

typical profile for the wafers is illustrated in Figure 6.2. Since freeze-dried products have extremely low moisture content, the depression in Tg cannot be attributed to the presence of a solvent. The lyophilisation process was shown to reduce the entanglement of polymeric chains. It was hypothesised that this may have led to a reduction in the Tg. However a study conducted by Simon and co-workers (2003) indicated that the reduction in entanglement is not responsible for the change in the Tg.

The reduction in the melting point (213˚C to 211˚C) of the wafer was anticipated and can be attributed to the decrease in water content after freeze drying (Sobral et al., 2001). The lower melting point may also be as a result of an interaction between the polymer and excipients.

A decrease in the height of the enthalpy recovery peak for the freeze-dried wafer, as compared to individual excipients may possibly be attributed to the decrease in thermal conductivity, due to the porous nature of the material (Simon et al., 2003).

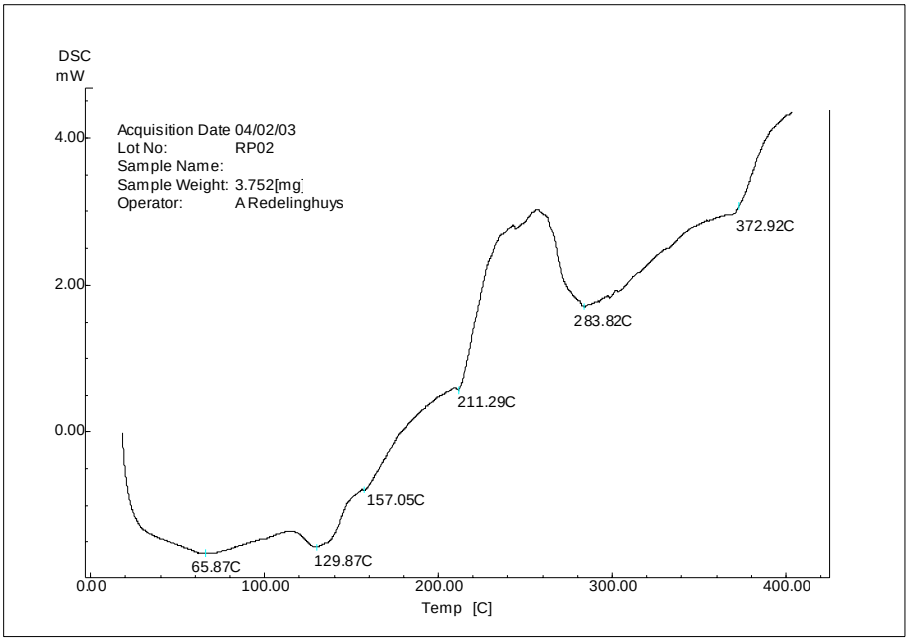


Figure 6.2 Typical Differential Scanning Calorimetry profile of a wafer formulation (F2).

6.4 Concluding Remarks

Although a reduction in the Tg occurred after the freeze drying process, the Tg of the wafers is still above the temperature of storage and processing, and thus is not of concern regarding product stability.

Chapter 7 Statistical Optimisation of Wafer Matrices

7.1 Introduction

Fonner and co-workers (1970) were among the first researchers to introduce the application of mathematical optimisation into the field of pharmaceutics, using the Lagrangian method as a constrained optimisation technique. Among the different methods that are available for solving constrained optimisation problems, the most common are Lagrangian and Simplex methods. The evolution of computer science has enabled the incorporation of the optimisation algorithm into the experimental design software.

A pure trial and error approach to optimisation would be extremely time consuming. Statistical software perform extensive analysis of the observed outputs and their rates of change as the inputs are varied, to guide the selection of new trial values. This study makes use of a generalised reduced gradient algorithm (GRG2) and Artificial Neural Networks (ANN).

The GRG2 is an algorithm that solves nonlinear optimisation problems by implementing a variation of the generalised reduced gradient method. GRG2 uses first partial derivatives of each function with respect to each variable. These are automatically computed by finite difference approximation. Once the initial data has been entered, the algorithm enters a two phase system for problem solving. Phase I objective function is the sum of the extent of constraint range violations including a fraction of the true objective. If a feasible solution is not found, this phase may terminate, indicating that the problem is not feasible.

Phase II begins with the feasible solution found in Phase I. A full optimisation cycle is run and a summary output is provided at the end of Phase II.

Another method of optimisation is the use of ANN. The application of ANN in advanced formulation design and development is being increasingly employed (Ibric et al., 2003; Leanne et al., 2003; Subramanian et al., 2004). In this study, a General Feedforward (GFF) model was selected to predict the rate of matrix disintegration, friability and resilience values using the statistical matrix generated from the CCF. Essentially a GFF is a generalisation of a Multilayer Perceptron (MLP) such that network connections can jump over one or more hidden layers (Figure 7.1). In theory, a MLP can solve any problem that a GFF network can solve. In practice, however, GFF networks solve the problem much more efficiently (Nelson and Illingworth, 1992; Principe et al., 1999). Such a network containing the same number of processing elements, as a standard MLP requires less training, hence increasing the efficiency of neurocomputing.

The objective of the experimentation undertaken in this Chapter was to optimise the properties of the wafer to generate an 'ideal' wafer formulation in terms of disintegration rate, friability and resilience.

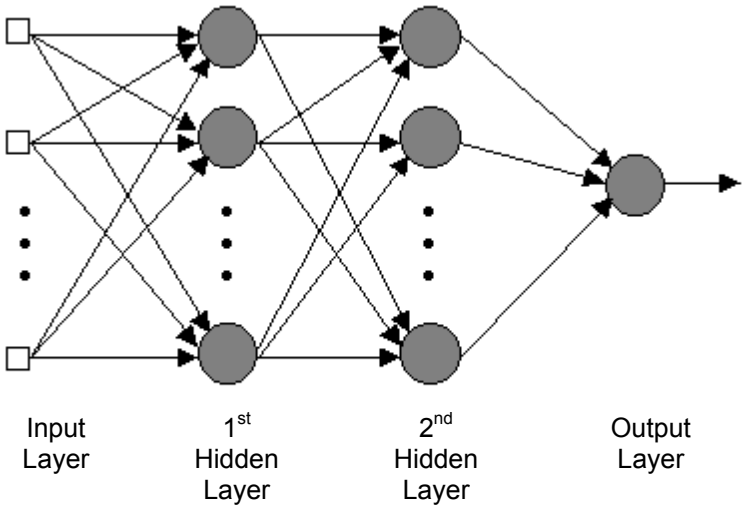


Figure 7.1 Schematic representation of a Multilayer Perceptron

7.2 Materials and Methods

Ingredients used in the production of the wafers were hydroxypropyl cellulose (HPC) (Klucel, EF Pharm, Hercules Inc., Wilmington, North Carolina, USA), lactose (Merck Lab Supplies Pty. Ltd., Midrand, Gauteng, South Africa), mannitol (Merck Lab Supplies Pty. Ltd., Midrand, Gauteng, South Africa), glycine (Aminoacetic Acid, Hopkin and Williams Ltd., Essex, England, UK) and diphenhydramine hydrochloride (Sigma-Aldrich, Steinheim, Germany) as a model drug.

All wafers described in this section were manufactured according to the method described in Chapter 5, section 5.3.2. The matrix disintegration rate, friability and matrix resilience studies were conducted as outlined in Chapter 5, section 5.3.3

7.2.1 GRG2 Optimisation

Based on the fact that the CCF follows a quadratic model, it was most appropriate to employ a GRG2 algorithm, solver technology (Frontline system ms, USA) to obtain the ideal response values for the optimal matrix disintegration rate, matrix resilience and friability using constraints that regulate the three-dimensional configuration of the wafer matrices.

The limitations placed on the independent input variables were as follows:

- 1≤ [HPC] ≤10 %w/v;
- Type of Diluent 0-1, 0= lactose and 1=mannitol;
- 1≤ [Diluent] ≤5 %w/v;
- 0≤ [Glycine] ≤0.6 %w/v; and
- 1≤ Fill Volume ≤2 mL.

Optimal responses for the desired training of data are depicted in Table 7.1. The rate of matrix disintegration was calculated such that the wafer would ideally disintegrate completely in 30 seconds (100%/30s = 3.33%/s). The parameters for friability were determined according to the USP 23 standard for conventional tablets. The matrix resilience was set to achieve the maximum feasible value.

Table 7.1 Desired values for the responses to develop an optimised formulation

Response	***Minimum***	***Maximum***	***Optimal***
Rate of Matrix Disintegration (%/s)	1	6	3.33
Friability (% loss)	0	0.8	0.1
Matrix Resilience (%)	95	100	Maximize

7.2.2 ANN Optimisation

For the hidden and output layers, a genetic algorithm with the SigmoidAxon transfer function and ConjugateGradient learning rule was employed respectively. A maximum of 10,000 epochs were run on NeuroSolutions Version 4.32 (NeuroDimension Inc., Gainsville, Florida, USA) to ensure optimal training of data.

7.2.3 Effect of Active Ingredient on Wafer Properties

The optimum formulation predicted by NeuroSolutions was prepared and 25mg of model drug diphenhydramine HCl (F_{nd}) was included into the wafer. Responses, namely, rate of matrix disintegration, friability and matrix resilience were measured and compared to those derived from the same formulation without drug (F_n).

7.3 Results and Discussion

7.3.1 GRG2 Optimisation

Table 7.2 below shows the formulation variables required to fulfil the desired values outlined in Table 7.1, based on the GRG2 algorithm. The desirability is an indication as to how achievable the desired response is, 1 being the maximum.

Results generated from the analysis of these wafers are shown in Table 7.3. The optimised formulations F_d and F_f were close to their target values. F_r on the other hand did not meet its target predicted value of 69.2% resilience. It is seen from the data in Table 7.3 that high disintegration values are associated with high friability and vice versa. This is undesirable.

Table 7.2 Generated values independent variables based on a selected range

	[HPC] (%w/v)	**Diluent Type*	*[Diluent] (%w/v)*	*[Glycine] (%w/v)*	*Fill Volume (mL)*	*Desirability Function*
High	10.0	1.00	5.00	0.60	2.00	-
Low	1.00	0	1.00	0	1.00	-
Matrix Disintegration Rate (F_d)	10.00	0.91	4.98	0.10	2.00	1.00
Friability (F_f)	1.19	0	4.38	0.60	2.00	1.00
Matrix Resilience (F_r)	1.00	0	5.00	0.00	1.00	1.00

**Diluent Type: 0=lactose, 1=mannitol and ratios indicate combinations*

Table 7.3 Responses measured for formulations optimised using GRG2 ± SD (N=3)

	F_d	F_f	F_r
Rate of Matrix Disintegration (%/s)	3.89 ± 0.19	0.91 ± 0.05	4.76 ± 0.19
Friability (% loss)	29.12 ± 0.37	0.45 ± 0.08	9.42 ± 1.96
Matrix Resilience (%)	2.16 ± 0.32	3.70 ± 0.15	3.07 ± 0.44

7.3.2 ANN Optimisation

The gradual levelling of the mean square error (MSE) with standard deviation boundaries for the 10 runs, indicating the sequential improvement of model predictability is illustrated in Figure 7.2. Table 7.4 reflects the average of the MSE values for all the training runs, the best network run out of 10,000 epochs, and the overall efficiency of the GFF model in the training process. Overall, it is evident that the training model employed was highly efficient. The parameters depicted in Table 7.4 are standard statistical indicators used by scientists involved in neuro-computing to quantitate the accuracy of model prediction and to subsequently select the optimal model (e.g. MLP vs. GFF algorithm). Results shown in Table 7.4 are highly satisfactory with a 100% fit for the variables HPC

concentration, diluent concentration and fill volume, and correlation coefficients of the variables are ≈0.9.

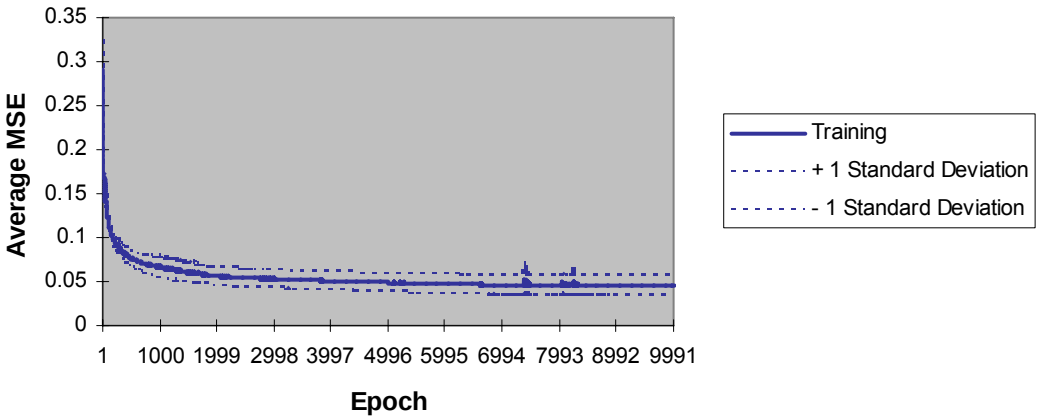


Figure 7.2 Average MSE with SD boundaries for 10,000 epochs

The formulation (F_n) determined by the ANN to satisfy the desired properties outlined in Table 7.1 was:

- [HPC] - 10.49 %w/v;
- Diluent composition – 0.88;
- [Diluent] – 5.22 %w/v;
- [Glycine] – 0.006 %w/v; and
- Fill volume – 0.94 mL.

To test the validity of the ANN an analysis of the responses illustrated that the values all fell within the desired range. The matrix disintegration rate was 4.95 %/s, friability 0 % loss and matrix resilience 11.81%. These results illustrate that the ANN was highly efficient in determining an optimised formulation.

Table 7.4 Neural Network indicators characterising the efficiency of data training

Averages of the Minimum Training Errors	***Training Mimimum***	***Training Standard Deviation***
Average of Minimum MSEs	0.04	0.01
Average of Final MSEs	0.05	0.01
Optimal Network Run Obtained From Data Training	***For Desired Responses***	
Run Number	9	
Epoch Number	10000	
Minimum MSE	0.03	
Final MSE	0.03	

Performance of Neural Network by Testing of Training Data					
Performance	*[HPC] (%w/v)*	*Diluent Composition*	*[Diluent] (%w/v)*	*[Glycine] (%w/v)*	*Fill Volume (mL)*
MSE	0.38	0.03	0.41	0.04	0.03
NMSE	0.03	0.20	0.19	0.73	0.15
MAE	0.47	0.09	0.39	0.11	0.11
Min Abs Error	0.001	0.003	0.017	0.001	0.004
Max Abs Error	1.62	0.15	2.22	0.29	0.43
Correlation coefficient	0.99	0.98	0.91	0.89	0.93
Percent Correct	100	No Convergence	100	No Convergence	100

7.3.3 Effect of Active Ingredient on Wafer Properties

It was more difficult to eject the wafers containing drug (F_{nd}) from the mould. Hence, it may be necessary to add more lubricant to this system. The results for F_{nd} are as follows:

- Matrix Disintegration Rate – 4.95%/s;
- Friability – 0 % loss; and
- Resilience – 3.34%.

The addition of drug to this system did not affect the disintegration rate, or the friability. The matrix resilience was substantially decreased from 11.81% to 3.35% with the addition of drug. This may be due to the fact that an increase in solid powder particles into the system will result in a decrease in the matrix's ability to form pores and hence a decrease in resilience.

7.4 Concluding Remarks

The statistical approach for formulation optimisation has proved to be useful when several variables require simultaneous evaluation. The mathematical model generated by regression analysis was used to predict and optimise the formulation variables, while the ANN provides an optimised solution. The prediction from the model and the experimental results in this study show a high degree of correlation, indicating the rigidity of the design employed.

Chapter 8 Modification of Wafer Technology to Design a Prolonged Release Oramucosal Device: Preliminary Studies

8.1 Introduction

In this study, a brief investigation was undertaken to modify the wafer technology developed thus far in an attempt to prolong the buccal delivery of the bioactive agent diphenhydramine hydrochloride. This research was conducted as part of a collaboration between The University of the Witwatersrand (South Africa) and The Medical University of Gdansk (Poland).

Advantages of prolonged release systems are well known. Briefly these include:

- Less frequent dosing;
- Reduced peak to trough fluctuations of drug concentration in the blood;
- Decrease in side effects; and consequently
- An increase in patient compliance.

Desirable attributes for prolonged buccal delivery will be a high drug loading capacity, ability to regulate drug release, ability to adhere to the buccal mucosa and the eventual erosion of the system that will avoid the need to remove the device after the dose has been delivered (Martin et al., 2002). These requirements can be met by using crosslinked hydrogels, which typically do not dissolve on exposure to the medium but rather only absorb saliva. As saliva penetrates the hydrogel matrix, chain relaxation occurs and drug is released through the spaces and channels within the network as well as through the dissolution/ disintegration/ disentanglement of the matrix (Shojaei, 1998). Pseudo-hydrogels on the other hand swell, and component molecules leach from

the surface of the matrix. In this case drug release occurs through the spaces or channels within the network as well as through dissolution/disintegration of the matrix.

To obtain an extended release buccal system, it is necessary that the system remains in contact with the mucosa to facilitate prolonged release. To fulfil this requirement, it is compulsory to incorporate a mucoadhesive polymer.

According to Lee and co-workers (2000), mucoadhesion may occur as a result of the following forces:

- Covalent bonding, e.g. cyanoacrylate;
- Hydrogen bonding, e.g. carbopol®, polycarbophil and acrylates; and
- Electrostatic interaction e.g. chitosan.

Interactions between chemical entities of the polymer and glycoproteins within the mucus or tissue are also responsible for adhesion. Polymer chains with high molecular weights and a large number of polar groups tend to develop more intensive mucoadhesive bonds. Hydrophilic polymers have stronger bioadhesive forces, compared to the hydrophobic components, due to their high swelling capacity (Choi and Kim, 2000).

8.2 Materials and Methods

Carmellose (Sol Sodowa, Poland), diphenhydramine hydrochloride (Pliva, Krakow, Poland), gelatine (I.G.G., Eberbach, Germany), pectin (Classic Cu 701, Herbstreith and Fox, Pforzheim, Germany), and zinc sulphate (Gliwice, Poland) were used as received.

8.2.1 Preparation of Prolonged Release Discs for Incorporation into Mucoadhesive Polymer

Crosslinking technology was used to decrease the solubility and hence retard the release of active ingredients from a polymeric disc. Zinc ions were used to crosslink pectin. This resulted in a three-dimensional network of pectin strands held together with ionic interactions. This is commonly described as the egg-box model (Grant el al., 1973). A diphenhydramine HCl concentration of 40mg/mL was achieved by mixing the drug in a 2%w/v solution of pectin in deionised water. Blisters (15mm diameter), were filled with 0.5mL of the suspension, frozen and then crosslinked. The polymer was frozen to ensure that the disc shape was maintained. Three methods of crosslinking were investigated:

- Method I: 0.5mL of 2.5%w/v zinc sulphate ($ZnSO_4$) solution was placed on the surface of the disc, and allowed to cure for 1 hour. Thereafter, the excess $ZnSO_4$ solution was removed by decanting. The discs lyophilised. Table 8.1 depicts the conditions of freeze dryer (Alpha 2-4, Christ, Osterode am Harz, Germany);
- Method II: The $ZnSO_4$ solution was applied to the surface of the frozen discs in the form of a spray and refrozen. Once frozen, the discs were turned and the procedure was repeated on the other side. These discs were frozen and then lyophilised; and

- Method III: Discs were frozen in liquid nitrogen, then transferred to blisters with a diameter of 20mm. 0.5mL of the $ZnSO_4$ solution was added to the discs and allowed to cure for 24 hours. Thereafter the $ZnSO_4$ salt solution was removed, discs were then lyophilised.

Table 8.1 Conditions and parameters of lyophilisation

Step	***Shelf temperature (ºC)***	***Time (hours)***	***Vacuum (mbar)***
1. Freezing	- 40	1	-
2. Primary drying	- 40	2	0.08
	-20	5	
	0	14	
	+20	4	
3. Secondary drying	+35	2	0.08

8.2.2 Incorporation of Crosslinked Granules into Mucoadhesive Wafer System

Discs were placed in wells containing 0.5mL of the mucoadhesive agent composed of 2%w/v pectin, 2%w/v carmellose sodium and 2%w/v gelatine (PCG), and frozen. A further 1mL of PCG was added and subjected to lyophilisation.

8.2.3 Preparation of Prolonged Release Granules for Incorporation into Mucoadhesive Polymer

During preliminary experimentation, an optimal composition of granules was determined to be 10g diphenhydramine, 10g pectin, 33.3g water, and 33.3g of 20% w/v $ZnSO_4$ solution.

Diphenhydramine HCl and pectin were manually mixed together with a mortar and pestle. Water was added in a dropwise manner to allow for the polymer to swell. The $ZnSO_4$ was added, causing the mixture to form a firm gel-like mass as a result of the crosslinks formed. The gel mass was extruded through stainless steel meshes of pore size: 0.1mm, 0.2mm and 1.0mm, forming granules that were dried at 37°C in an oven (Memmert, Bavaria, Germany).

To determine the drug entrapment efficiency of the granules, accurately weighed samples were dissolved in 10mL of a 2%w/v sodium citrate solution. Samples (N=3-5) were diluted with water and analysed using UV spectroscopy at a wavelength of 310nm.

8.2.4 Release Profile of Prolonged Release Discs and Granules

Discs or granules were placed in a beaker with 10mL of deionised water. The beaker was sealed using parafilm, and placed in a water bath set on very slow movement (110cpm) at a temperature of 37˚C. Samples were drawn after 30 minutes, 60 minutes and hourly thereafter.

8.2.5 Incorporation of Crosslinked Granules into Mucoadhesive Wafer System

Granules, were suspended in PCG, frozen and then lyophilised. Granules of diameter 0.2mm and 1.0mm were selected based on their release profiles for the production of wafers and will be termed W_2 and W_3 respectively from here on. For comparative purposes, an equivalent amount of diphenhydramine HCl was suspended in PCG, this suspension was used to prepare lyophilised wafers

termed W_1. The theoretical amount of drug present in each of the wafers was 20mg. To simulate the mechanical pressure and wetting of the system that occurs when the patient applies the wafer to the buccal region, wafers W_1 and W_3 were wet and a 200g weight was applied to them (W_{1p} and W_{3p} respectively). Release profiles were characterised as described in this Chapter, section 8.3.4 The portion of the system remaining intact after 24 hours was dissolved in a 2%w/v sodium citrate solution.

8.3 Results and Discussion

8.3.1 Prolonged Release Discs for Incorporation into Mucoadhesive Polymer

Discs prepared by the methods described in this Chapter, section 8.3.1 were easily removed from the blisters. Although all the discs had a porous surface, the discs prepared by methods I and II maintained the shape of the blister while those prepared by method III appeared flatter and harder as observed in Figure 8.1(c).

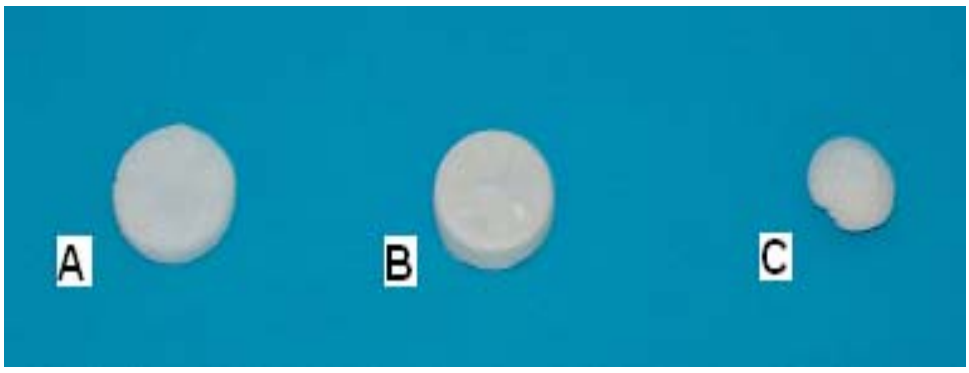


Figure 8.1 Lyophilised discs of pectin containing diphenhydramine HCl crosslinked using $ZnSO_4$ solution using method: (a) moistening, (b) spraying and (c) immersion

8.3.2 Drug Release Profiles

The release of diphenhydramine HCl from discs prepared using methods I and II was rapid (Figure 8.2), samples were withdrawn until no further changes were observed in the drug concentration liberated. The rapid liberation of the drug was due to incomplete crosslinking as a result of insufficient curing time. A cross-section of the discs showed that only the surface of the discs had been crosslinked. Drug release from the discs manufactured according to method III was satisfactory (Figure 8.2), however due to the change in shape, they were discarded from further studies.

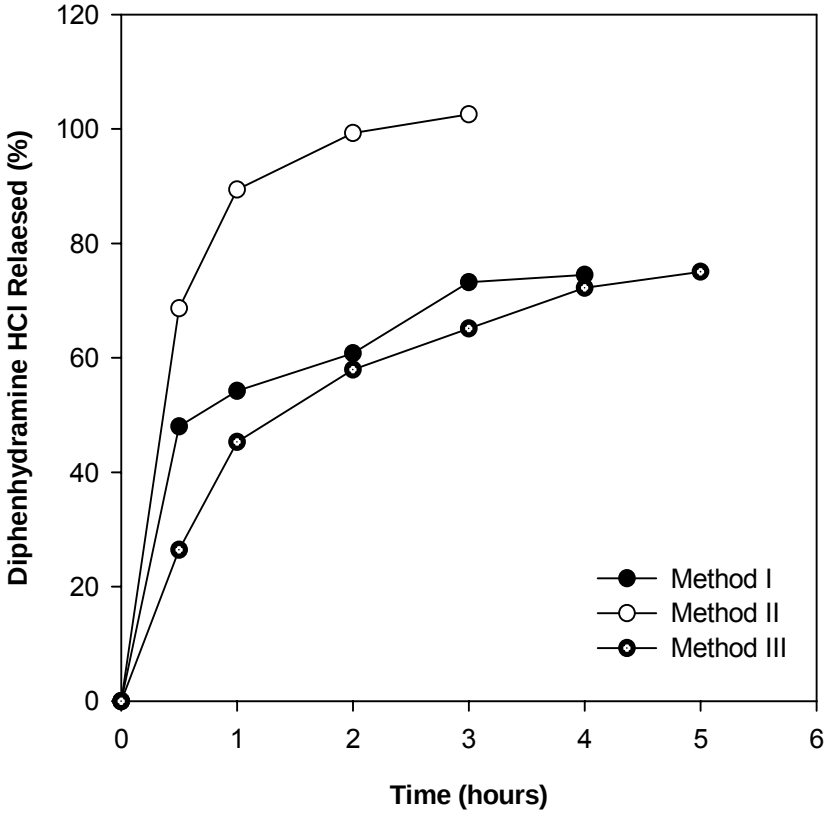


Figure 8.2 Release profiles of diphenhydramine HCl discs prepared by method I, II and III

A cross-section of the wafers was taken using a scalpel, showing that the discs did not change location during freezing or lyophilisation, thus remaining the

centre layer of the wafer as positioned at the onset as the middle layer of the wafer. Due to the presence of Zn^{2+} ions on the surface of the discs, crosslinks occurred with pectin present in the PCG mixture allowing firm attachment of the discs to the mucoadhesive.

8.3.3 Drug Release Profiles of Granules

It was found that the drug entrapment of the granules was ≈90% of the theoretical value. During the characterisation of the release profile of the granules, it was noted that after 24 hours, granules did not dissolve completely, therefore the remaining granules were dissolved in a 2%w/v sodium citrate solution to determine the amount of drug remaining. Similar release profiles (Figure 8.3) were observed for the 0.2mm and 1.0mm granules, with ≈50% of the active released at 3 hours and ≈70% after 5 hours. The smallest granules released diphenhydramine HCl very rapidly liberating close to 50% of its load within the first hour (Figure 8.3).

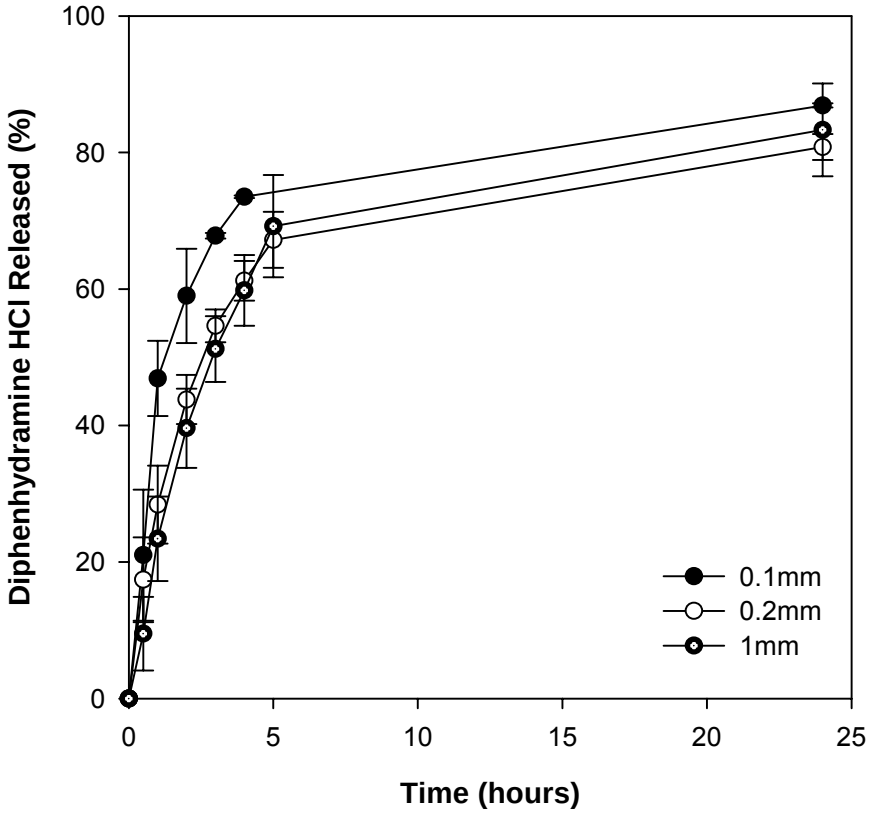


Figure 8.3 Drug release profiles of crosslinked granules varying in size (N =3-5)

8.3.4 Incorporation of Crosslinked Granules into the Mucoadhesive Wafer System

According to a study conducted by Romanowski (2004), a mixture composed of 2%w/v pectin, 2%w/v carmellose sodium and 2%w/v gelatine provided satisfactory mucoadhesion. This polymer mixture was used as the mucoadhesive base throughout this study.

The mucoadhesive polymer PCG, would ensure adherence of the system to the buccal mucosa, while the crosslinked granules provide prolonged release of the drug. Based on the successful slow release profiles of the 0.2mm and 1.0mm granules (Figure 8.3), these were selected for introduction into the wafer system.

The wafers that were produced had the following properties, as determined by visual inspection:

- Good mechanical strength;
- Porous; and
- Ease of removal from moulds.

The W_2 and W_3 systems had granules suspended throughout the matrix which appeared denser on the surface of the wafers as compared to those within the system. This was observed from cross-sections of the samples.

As a result of the pressure applied to formulations W_{1p} and W_{3p}, the wafer decreased to half of its original height, thus reducing the diffusion path for the drug. As a result of this phenomenon, it was anticipated that the drug would be liberated faster from these samples.

8.3.4.1 Drug Release Profiles

The inclusion of the granules to the wafer system retarded the release of diphenhydramine HCl even further. In the pure granule form, the 0.2mm and 1.0mm granules released ≈60% of the active (Figure 8.3), while the wafer system W_2 and W_3 containing the granules had only liberated 48% and 24% of drug respectively (Figure 8.4). Disintegration of the W_2 system was observed. This could be responsible for the similarity of this release profile with that of W_1 wafers. These wafers released diphenhydramine HCl the fastest among the unpressed wafers, while the pressed form (W_{1p}) provided the quickest release of the drug as compared to pressed and unpressed wafers. In contrast to this, the release profile of the W_{3p} wafers was similar to the W_3 wafers, indicating that the crosslinked granules within the system do control drug release.

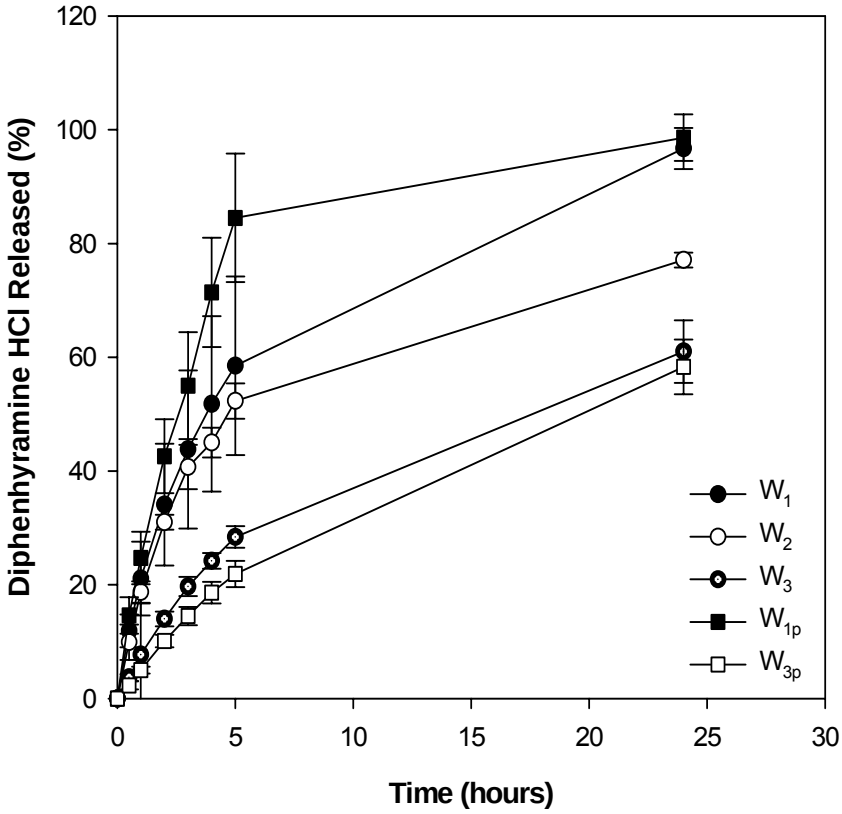


Figure 8.4 Percentage release of various diphenhydramine wafers intended for prolonged release (Wafers containing: only drug – W_1, granules of 0.2mm diameter – W_2, 1.0mm diameter granules – W_3. The subscript p indicates that pressure has been applied to these wafers)

8.4 Concluding Remarks

The preliminary studies into the development of these prolonged release wafers seem to be promising, indicating the feasibility of producing such a system. However refinement of the system is necessary. Further studies in the formulation of smaller granules, as well as modification of the crosslinking techniques will be essential. Combining crosslinking as well as dispersing pure drug within the matrix may be an approach to achieve the desired release profiles.

Chapter 9 Conclusions and Recommendations

9.1 Conclusions

The lyophilised wafer developed throughout this research is an effective and versatile drug delivery system for oramucosal application. This has been established from the extensive physicochemical and physicomechanical profiling conducted.

Through a screening and selection of polymers, HPC had the lowest gelation characteristics and was therefore suitable for the development of the wafer system. Suitable excipient and polymer combinations were established which allowed for the development of rapidly disintegrating and prolonged release wafer systems. The wafer system containing HPC, lactose, mannitol and glycine had the ability to disintegrate within 30 seconds. The modified wafer system, consisting of pectin crosslinked with zinc ions serving as the drug reservoir, and mucoadhesive polymer combination of pectin, carmellose and gelatine, provided effective release of model drug diphenhydramine hydrochloride over approximately six hours.

A successful, reproducible, manufacturing technique was established by the optimisation of the lyophilisation cycle, employing mineral oil as a lubricant and polystyrene moulds providing wafers of suitable characteristics.

Characteristics that were critical to the mechanistic functioning of the wafer, such as rate of matrix disintegration, rate of simulated saliva influx and friability, were extensively elucidated to determine the effects of the formulation variables using

ANOVA technology. A low concentration of polymer was associated with a high disintegration rate, friability and influx of simulated saliva. As predicted, an increase in the amount of diluent present increased both the disintegration rate and friability.

The ANOVA method was used to present a comprehensive profile of the physicomechanical properties such as matrix yield value, matrix tolerance, matrix absorption energy, matrix resilience and Brinell hardness number. A firm understanding of the effects of formulation variables on the responses formed the corner stone of the optimisation process.

Although the DSC did not form a component of the optimisation process, the information provided was integral in the determination of the effect of lyophilisation on the native ingredients. Through this analytical process, it was accepted that lyophilisation did not significantly alter the T_g.

The aim of this study, to consider formulation variables in the statistical optimisation of the lyophilised wafer system was achieved. The Design of Experiments and Artificial Neural Networks proved to be highly effective tools for the optimisation process, ultimately producing formulation characteristics within the desired range, disintegration 1-6 %/s, friability 0-0.8% weight loss and maximum resilience.

9.2 Future Prospects and Challenges

Whilst this study was successful with the use of diphenhydramine hydrochloride as the model drug, the compatibility of the wafer matrix with other drug classes would necessitate further studies.

Most commercially available rapidly dissolving open matrix systems facilitate rapid disintegration, most of the drug is absorbed via the GIT. The extent of penetration of drug through the buccal mucosa of the system developed in this study may be evaluated using *in situ* permeation studies in the pig model. The efficiency of drug absorption through the membrane may be increased with the inclusion of permeation enhancers in the formulation.

Thus far this study has supplied extensive data on the *in vitro* characterisation of the matrices. As *in vivo* studies provide valuable information relating to the disintegration and mechanical properties of the matrices, it may not completely mimic *in vitro* studies completely. *In vivo* studies should be performed, initially in animal models, followed by those in healthy human volunteers, to obtain the pharmacokinetic parameters. It will also be useful to develop an *in vitro-in vivo* correlation (IVIVC).

In addition, due to the well known fragility and hygroscopicity of lyophilised products, an appropriate packaging system for the wafers need to be developed to ensure that the dosage form reaches the patient and is administered intact.

The modification of this technology to provide a prolonged release mucoadhesive system seems promising. It is envisaged that this system will be applicable to many drugs requiring the extended release of bioactive material.

Therefore, the lyophilised wafer matrices developed in this study are highly effective in the rapid delivery of drugs, using the oral route as a site of administration. The manufacturing process is simple and reproducible. A number of unique opportunities are presented for the formulation of a controlled release drug delivery system.

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