NOVEL FORMULATION STRATEGIES FOR THE FABRICATION OF LYOPHILISED ORALLY DISINTEGRATING TABLETS

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Summary

Orally disintegrating Tablets (ODTs), also known as fast-disintegrating, fast-melt or fast-dissolving tablets, are a relatively novel dosage technology that involves the rapid disintegration or dissolution of the dosage form into a solution or suspension in the mouth without the need for water. The solution containing the active ingredients is swallowed, and the active ingredients are then absorbed through the gastrointestinal epithelium to reach the target and produce the desired effect. Formulation of ODTs was originally developed to address swallowing difficulties of conventional solid oral dosage forms (tablets and capsules) experienced by wide range of patient population, especially children and elderly.

The current work investigates the formulation and development of ODTs prepared by freeze drying. Initial studies focused on formulation parameters that influence the manufacturing process and performance of lyophilised tablets based on excipients used in commercial products (gelatin and saccharides). The second phase of the work was followed up by comprehensive studies to address the essential need to create saccharide free ODTs using naturally accruing amino acids individually or in combinations. Furthermore, a factorial design study was carried out to investigate the feasibility of delivering multiparticulate systems of challenging drugs using a novel formulation that exploited the electrostatic associative interaction between gelatin and carrageenan. Finally, studies aimed to replace gelatin with ethically and morally accepted components to the end users were performed and the selected binder was used in factorial design studies to investigate and optimise ODT formulations that incorporated drugs with varies physicochemical properties.

Our results show that formulation of elegant lyophilised ODTs with instant disintegration and adequate mechanical strength requires carful optimisation of gelatin concentration and bloom strength in addition to saccharide type and concentration. Successful formulation of saccharides free lyophilised ODTs requires amino acids that crystallise in the frozen state or display relatively high Tg', interact and integrate completely with the binder and, also, display short wetting time with the disintegrating medium.
The use of an optimised mixture of gelatin, carrageenan and alanine was able to create viscous solutions to suspend multiparticulate systems and at the same time provide tablets with short disintegration times and adequate mechanical properties. On the other hand, gum arabic showed an outstanding potential for use as a binder in the formulation of lyophilised ODTs. Compared to gelatin formulations, the use of gum arabic simplified the formulation stages, shortened the freeze drying cycles and produced tablets with superior performance in terms of the disintegration time and mechanical strength. Furthermore, formulation of lyophilised ODTs based on gum arabic showed capability to deliver diverse range of drugs with advantages over commercial products.

Key word: Dysphasia, gelatin, amino acids, gum arabic, multiparticulate system

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ABBREVIATIONS

ANOVA	Analysis of variance
˚C	Degree Celsius
Cr	Crystallisation
DF	degrees of freedom
DoE	Design of experiment
DSC	Differential scanning calorimetry
F	Fischer's ratio
FDA	Food and Drug Administration
g	Gram
g/mol	Gram/mole
GIT	Gastro Intestinal Tract
HCl	Hydrochloric Acid
HPLC	High Performance Liquid Chromatography
hr	Hour
IVIVC	In vitro-in vivo correlation
J	Joule
Kg	Kilogram
kV	Kilovolt
L	Litre
LODT	Lyophilised orally disintegrating tablet
log	Logarithm
mA	Milliampere
min	Minutes
mg	Milligram
µg	Microgram

mL	Millilitre
µL	Microlitre
mm	Millimetre
mTorr	Millitorr
MS	mean of square
MW	Molecular Weight
N	Newton
NSAIDS	Non-steroidal anti-inflammatory drugs
ODT	Orally disintegrating tables
p	Probability
P-gp	p-glycoprotein
QbC	Quality by control
R^2	Regression coefficient
s	Second
SD	Standard deviation
SS	Sum of squares
Tg	Glass transition temperature
Tg’	Tg of maximally freeze concentrate sample
TGA	Thermogravimetric analysis
Tm	Melting Temperature
USP	United State Pharmacopoeia
UV	Ultraviolet
v/v	Volume/volume
w/v	Weight/volume
w/w	Weight/ Weight
ΔHm	Change in Heat Flow of Melting

THESIS PUBLICATIONS

Peer-reviewed Journal Articles:

AlHusban, F., ElShaer, A., Jones, R., **Mohammed, A.** (2010). Recent patents and trends in formulation of orally disintegrating tablet. *Journal of Recent patents in formulation and drug delivery*. (Inpress).

AlHusban, F., Perrie, Y., **Mohammed, A.** (2010) Formulation and characterisation of lyophilised rapid disintegrating tablets using amino acids as matrix forming agents. *European Journal of Pharmaceutics and Biopharmaceutics,* 75, 2, 254-262.

AlHusban, F., ElShaer, A., Kansara, J., Smith, A., Grover, L., Perrie, Y., Mohammed, A. (2010) Investigation of formulation and process of lyophilised orally disintegrating tablet (ODT) using novel amino acid combination. *Pharmaceutics,* 2 (1), 1-17.

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Alhusban FA, Perrie Y, Mohammed A - A novel application for amino acids as matrix forming agents in lyophilised rapid disintegrating tablets. British Pharmaceutical Conference: September 2009, Manchester, UK.

Alhusban FA, Perrie Y, Mohammed A - Novel-Saccharide Free Rapid Disintegrating Tablets Containing Amino Acids. 36th Annual Meeting of the Controlled Release Society, July 2009, Copenhagen, Denmark.

Alhusban FA, Perrie Y, Mohammed A **-** Amino acids as novel matrix forming agents in lyophilised rapid disintegrating tablets. UKICRS Symposium April 2009, London, UK.

Alhusban FA, Perrie Y, Mohammed A - Influence of gelatin bloom strength and concentration on the hardness and disintegration time of rapidly disintegrating solid formulations. *Journal of Pharmacy and Pharmacology,* 2008; 60(Suppl. 1): A-95.

Chapter One: Introduction

Papers relating to this chapter

AlHusban, F., ElShaer, A., Jones, R., **Mohammed, A.** (2010). Recent patents and trends in formulation of orally disintegrating tablet. *Journal of Recent patents in formulation and drug delivery*. (Accepted).

Introduction

1.1. Project scope and significance

The gastro intestinal tract (GIT) is a highly specialized system in the body that is involved in secretion, digestion and absorption. All food nutrients required by the body must be ingested orally, processed by the GIT and absorbed into the bloodstream. Also, the GIT is responsible for preventing noxious materials from causing local irritation or systemic toxicity. Therefore the unique GIT physiology creates many barriers that face the systemic delivery of drug molecules. The major barriers include the presence of degradative enzymes and extreme pH conditions throughout the GIT, absorption efflux mechanisms (such as P-glycoprotein), first pass metabolism (hepatic), in addition to a number of hydrophilic/ lipophilic barriers. To address these barriers, while improving patient compliance, researchers have developed oral dosage forms (delivery systems) by combining drugs (active ingredients) with a variety of inert substances (excipients). Suitable oral delivery systems can be designed, depending on the physicochemical and pharmacokinetics characteristics of drugs, to provide control and accuracy of dosing, elegancy and stability in shelf and GIT, and to improve the dissolution and absorption profile of the drugs. Traditionally, oral delivery systems refer to tablets, capsules, solutions and suspensions that administered orally, swallowed and then transiting the gastrointestinal tract (GIT) to achieve the required drug release and absorption.

Despite phenomenal advances in the injectable, inhalable, transdermal, nasal, and other delivery systems, the oral route is still considered the most preferable for both patients and industry. Being natural, noninvasive, and safe method of drug delivery, oral delivery is, always, associated with high degree of patient compliance (Li and Robinson, 1987; Sastry et al., 1997; Fasano, 1998). On the other hand, oral delivery systems are able to accommodate various physicochemical properties of drugs, do not require strict sterile conditions and, therefore, less expensive to manufacture. Thus, even small improvements in oral drug delivery technology can make significant difference in enhancing patient compliance and drug delivery fields in general. Over the past decades, several novel technologies for oral delivery have been developed, examples include; oral rapid disintegrating (dissolving) tablets (segar, 1998), mucoadhesive buccal dosage forms (Poertero et al., 2006), site specific drug delivery (Liu et al 2003) and novel controlled release dosage forms (Dashevsky et al., 2004; Liu and Xu, 2007).

The launch of new technologies has expanded the market of oral drug delivery product significantly to generate $35 billion sales in 2004 with an expected annual growth of 10%. The main driver of this market growth is the rapid dissolve dosage forms and OTC market segment (Ghosh and Pfister, 2005). The worldwide market of rapid dissolved products was estimated of about $ 1.4 billion in 2005 (IMS data) (Muir, 2007).

Oral rapid disintegrating (dissolve) tablets (ODTs) are solid dosage forms that are placed in the mouth, rapidly disintegrate/dissolve when in contact with the saliva and then easily swallowed without the need for water (European pharmacopoeia, 2002). The basic idea behind the ODTs is to combine the benefit of solid (stable and easy to handle) and liquid (ingestible) oral dosage forms. ODTs provide practical solution for wide range of people who experience difficulty in swallowing (dysphasia). This includes paediatric and geriatric patients, as well as hospitalised or bedridden patients suffering from a variety of disorders like stroke, thyroid disorders, Parkinson's disease and other neurological disorders like multiple sclerosis and cerebral palsy (Sastry et al., 2000). It is estimated that 50% of the population is affected by this problem, which results in a high incidence of non-compliance and ineffective therapy (Segar, 1998). The convenience and ease of using ODTs is also important with normal consumers (Jeong and Park, 2008), as it offers convenient and practical dosage all the time especially in case of no access to water (Mizumoto et al., 2005). In addition to improving patient compliance, ODTs have been investigated for their potential in increasing the bioavailability of poorly water soluble drug, through enhancing the dissolution profile of the drug (Corveleyn and Remon., 1998; Ahmed and Aboul-Einien, 2007), and providing rapid onset of action, by avoiding the need for gastric disintegration and facilitating pre-gastric absorption (through the buccal and oesophageal mucosa) (Segar, 1998). Moreover, pharmaceutical companies also have commercial reasons for formulating ODTs. As a drug reaches the end of its patent, the development and formulation of the drug into new dosage forms allows pharmaceutical companies to extend the patent life and market exclusivity (Biradar et al., 2006).

1.2. Recent patents and trends in the formulation of ODTs

Patent databases are among the most important and up-to-date sources of technological information as every patent must contain an element of novelty. Hence, analysing and summarising patents of ODTs in one article is extremely important in building an overall

picture of latest developments and achievements in this field, which will be helpful for the art specialists and pharmaceutical companies who are interested in developing and patenting new technologies in ODTs. Accordingly, this article will present a review of recent advances in ODT formulations that have been patented over the last decade. The analysis of the patent documents has been carried out by searching in the free online patent worldwide database (http://www.freepatentsonline.com) of granted patents in orally disintegrating tablets and related fields. The manufacturing steps in addition to the excipients and active ingredients used in each patents were summarised in tables, whereas the motivations, major claims, inventive steps and significances were highlighted in the text according to the manufacturing approach.

Searching in the free online patent worldwide database (http://www.freepatentsonline.com) for ODTs patents resulted in finding 81 published patents over the period from 1999 to 2010.Quantitative analysis of these patents revealed various technologies that have been applied to manufacture ODTs namely compression based technologies, freeze drying, moulding, tablet loading and pulverisation. **Figure 1.1** shows the percentage of each approach from the total number of the analysed patents. Direct compression technologies have been most widely used over this period, and up to 85% of the filed patents utilised direct compression to manufacture ODTs. On the other hand, moulding technologies accounted for 9% in manufacturing ODTs, 4% for freeze drying, whilst only 2% of patents utilised tablet loading and pulverisation technologies.

Applications were filed from different countries including Japan, India, Canada, Great Britain, France, Belgium, Netherlands, Italy, Denmark, Germany, Korea, Portugal and USA. From the 81 patents studied, USA and Canada contributed the most (up to 44%) followed by Asia (37%) and Europe (19%), See **Figure 1.2.**

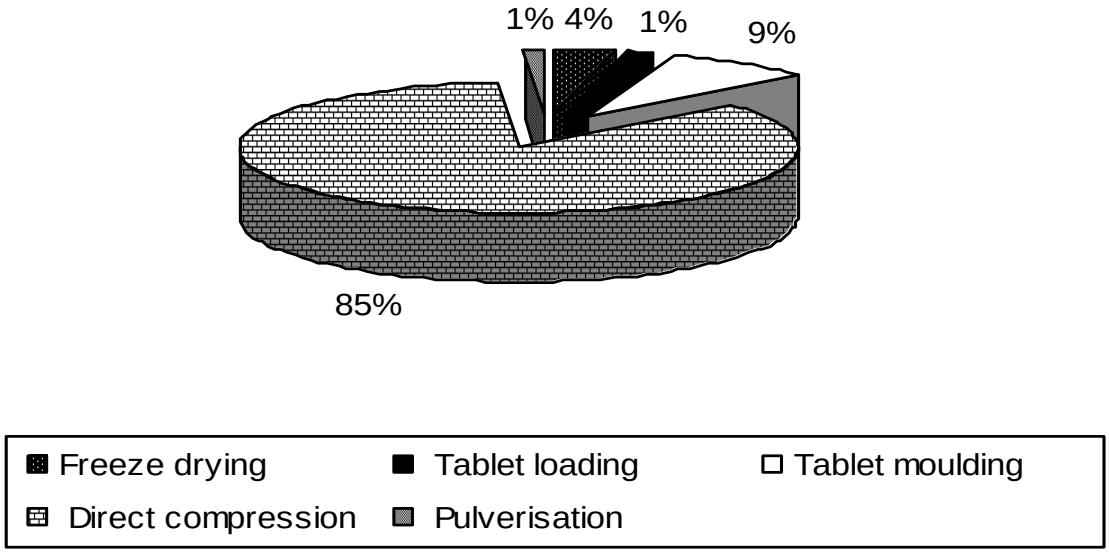


Figure 1.1 Various technologies used in manufacturing ODTs in the period 1999 to 2010.

Trend analysis of the number of patents published in each year was evaluated as well. **Figure 1.3** shows that only 2 patents were published in 1999 and the number of applications started to increase steadily between 2001 and 2007. Despite a minor decline in 2008 the following year (2009) witnessed the highest number of patent applications over the decade with up to 15 published patents in this year, which represents 18.5% of the total number of patents analysed. It is interesting to note that within the first quarter of the current year (2010) three patents were already published, indicating that this year might witness a high number of patents to be released.

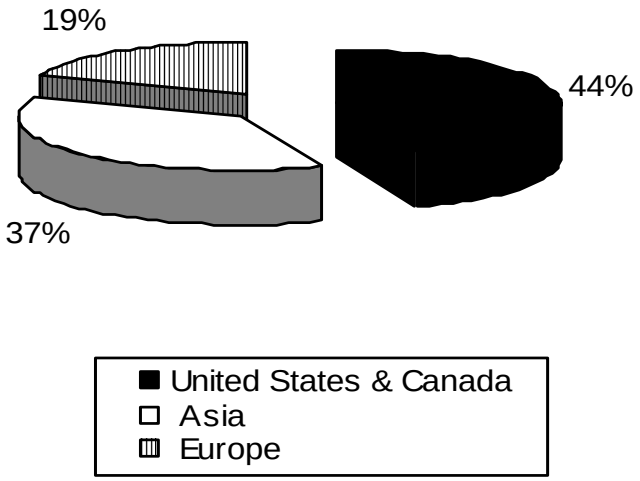


Figure 1.2 Geographical distribution of ODTs patents between 1999 & 2010.

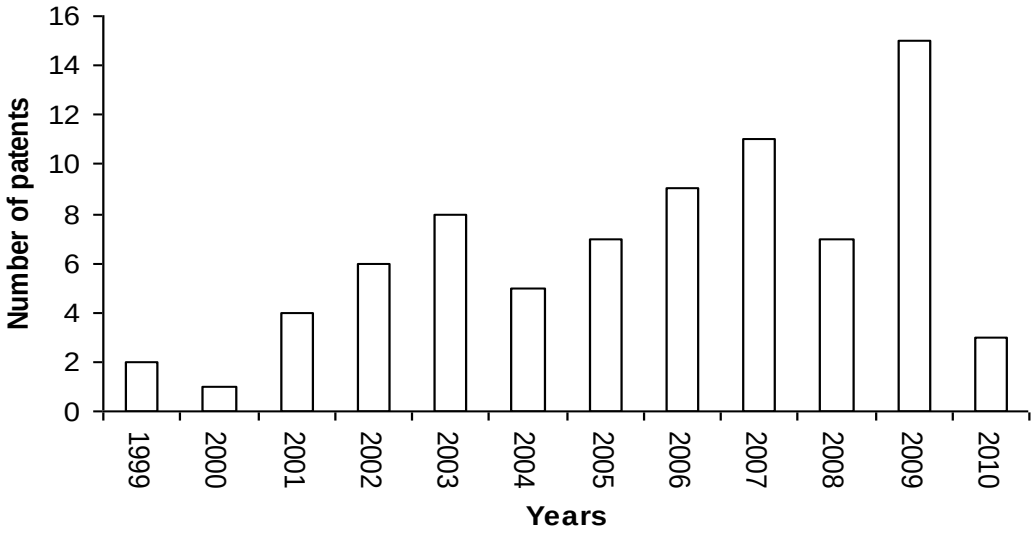


Figure 1.3 Number of patent applications filed in the period between 1999 to 2010.

1.2.1. Conventional tablet press

Manufacturing of ODTs using conventional tableting and packaging equipments is the simplest and most cost effective among other available techniques. Analysis of recent patents of ODTs produced by conventional tablet press methods, as shown in **Tables 1.1, 1.2 and 1.3,** revealed that the inventions were driven by two major motivations. Firstly, the need of innovative formulation strategies to modify the standard tableting procedure to provide tablets which disintegrate rapidly in the mouth with pleasant mouth feel and adequate mechanical properties. The second motivation is to extend the application of ODTs to more challenging drugs by overcoming some restrictions imposed by the nature of these drugs, such as their unpleasant taste, gastric acid sensitivity and instability during or after the manufacturing process, which limits their formulation in ODT dosage forms. Accordingly, the patents are discussed in detail below, based on the motivation of the invention and the employed formulation strategy.

1.2.1.1. Direct compression

Table 1.1 summarises patents that formulate ODTs by lightly compressing mixtures of active ingredients and excipients into tablets. These patents were based on using appropriate combinations of carefully selected excipients as the main components, without the need of further processing. The selected excipients provide rapid disintegration profile, pleasant mouth feel and adequate physical strength. All patents on direct compression have stated the necessity to use one or combinations of disintegrants to achieve fast disintegration with most of the work was dictated to the use of superdisintegrants which swell in contact with water and hence force the tablet to disintegrate. Sugars or sugar alcohols have been reported almost in all patents based on direct compression as they are highly water-soluble excipients which, in addition to their sweet taste, enhance the wettability of the tablet with water and consequently facilitates the disintegration (Chandrasekhar et al., 2009).

Table 1.1 Summary of patents that produced ODTs by direct compression.

Patent no.	Manufacturing Steps	Excipients	Drug	Comments	Reference
1	Involves; mixing the ingredients and meloxicam for 30 mins, followed by the addition of the lubricant. Blending again for 5 minutes then compression to form the tablets.	- Starch (20-50% w/w). - Glidants. - Water soluble excipient (40-80%) (ex. lactose).	Meloxicam or its pharmaceutically acceptable salts, such as meglumin, sodium, or potassium (1-25 mg/tablet).	Other auxiliary agents such as lubricants, sweeteners, souring and flavouring agents could be added.	Ohki et al., 2004
2	Involves; blending the active drug with excipients. Mixing the lubricant with the blend. And finally compression to form the tablets.	- Fillers (calcium sulfates). - Carbohydrates (Mannitol). - Starch clays (kaolin). - PEG (10-95% w/w).	Cox-2 inhibitors such as celecoxib and rofecixib.	Other conventional techniques such as wet, dry granulation and specialized techniques could be used but direct compression is used herein because of low cost.	Murpani et al., 2003
3	Involves; mixing of the ingredients, followed by direct compression to form the tablets.	- Soluble excipients (ex. sugar alcohol). - Lubricants. - Surfactants. - Liquefying solids (low melting point glycerides).	Poorly bioavailable drugs which degrade by enzymes or acid upon passing through GIT (ex. estrogens, progestins).	Very fast delivery of drugs giving blood levels similar to parenteral administration.	McCarty, 1999
4	Involves; sieving & mixing of all components (apart from the lubricant). Followed by direct compression to form the tablets.	- Spray dried mannitol. - Microcrystalline cellulose. - Humidity absorbing agents (syloid®) 0.1-0.5%. - Disintegration promoter (14-18.5%).		Spray dried mannitol is used because it is highly soluble in water, highly compressible, high dilution capacities and chemically stable.	Ferran, 2006
5	Involves; blending a mixture of a drug and excipients. Followed by compression to form the tablets.	- Binder (ex. starch). -Water-soluble excipient (ex. mannitol or lactose).	Any active ingredient or medicament.	A tablet preparation showing good sensory acceptability and yet having an adequate strength.	Nishii et al., 2003

6	Involves; mixing the drug with excipients. Followed by direct compression to form the tablets.	- Calcium carbonate (disintegrating agent and dental abrasive). - Sugar alcohol (ex. mannitol).	Therapeutic agents used for the prevention and treatment of dental caries and periodontal disease.	Friability of less than about 2% and disintegrates in less than about 60 seconds when immersed in water.	Withiam et al., 2005
7	Involves; mixing all formulation ingredients using a blender, adding the lubricant to the powder mixture, and finally direct compression to form the tablets.	- Titanium dioxide (water insoluble substance). - Sugar alcohol (ex. mannitol). - Superdisintegrant (ex. sodium starch glycolate).	Wide range of drugs suitable for ODT formulation.	The inclusion of titanium dioxide has dual functionality: Enabling rapid tablet disintegration, and providing tooth cleaning effect.	Mehra et al., 2005
8	Involves; dry mixing all formulation ingredients using a blender, adding the lubricant to the powder mixture, and finally direct compression to form the tablets.	- Filler (ex. microcrystalline cellulose). - Disintegrant (ex. low substituted hydroxypropyl-cellulose).	Epinephrine	The tablets are designed for buccal or sublingual absorption.	Rawas-Qalaji et al., 2007
9	Involves; mixing the compression blend, adding microcapsules and mixing. Followed by the addition of the lubricant to the final mixture, and finally compressing into tablets.	- Disintegrants (ex. starch). - Water insoluble inorganic excipient (ex. dibasic calcium phosphate). - Water-soluble filler (ex. lactose). - Surfactants.	Wide range of drugs suitable for ODT formulation.	Orally disintegrating tablets of microcapsules.	Dobetti, 2003
10	Involves; mixing the drug with the compression blend. Followed by the addition of a lubricant, and finally compressing into tablets.	- Disintegrants (ex. crospovidone). - Sugar alcohol (ex. mannitol). - Hydrophilic polymer (ex.	Ondansetron		Ahmed et al., 2008

		microcrystalline cellulose).			
11	Involves; mixing the drug with excipients. Followed by direct compression to form the tablets.	- Low surface area silica material (ex. Zeo® 49). - Sugar alcohol (ex. mannitol).	Pharmaceutical, nutraceutical or oral care active ingredients.	The inclusion of a low surface area silica material encourages quick disintegration in the oral cavity.	Withiam et al., 2007a
12	Involves; mixing the drug with excipients. Followed by direct compression to form the tablets.	- Low surface area titanium dioxide. - Sugar alcohol (ex. mannitol). - Disintegrant (ex. crospovidone).	Pharmaceutical, nutraceutical or oral care active ingredients.	The inclusion of a low surface area silica material encourages quick disintegration in the oral cavity.	Withiam et al., 2007b
13	Involves; mixing the drug with excipients. Followed by direct compression to form the tablets.	- Low surface area calcium carbonate. - Sugar alcohol (ex. mannitol). - Disintegrant (ex. crospovidone).	Pharmaceutical, nutraceutical or oral care active ingredients.	The inclusion of a low surface area silica material encourages quick disintegration in the oral cavity.	Withiam et al., 2007c
14	Involves; blending the active ingredients with the excipients. Followed by direct compression to form the tablets.	- Silicified microcrystalline cellulose. - Sweetening agent, flavouring agents, glidants.	Antibiotics (ex. amoxicillin alone or in combination with clavulonic acid).	Silicified microcrystalline cellulose allows the manufacture of high dose of amoxicillin/ clavulonic acid.	Skulji et al., 2006
15	Involves; separate mixing of two compressible mixtures. Followed by pre-compression of one mixture, and finally compressing of at least two layers.	- Binders (ex. PEG 8000). - Other inactive excipients (salivating agents, surfactants, super-disintegrants, and bulking agents).	Any drug which can be incorporated into the multi-layered ODT.	Multi-layered ODT (designed to avoid the limitations of mono-layered ODT associated with storage and handling).	Cherukuri, 2008
16	Involves; direct compression or granulating a mixture of a drug and excipients. Followed by	- Bitterness-reducing ingredient composed of an essential oil (ex. mint oil).	Bitter tasting drugs, namely Acetaminophen.	A tablet exhibiting little bitterness, when a bitter-tasting drug is comprised in the tablet.	Ohmri et al., 2003

	compression to form the tablets.	- High sweetness-sweetener (ex. stevia or aspartame). - Acidic phospholipid (ex. soybean lecithin).		
17	Involves; dry blending; of the drug, carrier, disintegrant, and lubricant. Followed by direct compression to form the tablets.	- Carrier: spray dried mixture of lactose monohydrate and microcrystalline cellulose.	Galanthamine hydrobromide	Gilis and De Conde, 2002

In addition to these two main mechanisms, some patents add another factor to promote the disintegration by inclusion of water insoluble excipients, such as microcrystalline cellulose (Ferran, 2006; Rawas-Qalaji et al., 2007; Ahmed et al., 2008) dibasic calcium phosphate (Dobetti, 2003) and titanium dioxide (Mehra et al., 2005), to generate repulsive forces with the readily soluble materials inside the tablet (Fukami et al., 2005). Humidity absorbing agents (permeabilizing agents) such as Syloid® have been used in directly compressed ODT to promote the disintegration by forming hydrophilic networks inside the tablet and hence facilitate saliva penetration (Ferran, 2006). Also, low surface area materials such as silica (Withiam et al., 2007a), titanium dioxide (Withiam et al., 2007b) and calcium carbonate (Withiam et al., 2007c) have been disclosed in patents to promote the quick disintegration properties of directly compressed ODTs. These materials must exhibit sufficiently low surface areas in order to improve the ability of the tablets to disintegrate quickly when placed in the oral cavity.

In distinction to the commonsensical use of superdisintegrants and saccharides as essential components to facilitate the rapid disintegration of directly compressed ODTs, Skulj et al (2006) used silicifed microcrystalline cellulose as a single component that can be mixed with high doses of active drugs and directly compressed the mixture into ODTs, suggesting that silicifed microcrystalline cellulose has multiple roles in the formulation, as a disintegrant, wicking agent, binder and filler (Skulji et al., 2006).

Other standard excipients are also included in all the patents to enhance the formulation process and the taste such as diluents, lubricants, glidants, binders, sweeteners, flavouring agents, preservatives and colorants. The claimed advantage of the direct compression approach is its low manufacturing costs due to the limited number of production steps (simple mixing and compressing).

1.2.1.2. Compression and preparation of rapidly dispersible granules

Granulation is any process which involves size enlargement (agglomeration) which converts small particles into physically stronger and larger aggregates. Methods available to granulate pharmaceutical powders can be broadly classified into wet and dry granulation, each having different strengths and weaknesses. Wet granulation, which is the most widely used process of granulation in the pharmaceutical industry, involves wet massing of the powder blend with a

granulating liquid, wet sizing and drying. There are various technologies available to merge all these steps into single and reproducible process, including high shear mixing granulation, fluid bed granulation, extrusion/ spheronisation, and spray drying granulation.

The process of granulation is traditionally applied in the pharmaceutical industry to enhance powder flow and cohesion properties of drugs and excipients that experience poor flowability and/or poor compactibility. Moreover, granulation prevents segregation of components, improves dissolution rate of hydrophobic drugs and leads to low dust generation, which results in decreasing cross contamination and airborne exposure. Hence, the development of rapidly dispersible granules suitable for the formulation of ODTs has received a great interest (Okuda et al., 2009).

Patents on ODTs that included granulation in their production methods as the inventive step, account for about 45% of compressed ODT patents, are summarised in **Table 1.2**. These patents have applied various procedures and materials in order to distinct their formulations. To achieve quick disintegration profile in the mouth, some patents (no. 18-27) dry mix highly hydrophilic excipients and disintegrants with the active ingredients and then carry out wet granulation on the mixture to produce rapidly dispersible granules that can be compressed to produce ODTs. Due to the presence of highly water-soluble excipients and superdisintegrants in these formulations, the mechanism of disintegration of such tablets is probably a combination of the wicking effect of hydrophilic components and swelling of disintegrants. Upon contact with water, the hydrophilic components dissolve quickly, allowing more water to penetrate into the tablets which causes the disintegrants to swell and consequently break the tablets into small particles. In addition to the standard ODT excipients such as sugars, sugar alcohols, disintegrants and flavours, various hydrophilic materials have been reported as main excipients, as shown in **Table 1.2**.

Ohta et al (2005) developed ODTs containing a large quantity of amino acids, by granulating a mixture of an amino acid and a disintegrant with an aqueous solution of saccharide, drying the granules and compressing the granules into tablets.

Table 1.2 Summary of patents that produced ODTs by compression of rapidly dispersible granules.

Patent no.	Manufacturing Steps	Excipients	Drug	Comments	Reference
18	Involves; wet granulation of ODT components with water or ethanol. Followed by drying and mixing with a lubricant, and finally compression to form the tablets.	- Sugar alcohol (D-mannitol). - Saccharrides (lactose). - Disintegrants (ex. crospovidone).	Wide range of pharmaceutically active ingredients.	Both with average particle diameter not more than 30µm.	Ohta et al., 2001
19	Involves; mixing the drug with components 1 and 2. Followed by; wet granulating, sieving, and drying, and finally compression at low pressure to form the tablets.	- Component 1: highly plastic materials (ex. fructose, maltodexrin). -Component 2: water penetration enhancers (ex. carbohydrates). - Binder polymers.	Wide range of active ingredients can be used such as loratidine, aspirin, and acetaminoph en.	Patent claims high plasticity of the tablets, and therefore only small force is necessary to reach the plastic deformation stage during compression.	Fu et al., 2005
20	Involves; granulating a mixture of an active drug with excipients. Followed by compression to form the tablets.	Corn starch, lactose, crystalline cellulose, hydroxypropyl-cellulose, and light anhydrous silicic acid.	Micronised AS-3201	Patent claims an improvement in the dissolution characteristics and bioavailability of AS-3201.	Ohashi et al., 2006
21	Involves; granulating a mixture of a drug and excipients. Followed by compression to form the tablets.	- Saccharides (D-mannitol). - Filler (crystalline cellulose).	Applies to any pharmaceutic-ally effective drug of choice.		Higuchi et al., 2009
22	Involves; granulation of a mixture of amino acids and excipients. Followed by compression to form the tablets.	- Disintegrating agent (ex. crospovidone). - Binder (ex. lactose or mannitol).	Amino acid and/or amino acid derivative.	ODTs containing an amino acid as a principal agent.	Ohta et al., 2004

23	Involves; granulating a mixture of a drug and excipients. Followed by compression to form the tablets.	- Saccharide having low mouldability (ex. maltiol).	N –acetylglucosamine, β-carotene and/or vitamin A.	N -acetylglucosamine based tablets, with excellent disintegration property in the oral cavity and adequate hardness.	Kamisono et al., 2007
24	Involves; granulating a mixture of a drug and excipients. Followed by compression to form the tablets.	- Sugar alcohols (ex. mannitol). - Flow agent (ex. silicon dioxide).	An active ingredient or a pharmaceutically acceptable salt.	The ODT has a non-filamentous microstructure of at least two sugar alcohols.	Amin et al., 2008
25	Involves; granulating a mixture of a drug and excipients. Followed by compression to form the tablet.	- Matrix forming agent (Silicified micro-crystalline cellulose).	Simvastatin	Improved tablet stability by using a non-alkaline lubricant.	Jansen , 2007
26	Involves; granulating a mixture of a drug and excipients. Followed by compression to form the tablet.	- Binder (ex. cellulose-based polymer). - Lower critical solution temperature (LCST) modifier (ex. an electrolyte).	An organic or inorganic compound that is physiologically or pharmacologically active.	Ensuring fast disintegration by using a lower LCST modifier to reduce the LCST of the polymer below or about 37ºC.	Dong, 2010
27	Involves; granulating a mixture of a drug and excipients. Followed by compression to form the tablets, and finally aging is performed on the tablets.	- Saccharide (ex. glucose). - Water-soluble binder (ex. polyvinylpyrrolidone). - Additional excipient (ex. mannitol).	Any medicament which can be formulated by a conventional wet granulation process.	A simple method for producing intrabuccally disintegrating tablets in large scale.	Shirai et al., 2002
28	Involves; sieving and mixing of the active ingredient and excipients. Followed by wet granulation of the active ingredient, starch and effervescent base. Followed by the addition of an effervescent acid and	- Effervescent couple: effervescent base (sodium carbonate) and effervescent acid (malic acid). - Lubricant, bulking & disintegrating agent.	Preferably antacids such as calcium carbonate and magnesium hydroxide at 25-50% w/w.	An effervescent couple generates a gas evolving reaction when in contact with saliva, which enhances tablet	Ouali, 1998

	lubricant, and finally compression is performed to form the tablets.			disintegration. A high concentration of corn starch synergises ODT disintegration.	
29	Involves; granulation of the active drug with the excipients. Followed by compression to form the tablet.	- Low density alkali earth metal salts (ex. calcium carbonate). - Water-soluble carbohydrates (ex. sorbitol).	Wide range of pharmaceutically active ingredients.	Spray drying or pre-compaction of low density alkali earth metal salts or water soluble carbohydrates enhances their compressibility.	Eoga and Valia, 1999
30	Involves; dry blending the drug with a; disintegrant, diluent and glidant. Followed by compressing the resultant mixture in the dry state to form the tablets. Tablet film coating can be applied at the end.	- Spray dried mixture of lactose monohydrate & microcrystalline cellulose (75:25). - Disintegrant. - Film forming polymer.	Galanthamine hydrobromide (1:1) 2-10%.	Film coated tablets are easier to swallow, but their weight should range between 3-8% in order not to adversely affect the disintegration time.	Gilis and De Conde, 2002
31	Involves; mixing of the components, followed by direct compression to form the tablets. Pre-treatments such as wet granulation and coating may be applied.	- Silicified microcrystalline cellulose.	Pharmaceutically active agent, nutrient, nutraceutical or cosmetic.	Other auxiliary excipients are not necessary but may be used (ex. disintegrants, lubricants, masking agents and sugars).	Platteeuw and Heuvel, 2004
32	Involves; wet granulation of the active drug and excipients. Followed by drying the resultant wet granules, and finally compression to form the tablets.	- Carbohydrates (ex. spray dried mannitol). - Water insoluble filler (ex. microcrystalline cellulose).	Any suitable ingredients which could be pharmaceutically active.		Grimshaw et al., 2007
33	Involves; granulating a mixture of the drug and excipients. Followed by	- Dissolution retardant (ex. polymethacrylate).	Valdecoxib	Useful in the treatment or prophylaxis of	Le et al., 2003

	compression to form the tablets.	- Rapid dissolution excipient (ex. mannitol).		cyclooxygenase-2 mediated conditions and disorders.	
34	Involves; spray drying a homogenised aqueous mixture of water soluble and insoluble excipients. Followed by the addition of an active ingredient (powder, granules, pellets or beads) and the compression blend. Finally, direct compression is performed to form the tablets.	- Water insoluble excipient (calcium silicate). - Water-soluble excipient (ex. carbohydrate). - Compression blend: binders, disintegrants, diluents, salivating agents, sweeteners, lubricants and stabilizers.	Wide range of drugs suitable for ODT formulation.	The patent covers any method that produces particles with intimate contact of water-soluble and insoluble excipients (for e.g. wet mixing, spray congealing, and precipitation).	Gandhi et al., 2009
35	Involves; heating an aqueous solution of carbohydrate. Followed by the addition of calcium silicate with continuous stirring. Drying the mixture in a heated air stream is then performed, followed by the addition of an active ingredient (powder, granules, pellets or beads) and the compression blend. Finally, direct compression is performed to form the tablets.	- Calcium silicate. - Carbohydrate (ex. mannitol). - Compression blend: binders, disintegrants, diluents, salivating agents, sweeteners, lubricants and stabilizers.	Wide range of drugs suitable for ODT formulation.	Any process that ensures the complete coating of calcium silicate with the carbohydrate can be employed (for e.g. spray drying and fluidized bed process).	Pilgaonkar et al., 2009
36	Involves; wet-granulating a mixture of water-soluble and insoluble excipients with a drug. Drying the wet granules is then performed, followed by the addition of disintegrants, lubricants, water-soluble and insoluble fillers, and the addition of other excipients. Finally, compression is carried out to form the tablets.	- Water-soluble carbohydrate (ex. mannitol). -Water insoluble filler (ex. microcrystalline cellulose). -Disintegrant (ex. sodium carboxymethyl-cellulose). - Other excipients: flavouring agents sweeteners, preservatives	Pharmaceutically active, nutraceutically active, or breath fresheners.		Grimshaw et al., 2008

		and colorants.			
37	Involves; blending the excipients together. Followed by co-processing the mixture (milling or wet granulation), and the addition of a lubricant. Finally, compression is performed to form the tablets.	- Compression excipients; ethylcellulose (water insoluble binder), disintegrants, fillers, and flow aids.	Wide variety of active ingredients.	This patent relates to the use of ethylcellulose in orally disintegrating tablets.	Durig, 2008
38	Involves; preparing agglomerates of one or more superdisintegrants. Followed by mixing the prepared agglomerates with the active ingredient and the excipients. Finally, compression is performed to form the tablets.	- Superdisintegrants (ex. sodium starch glycolate and croscarmellose cellulose).	Wide range of pharmaceutically active ingredients.	It was demonstrated that disintegration time of the tablets containing agglomerates is faster than tablets prepared by dry blending.	Tian et al. 2005
39	Involves; melt-granulation of low melting point and water soluble ingredients. Followed by congealing using spray drying, milling, or mixing. Finally, compression to form the tablets is carried out.	- Low melting point compounds (ex. polyethylene glycol, monoglycerides). - Water-soluble excipients (ex. saccharides, amino acids).	Wide range of pharmaceutically active ingredients.		Abu-Izza et al. 2002
40	Involves; granulating a mixture of the excipients. Followed by mixing the granules with the active drug, and finally, compression is performed to form the tablets.	- Microcrystalline cellulose. - Saccheride (D-mannitol). - Binder (maize starch gum).	Domperidone	Convenient administration and the delivery of Domperidone for gastrokinetic and antiemetic activity.	Ramalho et al. 2005
41	Involves; granulation of a mixture of a medicament and sugar (the core). Followed by granulating the core with a disintegrating agent, and finally	- Sugar (ex. mannitol). - Disintegrating agent (ex. crystalline cellulose).	The medicament is not particularly limited.	The tablets are produced by tableting drug cores coated with a pharmaceutical	Suga and Nakano, 2006

	compression is performed to form the tablets.			disintegrating agent.	
42	Involves; preparing microgranules from a drug, a sugar alcohol and an ODT binder. Followed by preparing rapidly dispersing microgranules from saccharides and a disintegrant. Blending of the drug microgranules and rapidly dispersing microgranules is then performed, and finally direct compression is carried out to form the tablets.	- ODT binder (ex. PVP, HPMC, corn starch). - Sugar alcohol (ex. mannitol). - Disintegrant (ex. crospovidone). - Other excipients: flavouring agents, preservatives, wetting agents, coloring agent, and taste-masking agents.	Wide range of drugs suitable for ODT formulation.		Venkatesh et al. 2009a
43	Involves; preparing granules of a disintegrant with a sugar alcohol and/or a saccharide. Followed by coating the granules with a disintegrant and mixing the coated granules with an active ingredient and other excipients. Finally, compression is carried out to form the tablets.	- Disintegrant (ex. crospovidone). - Sugar alcohol and/or a saccharide (ex. mannitol). - Coating polymer (ex. crospovidone solution). - Other excipients: lubricants, taste-masking agents, and sweeteners.	Wide range of drugs suitable for the direct compression process.	The granule comprises a disintegrant inside and coated outside. The active drug is added during granule preparation and/or during the mixing step.	Akutagawa and Narasaki, 2009
44	Involves; wet-granulating a mixture of a disintegrant and a sugar alcohol. Followed by coating the granules with a disintegrant and mixing the coated granules with an active ingredient and other excipients. Finally, direct compression is performed to form the tablets.	- Disintegrant (ex. crospovidone). - Sugar alcohol (mannitol or erythritol). - Other excipients: lubricants, taste-masking agents, sweeteners, colorants, and binders.	Wide variety of active ingredients.	The granule comprises a disintegrant inside and coated outside. To insure rapid disintegration, the active drug is preferably added to the outside of the coated granules.	Akutagawa and Narasaki, 2010

45	Involves; mixing the unsuppressed bitter tasting drug granules, granules containing water-soluble excipients, and compression blend excipients. Followed by compression to form the tablets. Finally, an alcohol based solvent is applied to the compressed tablets and allowed to evaporate.	- Drug granules: drug and fillers. - Water-soluble granules: a saccharide (ex. mannitol) and a binder which is soluble in water and in alcohol solvent (ex. polyvinyl pyrrolidone).	Drugs with a bitter taste.	Treating the tablets with an alcohol based solvent after compression enhances their hardness.	Uemura et al., 2009
46	Involves; preparing a microporous binder. Followed by mixing the microporous binder with a drug and other excipients using a blender. Finally, compression is carried out to form the tablets.	- Microporous binders: ionisable or non-ionisable cellulosic polymer (ex. hydroxypropyl-methyl-cellulose phthalate) and wicking agent (ex. sugar alcohol, saccharide). - Other excipients: diluents, lubricants, glidants, binders, sweeteners, preservatives and colorants.	Wide variety of active ingredients.	The microporous binder is prepared by causing liquid-liquid or solid-liquid phase separation for a single phase solution of the polymer and wicking agent, prior to drying to the solid particle.	Ray et al., 2008
47	Involves; granulating a mixture of a cyclic GMP phosphodiesterase inhibitor and the excipients. Followed by compression-moulding to form the tablets.	- Filler (ex. crystalline cellulose). - Surfactant (ex. sodium lauryl sulphate). - Water soluble polymer (ex. methylcellulose). - Saccharides (ex. mannitol).	Cyclic GMP Phosphodieste-rase Inhibitors.	The ODT can be easily taken, swallowed and handled, with an improvement in solubility of the drug	Grenier et al., 2007
48	Involves; granulating a mixture of a drug and excipients. Followed by compression to form the tablets.	- Disintegration agents (ex. type-c methacrylic acid copolymers and crospovidone). - Diluent (ex. mannitol).	The medicament is not particularly limited.	Using combinations of the disintegrants produces ODTs that disintegrate in less than 30 seconds.	Furitsu et al., 2004

Fu et al (2005) described a method of preparing highly plastic granules that can be compressed into ODTs, which involves granulating a mixture of a porous, plastic substance (ex. LYCATAB®, MALTRIN®, GLUCIDEX®), a water penetration enhancer (saccharides), active ingredients and a binder, sieving and/or drying the granules and lightly compressing into tablets that are characterised by fast disintegration and low friability profiles.

Kamisono et al (2007) described a method of incorporating high doses of N-acetylglucosamine into ODT dosage forms, by preparing granules from a mixture of N-acetylglucosamine and a low mouldable saccharide, mixing the granules with a high mouldable saccharide, and compressing into tablets.

Other patents add effervescent agents to the mixture before the granulation process in order to synergise the fast disintegration profile by generating a gas evolving reaction when in contact with saliva which enhances tablet disintegration (Ouali, 1998 ; Eoga and Valia, 1999).

In contrary to the conventional concepts of using highly water-soluble excipients to formulate rapidly dispersible granules, the use of water insoluble excipients have also been cited in a number of patents as the main excipients (patents no. 30 -37). Various water insoluble materials are disclosed in ODTs patents, including calcium silicate (Gandhi et al., 2009; Pilgaonkar et al., 2009), microcrystalline cellulose (Shirai et al., 2002; Le et al., 2003), silicified microcrystalline cellulose (Platteeuw and Heuvel, 2004), ethylcellulose (Durig, 2008) and polymethacrylate (Suga and Nakano, 2006). The granules are produced by granulating a homogenous mixture of the water insoluble component, a highly water soluble excipient (for example a saccharide or sugar alcohol) in addition to the active drug and other auxiliary ODT excipients. The presence of highly water-soluble excipients in close proximity with the insoluble materials is crucial to allow rapid disintegration, which, as mentioned earlier, generates repulsive forces between the two excipients inside the granules and consequently breaks down the tablets.

Additional disintegration factors are usually employed to promote disintegration by inclusion of superdisintegrants in the formulation. However, granules manufactured from silicified microcrystalline cellulose (SMCC) do not require the inclusion of highly water-soluble excipients or disintegrants to display rapid disintegration (Jansen , 2007; Platteeuw and Heuvel, 2004), possibly due to its intrinsic ability to absorb water that can initiate self

subsequent swelling activity (Kachrimanis et al., 2003) which results in complete disintegration of the tablet.

Alternative approaches, where the active drug and auxiliary excipients are not included in the granulation mixture and are added to the rapidly dispersing granules just before the compression step, have been also patented (no. 38-40). Ramalho et al (2005) described a method of manufacturing ODTs of domperidone by lightly compressing a mixture of the drug with pre-prepared rapidly dispersing granules, which comprises of mannitol and maize starch gum (a binder). Tian et al (2005) prepared agglomerates, comprising of one or more superdisintegrants which can be mixed with active drugs and other auxiliary excipients and then compressed into ODTs. Abu-Izza et al (2002) mixed the active drug with fast dissolving granules, comprising a low melting point compound such as polyethylene glycol, hydrogenated oil (Wecobee M) and a water-soluble excipient such as a sugar alcohol (mannitol).

Furthermore, other patents have employed complicated procedures to formulate ODTs by compressing granules (no. 41-45). Suga and Nakano (2006) developed a method to produce ODTs which involves secondary granulation or coating of disintegrating agents (superdisintegrants) on the primary granules (cores), which comprises an active drug and a saccharide. A similar method, but where the active drug is added during granules preparation and/or during the compression moulding stage, are patented elsewhere (Akutagawa and Narasaki, 2009; Akutagawa and Narasaki, 2010. Venkatesh et al (2009) formulated ODTs of temazepam by compressing a mixture of two groups of granules, in which one contains the active drug (temazepam) with a saccharide and an ODT binder (drug microgranules), while the second comprises saccharides and disintegrants (rapidly dispersed microgranules). A similar method of compressing a mixture of two separately prepared granules of a drug and water-soluble saccharide was applied to suppress the bitter taste of a drug when prepared as ODTs (Uemura et al., 2009). Ray et al (2008) described a method of preparing fast disintegrating microporous binder particles that can be mixed with active drugs and compressed into highly porous ODTs. The microporous binder, which comprises an aqueous soluble cellulosic polymer and a wicking agent, is prepared by causing liquid-liquid or solid-liquid phase separation of a single phase solution of the polymer and wicking agent prior to drying the solid particle.

1.2.1.3. Compression of multiparticulates into ODTs

As mentioned earlier, the fast disintegrating behaviour of the ODT in the mouth limits the number of active drugs that can be incorporated, due to their bad taste, slow onset of action, short half life and/or instability in gastric fluids. Tableting of multiparticulates into ODTs has attracted scientists to overcome these limitations and widen the application of ODTs. The basic idea is to prepare ODTs that disintegrate rapidly in the mouth into easily swallowing small particles that mask and protect the active drugs until released at appropriate sites in the gastrointestinal tract. Recent patents have described several approaches of preparing multiparticulate systems that can be compressed into ODTs, see **Table 1.3**.

Tableting of coated drug particles is the most common technique cited in recent patents to mask the bad taste of active drugs in ODT formulations (patents no. 49-55 in **Table 1.3**). Mimura et al. (2009) formulated ODTs of the bitter-tasting mitiglinide calcium hydrate by simple coating of the drug granules with water insoluble polymers such as ethyl acrylate-methylmethacrylate copolymer or acid-soluble polymers such as aminoalkyl methacrylate copolymer E that delays the dissolution of the drug in the mouth. Whereas, other patents have described coating the drug particles with a mixture of water insoluble polymer such as ethylcellulose and gastro-soluble pore-former such as calcium carbonate to prevent their bad taste from developing in the mouth, while ensuring complete release in the stomach (patents no. 50-53, in **Table 1.3**). In distinction with polymeric materials, lipids have been used to coat drug particles and hence mask their unpleasant taste (Szamosi et al., 2007; Harland, 2003).

Moreover, multilayer coating has been developed to overcome formulation and stability issues associated with tableting of conventional coated granules into ODTs. For instance, oxycodone was formulated into ODTs as taste masked granules by applying a subcoat of a gastric-soluble compound such as polyvinyl alcohol before coating with a conventional taste-masking polymer (for e.g. Eudragit®). The subcoat was applied to prevent possible interaction between the taste-masking polymer and oxycodone that leads to oxidatitive degradation of the drug. Another advantage of the multilayer coating system is the ability to incorporate acetaminophen along with oxycodone within the ODT, which is usually difficult as their direct contact promotes the degradation of oxycodone (Hoarau, 2009; Oury et al., 2009).

Table 1.3 Summary of patents that disclosed the compression of multiparticulates into ODTs.

Patent no.	Manufacturing Steps	Excipients	Drug	Comments	Reference
49	Involves; granulating a mixture of a drug and excipients. Followed by coating with taste masking polymer and mixing with the excipients. Compression is then carried out to form the tablets.	- Microcrystalline cellulose. - Masking agent (ex. aminoalkyl methacrylate copolymer E). - Sugar or sugar alcohol (ex. lactose).	Mitiglinide calcium hydrate	A bitterness-masked tablet.	Mimura et al., 2009
50	Involves; coating drug particles with the first coat. Followed by second coating with flavouring agents or sweeteners and mixing with the compression blend. Finally, direct compression is performed to form the tablets.	- First coat: water insoluble polymer (ex. ethylcellulose) and gastrosoluble pore-former (ex. maltodextrins). - Compression blend: saccharide and/or sugar alcohol granules with a disintegrant.	Ranitidine salt, solvate or ester.	Orally disintegrating tablets of taste-masked microcapsules.	Venkatesh et al,. 2009b
51	Involves; coating particles of lamotrigine with a taste-masking layer. Followed by mixing the coated particles with rapidly dispersing granules. Finally, direct compression is carried out to form the tablets.	- Taste-masking layer: water insoluble polymer (ex. ethylcellulose) and gastrosoluble pore-former (for e.g. calcium carbonate). - Rapidly dispersing granules: a disintegrant and a sugar alcohol and/or a saccharide.	Lamotrigine	Orally disintegrating tablets of taste-masked microcapsules.	Venkatesh et al,. 2009c
52	Involves; granulating diphenhydramine with fillers and a binder. Followed by coating the particles with a taste-masking layer and mixing the coated particles	- Taste-masking layer: water insoluble polymer (ex. ethylcellulose) and gastrosoluble pore-former (ex. Sodium chloride). - Rapidly dispersing granules: a	Diphenhydramine	Orally disintegrating tablets of taste-masked microcapsules.	Venkatesh et al,. 2009d

	with rapidly disintegrating granules and the compression blend. Compression is then performed to form the tablets.	disintegrant and a saccharide.			
53	Involves; granulating a mixture of the drug, a binder and a diluent. Followed by coating the granules with a water insoluble polymer and a water soluble substance. This is followed by granulating a mixture of the coated microcapsules and mannitol with an aqueous solution of maltose. Finally, compression is carried out to form the tablets.	- Binder (ex. Hydroxypropyl-methyl-cellulose). - Diluent (ex. crystalline cellulose). - Water insoluble polymer: (ex. ethylcellulose). - Water soluble substance (ex. Hydroxypropyl-methyl cellulose).	Wide variety of active ingredients.	Orally disintegrating tablets of taste-masked microcapsules.	Kurimoto et al., 2005
54	Involves; coating the active drug with a lipid base solution. Followed by mixing with silicified excipients. Compression is then performed to form the tablets.	- Lipid (ex. fatty acid glycerol ester). - Silicified excipient (ex. silicified micro-crystalline cellulose).	Any compound that provides a therapeutic effect.	Silicified excipients and lipid coating of active agents prevent unpleasant taste, and provide better chemical and mechanical stability of the coated active substrate.	Szamosi et al., 2007
55	Involves; coating the active material by a hot melt fluid bed process. Followed by mixing with the other excipients. Finally, compression is performed to form the tablets.	- Lipid-based coating material (ex. an ethoxylated fatty acid). -Bulking agent (ex. mannitol). - Binder (ex. starch).	Any biologically active material.		Harland, 2003
56	Involves; spray drying a suspension of oxycodone and a binder.	- Binder (ex. cellulose based polymers).	Oxycodone and acetaminophen.	The solvent used in spraying the drug pellets	Hoarau et al., 2009

	Followed by applying a subcoat onto the drug pellets and coating with a taste-masking layer. This is then followed by mixing the coated pellets with acetaminophen, a disintegrant and a soluble diluent. Finally, compression is carried out to form the tablets.	- Subcoat: gastric soluble compound (ex. polyvinyl alcohol). - Taste-masking layer: (ex. Eudragit®). - Disintegrant (ex. crospovidone). - Soluble diluent (ex. a polyol).		and subcoating is hydroalcoholic which is claimed to reduce oxycodone degradation.	
57	Involves; coating the neutral core with an opioid and binder solution. Followed by subcoating the coated pellets and applying additional coating with a taste-masking layer. Mixing the coated pellets with a compression blend is then performed, followed by the addition of coated crystals of acetaminophen with a compression blend. Precompression of the acetaminophen mixture is carried out, followed by the addition of the opioid mixture above the precompressed tablet and finally compression is performed again.	- Neutral core (ex. a sugar). - Binder (ex. cellulose based polymers). - Subcoat: gastric soluble compound (polyvinyl alcohol). - Taste-masking layer: water insoluble polymer (ex. Eudragit®) and pore forming agent (ex. polyol). - Compression blend: diluents, lubricants glidants, binders sweeteners, preservatives and colorants.	Oxycodone and optionally acetaminophen.	Multilayer orally disintegrating tablet.	Oury et al., 2005
58	Involves; granulating a mixture of a drug and excipients. Followed by sub-coating with a film and enteric	- Basic inorganic salt; a salt of magnesium and/or a salt of calcium.	An acid-labile physiologic-ally active substance.	Orally disintegrable tablet consisting of an enteric coated acid-labile	Shimizu et al., 2001

	coating of the core. Mixing of the coated pellets with rapidly dispersible granules is carried out, and finally compression is performed to form the tablets.	- Sugar alcohol (ex. erythritol). - Crystalline cellulose and sustained-release agent/enteric polymer agent (ex. methacrylate copolymer).		physiologically active substance.	
59	Involves; coating a neutral core with the drug and basic inorganic salt. Followed by coating with a water- soluble polymer and enteric coating. An additional coat with mannitol is applied. Blending the granules with a comprssion blend is performed, and finally compression is carried out to form the tablets.	- Neutral core (ex. crystalline cellulose). -Basic inorganic salt (ex. magnesium carbonate). - Water-soluble polymer (ex. HPC). - Enteric polymer agent (ex. methyl acrylate copolymer). - Compression blend: crystalline cellulose, sugar alcohol (ex. mannitol), binder (ex. HPC) and disintegrants.	Lansoprazole	Orally disintegrating tablets of enteric coated granules of acid-labile drug.	Shimizu et al., 2008a
60	Involves; coating of active-loaded beads with sustained or enteric coating. The manufacture of cushioning components is then carried out. Followed by the co-processing of active-loaded beads with cushioning components into Cushion Beads™. Freeze drying is then carried out, and finally compression is performed to form the tablets.	- Coating polymers (ex. Eudragit). - Highly compactable filler (ex. microcrystalline cellulose) to synthesize cushioning components.	Dietary supplements, Pharmac eutically active drugs, or prodrugs.	Milling of Cushion Beads™ to a particle size of between 10-50 mesh results in immediate dispersion of tablets in the mouth without losing the ability to protect coated particles during compression.	Do et al., 2004
61	Involves; coating the active ingredients. Followed by mixing with disintegrating agents, soluble	- Soluble diluent agents (30-90% w/w) (ex. Polyols). - Disintegrants (ex.	Ibuprofen, paracetamol and aspirin.	Permeabilizing agents enhance the formation of hydrophilic networks	Chauveau et al., 2006

	diluents, permeabilizing agents and lubricants. Homogenising by a dry mixer is then carried out. Finally, compression is performed to form the tablets.	Croscarmellose). - Lubricants. - Permeabilizing agents (ex. Syloid®).		which facilitates saliva penetration and in turn tablet disintegration.	
62	Involves; wet granulating a mixure of ion-exchange resin/active drug complex with the excipients. Sieving and drying is then carried out. Finally, compression is performed to form the tablets.	- Ion-exchange resin (ex. Duolite AP™ 143). - Coating polymers (ex. methacrylate). - Binder (ex. maltodextrin). -Diluent (ex. Lactose).	Any ionic active ingredients (ex. Diphenhydramine hydrochloride, cetirzine hydrochlor-ide).	The fast melting properties of the tablets is achieved by using highly plastic granules.	Jeong et al., 2006
63	Involves; dispersing the active ingredients in a hydrogel. The matrix hydrogel is then hardened to form microcapsules. Surfactant is then added. Granulating the microcapsules with the excipients is then carried out. Finally, compression is performed to form the tablets.	- Hydrogels (ex. gelatin, albumin, alginates). - Surfactants (ex. lecithin). - ODT excipient (ex. sugar alcohol).	Drugs which cause irritation to GIT such as antacids, anti-ulcer, cimetidine, ranitidine, nizatidine.	Surfactant is added to prevent aggregation of microcapsules.	Yang et al., 2005
64	Involves; forming nanoparticles of poorly soluble drugs and a surface stabilizer. One or more water-soluble or water dispersible excipients are then added. Finally, compression is performed to form the tablets.	- Surface stabilizer (ex. lecithin or gelatin). -Water dispersible excipient (ex. sugar or sugar alcohol). - Other excipients for e.g. binders, fillers, buffers, sweeteners.	Wide range of drugs; preferably poorly soluble active agents (ex. penicillins), ketoprofen, nifidipine).	Nanoparticulate compositions are characterised by large surface area and hence rapid disintegration.	Jain et al., 2001

Enteric coating has also been used to deliver acid-labile drugs as compressed ODTs. Shimizu et al (2001) and Shimizu et al (2008a) described a multi step method to prepare enteric coated pellets of lansoprazole suitable for direct compression into ODTs, in which the pellets comprise of coating a neutral core with the drug and basic inorganic salt, undercoating with a water-soluble polymer (ex. hydroxypropyl methylcellulose), enteric coating and finally applying an additional coat of a sugar alcohol (mannitol or erythritol). The tablets disintegrate completely in the mouth to allow easy swallowing of enteric coated pellets that can maintain their integrity in the acidic environment of the stomach to protect the drug from degradation and then dissolve upon contact with the basic environment of the intestine to release the active form of the drug.

Furthermore, some patents have addressed various formulation difficulties associated with the inclusion of coated multiparticulates in an ODT. One of the major challenges of compressing coated pellets into tablets is the ability of the coating layer to withstand the compression force which is applied to produce tablets with acceptable mechanical properties (Bodmeier, 1997). In a trial to address this issue in ODT formulations, Do et al (2004) proposed a method to cover the coated pellets with cushioning components, consisting of highly compactable filler, highly water absorbing material and disintegrants. The cushioning component allows the coated pellets to be compressed into tablets, protecting against any possible rupture of the coating layer during compression and providing rapid disintegration behavior in the mouth. The manufacturing steps are summarized in **Table 1.3** (patent no. 60). Szamosi et al (2007) have reported the use of silicified excipients such as silicified microcrystalline cellulose as part of the ODT compression mixture to aid in retaining the beneficial properties of the coated particles.

Achieving short and smooth disintegration in the mouth is another challenge of incorporating coated pellets in an ODT system. Chauveau et al (2006) reported the use of permeabilizing agent such as the precipitated silica (Syloid® FP244) in addition to standard ODT excipients (saccharides, superdisintegrants and hydrophilic binders). The permeabilizing agent allows the formation of hydrophilic networks which facilitates the penetration of the saliva and consequently encourages quick oral disintegration.

Ion exchange resins have been employed to overcome the uncontrolled burst effect and limited drug loading of the coated pellets. Jeong et al (2006) described a method of preparing ODTs based on ion exchange resins and active drug complexes with sustained release, enteric coating and taste masking properties (patent no. 62 in **Table 1.3**).

Incorporating microcapsules in ODT formulations has been employed to deliver drugs which cause irritation to the gastrointestinal tract when introduced directly to the mucosa as a solid such as antiacids (cimetidine, ranitidine, nizatidine), non steroidal anti-inflammatory drugs (NSAIDs) and calcium channel blockers (Yang et al., 2005).

Nanoparticles of poorly soluble drugs have been incorporated in a compressed ODT formulation to provide fast onset of action through combining the rapid disintegrating ODTs and the rapid dissolution profiles of nanoparticles (Jain et al., 2001).

1.2.1.4. Post-compression treatment

Treatment of compressed ODTs after their removal from the compression dies has been disclosed in patents to enhance the mechanical properties and improve the disintegration profile of the ODT, **Table 1.4.** Moistening and subsequent drying treatment of the compressed tablets has been applied to improve the mechanical property of ODTs (Fu et al., 2006; Kajiyama et al., 2003). The moistening process is carried out by introducing the compressed ODTs to a relative humidity value above the critical relative humidity of the compressed mixture for a predetermined time sufficient to form liquid bridges between the particles inside the tablet (Fu et al., 2006; Kajiyama et al., 2003). Subsequent drying solidifies the liquid bridges and hence the tablet strength is increased substantially (Lee et al., 2002). Aging the tablets by allowing them to stand at room temperature for several hours to several days is another method to enhance mechanical properties of highly porous compressed ODTs that has been patented, see **Table 1.2** patent no 27 (Shirai et al., 2002). An alternative approach in case of humidity sensitive drugs has also been disclosed in patents. Uemura et al (2009) applied alcohol solvent on the surface of the compressed tablets to enhance the hardness of ODTs without deteriorating the disintegration time. Treatment with alcohol liquefies the binder and consequently builds bridges between the granules that are solidified after evaporating the alcohol (patent no 45 in **Table 1.2**).

To improve the disintegration profile of compressed ODTs, various techniques for post compression treatment have been developed. The idea is to create highly porous structure for the tablet that promotes fast penetration of the disintegrating medium inside the tablets and consequently shorter disintegration time. Lee et al (2002) proposed a method to prepare highly porous ODTs by compressing a mixture of spray dried particles containing an active drug

Table 1.4 Summary of Patents that employed post compression treatment to manufacture ODTs.

Patent no.	Manufacturing Steps	Excipients	Drug	Comments	Reference
65	Involves; mixing the drug with the excipients. This is followed by direct compression to form the tablets. Moisture treatment is then performed, and finally the tablets undergo drying.	- Mannose as a principal component (structure-former).	Any pharmaceutically effective drug of choice.	The incorporation of mannose imparts both structure-forming and fast-dissolution properties to the tablets.	Fu et al., 2006
66	Involves; granulating a mixture of a drug and excipients. Compression is then carried out to form the tablets. This is followed by moistening and drying of the tablets.	- Saccharide (ex. mannitol). - Pharmaceutical preparation carrier (ex. aqueous ethyl cellulose).	Any pharmaceutically active component with an unpleasant taste and inferior fluidity.	Masking bitter tasting drugs and improving the fluidity of inferior fluidity drugs.	Kajiyama et al., 2003
67	Involves; a sublimable substance being tableted together with a spray-dried particulate containing an active ingredient, a poly (ethylene glycol) and other excipients. The tablet is then dried at 42-48ºC by sublimation until the tablet becomes porous.	- Binder (ex. Polyvinylpyrrolidone). - Inorganic substance (ex. silicon dioxide). - Sublimable substance (ex. menthol). -Poly (ethylene glycol). -Saccharide (ex. Mannitol).	Any pharmacologically active ingredient.	A tablet having an enhanced strength as well as a high disintegration rate in the oral cavity.	Lee et al., 2002
68	Involves; granulating a mixture of a drug and excipients. This is followed by compression to form the tablets. The tablets then undergo moisturizing and drying.	- Water-soluble saccharide (ex. Mannitol).	Any medicinal agent.	A tablet that can disintegrate in the oral cavity typically between 3 and 5 seconds.	Tatara et al., 2001
69	Involves; granulating a mixture of a drug and excipients. Compression of the wet granules then takes place. The compressed tablets are then dried.	- Saccharide (ex. mannitol). - Binder (ex. polyvinyl alcohol).	Any medicine.	A tablet which does not have uncomfortable tastes, which is superior in stability.	Morita et al., 2003

and a sublimable substance suitable for oral administration such as menthol. Introducing the compressed tablet to sublimation conditions, vacuum and/or temperature, remove the sublimable substance and consequently creates highly porous tablets. Tatara et al (2001) added moisturizing and drying steps in the manufacturing step of ODTs after removing from the compression dies to reshape the compressed tablet into highly porous matrix. Whereas, Morita et al (2003) prepared ODTs with high porosity by compressing wet powder into tablets and then drying them in the mould.

1.2.2. Moulding

Moulding technology is used mainly to prepare ODTs using water-soluble ingredients such as saccharrides. The powdered form of these ingredients is moistened with water or ethanol and moulded under pressure (usually lower than the conventional tablet compression pressures). Moulded forms can be prepared by heat moulding; by dissolving or dispersing the drug into a molten matrix, or by no-vacuum lyophilisation, in which the solvent is evaporated from the drug suspension or solution at standard pressure (Fu et al., 2004).

Some moulded ODTs are solid dispersions; as the drug does not dissolve completely in the molten carrier or could exist as micro-particles or discrete-particles. Despite the fast disintegration time of the moulded ODTs, they do not posses high mechanical strength, and therefore break easily upon handling or opening of blister pockets. Recently, non-conventional equipments and multistep processes have been used to improve the mechanical properties of moulded tablets.

Table 1.5 summarises recent patents of ODTs manufactured by moulding technology. Some patents focused on formulating moulded ODTs with high mechanical properties (hardness of 4kp) and without prolonging the disintegration time (less than 1 minute). Takaishi et al (2005) used low melting point saccharides such as mannitol and erythritol which upon heating result in melting of the excipients and formation of bridges between different excipients and the drug to improve the mechanical properties. On the other hand, Bunick and Luber (2009) utilised hydrated salts with dehydration temperatures between 20-120^{o}C as binders. Heating these hydrated salts promotes their fusion with other ingredients to form aggregates with better mechanical properties. In 2010, Bunick and Luber (2010) selected binders from groups of fats, waxes or water-soluble polymers with melting points less than 160^{o}C.

Table 1.5 Summary of patents that produced ODTs by moulding technology.

Manufacturing Steps	Excipients	Drug	Comments	Reference
Involves; moulding the diluent, drug and saccharides at low pressure. This is followed by heating until the saccharides melt. Cooling then takes place to re-solidify the saccharides.	- Saccharides (0.5-25% w/w) (ex. xylitol or maltose). - Diluent (ex. crystalline cellulose). - Polymers for bitter-tasting drugs (ex. ethylcellulose).	Wide range of active ingredients can be used.	Saccharides should have a melting point lower than the other excipients.	Takaishi et al., 2005
Involves; materials being dispensed into a recess. The recess is then sealed and the materials are heated above the dehydration temperature of the hydrated salt to form aggregates. The preparation is then cooled to form solid tablets.	- One hydrated salt (5-40%) like sodium sulphate hydrate. - Carbohydrates (40%) such as dextrose. - Effervescent couples (ex. calcium carbonate and citric acid).	Wide range of active ingredients.	Should be free from directly compressible water insoluble fillers such as cellulose and starch.	Bunick and Luber, 2009
Involves; preparing a liquefied mixture of the excipients. This is followed by filling a pre-measured volume of tableting material into a tablet package having an open-ended cavity. Finally, the tableting material is heated to form the desired tablet.	- Binder (ex. cocoa butter). - Carbohydrate or carbohydrate alcohol (ex. dextrose or mannitol). - Filler (ex.cellulose derivative). -Flavouring agent (ex. menthol). - Other auxiliary excipients.	Pharmaceuticals, minerals, vitamins and other nutraceuticals.	A method and apparatus for forming an orally disintegrating dosage unit directly in the package, without the use or with the minimal use of solvents.	Bunick and Luber, 2010
Involves; blending the dry constituents, and subsequent addition of a solution comprising of; water and ethyl alcohol to the dry components. This is followed by blending to form a wet blend. Finally, moulding is performed to form the tablets.	- Mannitol 75-95%. - Disintegrating agent 1-10% (ex. sodium starch glycolate). - Other excipients, such as; diluents binders and lubricants.	Olanzapine		Chungi et al., 2006

Involves; preparing diphenhydramine particles. This is followed by coating with a taste-masking layer, and mixing the coated particles with rapidly disintegrating granules. Finally, compression is carried out to form the tablets.	- Film-forming binder (0.5-10%) (ex. PVP). - Polymeric binder (1-10%) (ex. povidone). - Taste-masking water insoluble polymers 5-30% (ex. cellulose acetate). - Rapidly dispersing granules comprising 1-10% disintegrant and 90-99% sugar alcohol.	Diphenhydramine		Venkatesh et al., 2009d
Involves; core preparation and subsequent coating of the core with a water-soluble polymer. This is followed by enteric coating and blending. Moulding is then performed to form the tablets.	- Enteric coating polymers (ex. cellulose acetate phthalate). - Basic inorganic salt (sodium carbonate). - Water-soluble polymers (for e.g. hydroxypropyl-cellulose). - Water-soluble sugar alcohol (ex. mannitol). - Crystalline cellulose (3-50%).	Benzimidazoles (ex. lansoprazole).		Shimizu et al., 2008b
Involves; mixing the uncured shearform matrix and active ingredients. This is followed by moulding the mixture using compression to form the tablets. Finally, curing of the uncured shearform matrix takes place to yield crystalline stable tablets.	- Carrier material (ex. sugar combinations or maltodextrins). - Crystallization modifier (ex. Spans™ and Tweens™). - Effervescent disintegration agent.	Wide range of active ingredients can be used.	The patent disclosed an apparatus for preparing ODTs consisting of a mixing station, moulding station, and curing station.	Myers et al., 1999

Other patents focused on incorporating drugs into moulded ODTs; Chungi et al (2006) prepared Olanzapine ODTs and provided the appropriate relative percentage by weight to prepare such formulations. Venkatesh et al (2009d) succeeded in preparing diphenhydramine ODTs using moulding technique.

Shimizu et al (2008b) designed a method to prepare coated benzimidazole ODTs by moulding. Firstly, a core of crystalline cellulose, lactose, acid-labile active ingredient and basic inorganic salt was prepared followed by coating with a water-soluble polymer. The resultant composition was enteric coated (2-3 layers) with polyethylene glycol, triethyl citrate and finally mannitol to form granules. The resultant granules were then blended with additives and moulded at low pressure (0.5-3ton/cm^2), to form tablets. Only one patent, filed by Myers et al (1999), had designed an apparatus for formulating moulded ODTs. The apparatus implements the mixing, filling, tamping and curing procedures in a continuous process and consists of three stations; mixing station, tamping/forming station which applies low pressures to form the dosage forms and a curing station where the formed matrix is bound and crystallized by subjecting to heat or controlled moisture.

1.2.3. Freeze-drying

Freeze-drying or lyophilisation is a process of removing solvent (water) at a temperature below its freezing point under the influence of high vacuum, by a physical process called sublimation. Sublimation occurs when a frozen liquid goes directly to the gaseous state bypassing the liquid state. Thus, removing the solvent at the solid state retains the structure of the formulation and consequently creates a highly porous structure (Mascarenhasa et al., 1997).

Freeze-drying has been used extensively in the drying process of thermo-labile active proteins and biological drugs, as the drying is carried out at low temperatures. However, the ability to form highly porous structures has attracted scientists to apply freeze-drying in fabricating tablets that allow faster penetration of disintegrating medium and disrupt the structure quickly, causing complete disintegration (Segar, 1998). Compared to other freeze-dried products such as lyophilized proteins and small molecules, tablets are exposed to many mechanical stresses during packaging, shipping and handling by patients, therefore a binding agent should be included in the formulation, which upon drying forms a continuous matrix that has definite shape (Chandrasekhar et al., 2009). The binder should be hydrophilic in

nature, to allow fast dispersion in the saliva, and, preferably with low glass transition temperature (37°C), which helps in the disintegration, and gives a smooth texture after disintegration (Segar, 1998). In addition, the formulation includes highly hydrophilic small molecules that disperse in the binder solution, cementing the porous structure in the dry state and dissolve upon hydration with the saliva and consequently disrupt the structure of the tablet. This type of material is usually referred to as matrix supporting/disintegration enhancing agents (AlHusban and Mohammed, 2010).

Other additives incorporated in the formulation include taste masking agents, colorants, in addition to stability promoting agents that ensure the integrity of the formulation during and after freeze-drying (Segar, 1998). Active drugs with varied physicochemical properties and at varied doses can be incorporated within the formulation as a solution, suspension or emulsion provided the drug has sufficient stability in an aqueous environment. However, for water-soluble drugs a limitation in the maximum dose is imposed by the plasticising effect of the drug molecules on the matrix system that results in lowering of the glass transition temperature or eutectic melting temperature and consequently lowers the collapse temperature resulting in longer freeze drying regimes. This limitation can be solved by adding crystallising agents within the formulation which gives rigidity and stability to the formulation against possible collapse. Another strategy to increase drug loading of water-soluble drugs, is to promote complex formation between the drug molecules and ion exchange resins, to conceal their plasticising behavior, which also provide an additional benefit of masking the taste of bitter drugs (Segar, 1998). Insoluble drugs can be incorporated in freeze-dried tablets without complications, by preparing aqueous suspensions or emulsions of the drug, which might need the addition of suitable thickening or emulsifying agents that does not deteriorate the properties of the tablets (Sastry et al., 2000).

After preparing the liquid system of the drugs and the required excipients, the formulation is filled into blister cavities, frozen at low temperature and then freeze-dried in suitable conditions. The resultant tablet requires special packaging to provide extra protection from external pressure and moisture, as the tablets can fracture and absorb moisture easily because of their spongy and highly porous nature (Dobetti, 2001).

Recent patents in lyophilised ODTs are principally different only in their disclosed excipients, mainly the polymeric binder and matrix supporting/disintegration enhancing agents, as the

general procedure for manufacturing lyophilised ODTs (see above) is common in all the patents.

Remon et al (2000) described a lyophilised ODT which is able to deliver a wide range of active ingredients utilizing maltodextrin, having a DE (dextrose equivalent) value between 12 and 40, isomalt or mixures of both as matrix forming agents, and water-soluble polymers such as xanthan gum, methylcellulose and hydroxypropyl methylcellulose as binding agents.

Johnson et al (2002) disclosed the use of a wide range of polymeric materials from animal, plant or synthetic origin as binding agents such as; gelatin, dextrin, acacia, guar, agar, xanthan, polysaccharides, alginate, dextran and polyvinylpyrrolidone in addition to sugars, sugar alcohols and/or amino acids as matrix supporting/disintegration enhancing agents to manufacure ODTs of a dopamine agonist and testosterone.

Li et al (2007) described a lyophlised ODT composition intended to solve some ethical and formulation problems associated with using gelatin as a main excipient. The composition which comprises of pullulan as a binder and amino acids as matrix supporting/disintegration enhancing agents is claimed to have easier formulation steps and shorter freeze-drying time than gelatin based systems.

1.2.4. Tablet loading

Recently, a patent was granted which refers to disintegrating loadable tablets (Holm and Slot, 2009). Disintegrating loadable tablets in compressed form, comprise of at least 60% w/w of a sorbent material selected from metal oxides (for e.g. magnesium oxide) and metal silicates (for e.g. sodium silicate) having a specific surface area of at least $50m^2/g$ or mixtures of such sorbent materials, hydrophilic substances and a disintegrant or a mixture of disintegrants (0.5-15% w/w). A hydrophilic substance (15% w/w) for example glucose functions as a wetting agent or a humectant. A suitable superdisintegrant was sodium carboxymethyl cellulose.

Prior to loading, the tablet in compressed form has; a porosity of 45% v/v or more, a hardness of at least 20 Newton, and a loading capacity of at least 30% of a liquid. The loading of the tablet with the active substance (in pharmaceutically acceptable liquid formulation), involves spraying the liquid onto the tablet or by placing the tablet in an excess of the pharmaceutically acceptable liquid formulation to saturate the tablet.The pharmaceutically acceptable liquid

formulation can comprise; an oil/oily-like material (for example a vegetable oil), or a pharmaceutically acceptable solvent, which can be in the form of an emulsion, microemulsion or a suspension. Loadable tablets may also contain other pharmaceutically acceptable excipients, for example; fillers, diluents, binders etc.

This method of preparing an ODT is particularly suitable for the loading of tablets with substances having low water-solubility, as these substances can be dissolved in oil/oily-like materials and especially in such cases where the substance is desired to be delivered in microcrystalline and/or amorphous form to increase release and absorption (Holm and Slot, 2009).

1.2.5. Compression of pulverized components

In 2007 a patent was published which relates to a novel method of manufacturing ODTs, by compressing components which are in a pulverized form. It is claimed that tablets produced from this method, have a similar porous structure as usually that results from freeze-drying processes (Bauer and Rohrer, 2007).

The manufacturing process begins with preparing a dry mixture comprising a suitable binding agent, such as acacia, active ingredients, fillers (ex. Mannitol) and other components (lubricants). Liquefied or compressed gases (ex. fluoroalkanes) or gas mixtures (ex. azeotropic mixtures) under high pressure, optionally in the presence of low-boiling solvents (ex. methanol), is used to moisten the dry mixture. This is followed by stirring, homogenisation and the production of the mouldable plasticized mass in an autoclave, where the high pressures can be tolerated. The tablet is produced by filling the wetted mixture into a mould under pressure (between normal pressure and up to 100 bar). Decompression process is applied to remove the gaseous component and consequently creating highly porous ODTs (Bauer and Rohrer, 2007).

1.2.6. Factors affecting the selection of technology

1.2.6.1. Manufacturing cost

The general cost of manufacturing ODTs, varies considerably from one technology to another. Freeze-drying technology is considered an expensive method of manufacturing ODTs. Long freeze-drying cycles, complex and specialist industrial plants, processes and equipment, are responsible for the high production costs (Tang and Pikal, 2004). Compression of pulverized components technology can also be considered as an expensive method of manufacturing ODTs, as specialist equipment is required which can tolerate the high pressures used during the manufacturing process. Meanwhile, the use of direct compression and granulation-compression methods to manufacture ODTs is considered a more cost effective method, where production costs are much lower, as standard equipment and materials are used.

1.2.6.2. Active ingredient dose

The dose of drug which can be incorporated into an ODT relies heavily on the technology used to manufacture the tablets. High doses of active ingredients can be incorporated into tablets prepared by moulding and standard tableting methods, up to 1000mg per tablet. However in freeze-drying, incorporation of high doses of water-soluble active ingredients can be challenging, typically up to 60mg of water-soluble drugs a tablet (Lee et al., 2002).

1.2.6.3. Physicochemical properties of active ingredients and excipients

The physicochemical properties of active ingredients and excipients can be factors which determine the technology to manufacture ODTs. Technologies such as direct compression and moulding appear flexible and versatile when it comes to the physicochemical properties of active ingredients and excipients. They generally include a mixture of excipients which exhibit high aqueous solubility and good mouldabilility, which ensure the formation of robust tablets with rapid disintegration profiles.

Meanwhile, technologies such as freeze-drying and tablet loading appear more selective when it comes to the physicochemical properties of the active ingredients and excipients. The active drug should exhibit sufficient stability in solution to allow efficient incorporation in to the final dosage form. Also, excipients for freeze dried ODTs should accomplish stringent characteristics

such as reasonable drying time, stability during freeze-drying process, as well as formation of elegant tablets with short disintegration time and adequate mechanical properties (Chandrasekhar et al., 2009). Nevertheless, freeze drying is principally more suitable than other technologies in cases of heat sensitive active drugs as the manufacturing is carried out at low temperatures.

1.2.6.4. Required performance and properties of the ODTs

The performance and properties of ODTs varies considerably, based on the technology used to prepare the tablets. For example the use of freeze-drying technology produces highly porous tablets which disintegrate and dissolve smoothly in the oral cavity in a matter of seconds, but these tablets show poor physical properties in terms of tablet hardness and fracturability (Fu et al., 2004). Whilst the use of direct compression and standard tableting technologies produce tablets which exhibit better physical properties than the freeze dried tablet, but generally most of them need a minute to disintegrate/dissolve completely in the oral cavity (Lee et al,. 2002).

1.2.7. Current and future developments

The review of ODT patents from 1999 to 2010 has shown that current technologies namely; compression-based methods, moulding and freeze-drying, have been extensively researched, developed and modified. In particular, compression-based methods (direct compression and granulation-compression) are the technologies which have seen the most extensive development and modification, as these methods are more easily adapted and developed. Areas of these technologies which have been developed and modified include; method modification, selection of specific excipients and post compression treatment of the tablets. Other areas of these technologies which have been developed include; development of ODTs for a specific active ingredient or group of active ingredients, various taste-masking approaches, in case of bitter-tasting active ingredients, and coating technologies.

The last ten years has seen the emergence of novel technologies and methods of manufacturing ODTs, such as tablet loading, compression of components which are in pulverized form and sublimation. Based on the review of patents of the last ten years, the

future development of ODTs appears to lie with the emergence of new technologies to produce ODTs which exhibit both rapid oral disintegration and improved physical properties (hardness and fracturability).

In terms of freeze-drying technology, developments in method modification and/or material selection is required in order to enhance the physical strength of the tablet and minimise the primary drying time of the freeze-drying cycle, as this will result in a shorter and more efficient manufacturing process. In terms of compression-based technologies, developments need to take place which will lead to an increase in tablet porosity, as this is the limiting factor in the fast disintegration of tablets manufactured from compression-based methods, and to simplify the manufacturing steps especially when incorporating multiparticulates into compressed ODTs.

Development of taste-masking technologies to ensure that bitter-tasting active ingredients can be administered conveniently to patients is also required. Finally, the development of ODTs which exhibit sustained, modified or controlled release/delivery of active ingredients will ultimately improve the therapeutic efficiency and treatment of a variety of medical conditions, through reducing frequency of dosage administrations.

1.3. Rationale and aim of the research project

Among the existing approaches to prepare ODTs, lyophilisation (freeze drying) has been considered the most successful in terms of sales value, sales volume and number of products available on the market (Muir, 2007). As mentioned previously, the disintegration time for the lyophilised tablets is very short (the shortest among other technologies) due to their highly porous and hydrophilic matrix. However, the formulation still suffers from some disadvantages that need to be addressed. The tablets usually have very poor mechanical properties (Kuno et al., 2005; Narazaki et al., 2004; Fukami et al., 2006) and require protection in the form of specialized packaging like the ZYDIS blister peel back packing. Furthermore, the formulation of lyophilised ODTs is usually restricted by the dose and characteristics of the drug that can be incorporated. For instance, the maximum dose of water insoluble drug is less than 400mg and for water soluble is 60 mg. Also, the drug should be chemically stable with acceptable taste and particle size smaller than 50 µm (Segar, 1998).

Although there are many patents describing the preparation of ODTs by lyophilisation very scanty literature is available detailing factors that control mechanical properties and disintegration time of these formulations (Ahmed and Aboul-Einien, 2007; Corveleyn and Remon, 1998). Accordingly, the current research aims to investigate the role of formulation excipients and processes in the development of lyophilised orally disintegrating tablets (ODTs) and use this knowledge to achieve further advances in the field. The research strategy is rationalised as follows:

- Chapter two: to investigate the role of the most common excipients used in commercial products (gelatin and saccharides) for their influence on the manufacturing process and performance of the lyophilised ODTs.

- Chapter three: to develop saccharide free ODTs by investigating the feasibility of using naturally occurring amino acids, individually, as a matrix supporting/ disintegrating enhancing agent.

- Chapter four: to investigate the feasibility of novel combinations of two amino acids to combine the benefits of the incorporated amino acids and minimize their drawbacks, and to determine the influence of the freezing protocol on tablets characteristics and primary drying time.

- Chapter five: to optimise ODT formulations suitable for the delivery multiparticulate systems of challenging drugs using a novel formulation that exploite the electrostatic associative interaction between gelatin and carrageenan

- Chapter six: to explore advantageous natural polymers for their use as a binder in the formulation of lyophilised ODTs to replace gelatin with ethically and morally accepted components.

- Chapter seven: to study and optimised the application of gum arabic as a binder in the formulation of lyophilised ODTs to deliver highly water soluble, slightly soluble or insoluble active drugs using factorial design studies.

Chapter Two: Preparation, Optimisation and Characterisation of Lyophilised ODTs Based on Gelatin and Saccharide

Papers relating to this chapter

Alhusban, F., Perrie, Y., Mohammed, A. (2010) Preparation, optimisation and characterisation of lyophilised rapid disintegrating tablets based on gelatin and saccharide. *Current Drug Delivery,* 7, 65-75.

Alhusban FA, Perrie Y, Mohammed A - Investigation of formulation factors on the sublimation rate of orally disintegrating tablets. **37th Annual Meeting of the Controlled Release Society**, July 2010, Portland, USA.

Alhusban FA, Perrie Y, Mohammed A - Influence of gelatin bloom strength and concentration on the hardness and disintegration time of rapidly disintegrating solid formulations. *Journal of Pharmacy and Pharmacology,* 2008; 60(Suppl. 1): A-95.

Preparation, Optimisation and Characterisation of Lyophilised ODTs Based on Gelatin and Saccharide

2.1. Introduction and Aims

Despite recent success, many orally disintegrating tablets (ODTs) still face problems of low mechanical strength and therefore require protection in the form of specialized packaging (Abdelbary et al., 2004; Kearney, 2002).

This chapter aims to find a practical balance between the mechanical properties and disintegration time of lyophilised ODTs based on gelatin and saccharides through careful optimisation of gelatin bloom strength and concentration in addition to type and concentration of the saccharide. Tablets containing gelatin with different bloom strength value, low (60 bloom) and high (225 bloom), at concentrations of 2, 5, 7.5, and 10 %w/w were formulated and characterised to determine the ideal gelatin concentration and bloom strength to be used for further studies. Moreover, the effect of gelatin stock solution concentration on the sublimation rate and product temperature during the primary drying process was investigated. Five saccharides, xylitol, glucose, trehalose, maltotriose and mannitol, at concentrations ranging from 10 to 80 % w/w (of total solid material) were incorporated in the optimised gelatin solutions and the resultant formulations were characterised in terms of thermal properties, physical appearance, mechanical strength and disintegration time. Clonidine HCl ($C_9H_9Cl_2N_3$. HCl, MW: 266.55 g/mol) was incorporated in the optimised formulation as it is one of the off patent drugs included in the priority list published in 2007 by European Medicines Agency for development of paediatric formulation. It is a centrally acting alpha2-adrenoceptors agonist, used in management of mild to moderate hypertension, and it is available as tablets for oral administration in three dosage strengths: 0.1 mg, 0.2 mg and 0.3 mg (Katzung, 2005).

2.2. Selection of excipients

All the excipients used in this study are well known as safe materials for human use and have been used or investigated in many pharmaceutical applications. Gelatin is a pure protein that is obtained by thermal denaturation of collagen. It is widely used in solid oral dosage forms, i.e. hard and soft gelatin capsules, as it forms thermo-reversible gels upon hydration with melting points around 35-37 °C (just below body temperature). It occurs in different bloom strength according to its gel rigidity, with the higher bloom strength value forming more rigid gel (Segtnan and Isaksson, 2004). In the formulation of lyophilised ODTs, gelatin has been used extensively as a binder in ZYDIS® products to give shape and resilience to the table after freeze drying (Seager, 1998). The saccharides with varied physicochemical properties and structural features were studied to determine their effect on the formulation of ODT.

Xylitol, $C_5H_{12}O_5$, is a naturally occurring non-reducing sugar alcohol with a molecular weight of 152.15 g/mole. Due to the sweet taste, cooling sensation in the mouth and non-cariogenic profile xylitol has been used extensively in orally administered products such as chewing gums (Moss, 1999). However, its highly hygroscopic nature may affect the stability of the product (Ciper and Bodmeier, 2005).

Glucose is a reducing monosaccharide that is globally used as intravenous diluent. The empirical formula of glucose is $C_6H_{12}O_6$ and the molecular weight is 182 g/mole. Glucose has been recently investigated as a lyoprotectant in many pharmaceutical formulations (Shahgaldian et al., 2003; Zhang et al., 2008).

Trehalose is a non-reducing disaccharide consisting of two glucose unit linked by an α,α-1,1-glycosidic linkage. The empirical formula is $C_{12}H_{22}O_{11}$ and the molecular weight is 342.31 g/mole. Trehalose occurs as white crystals with low hygroscopic profile (Richards et al., 2002; Elbein et al., 2003). It has been included in many pharmaceutical formulations as a lyoprotectant, such as cellular, protein (Elbein et al., 2003) and liposomal (Christensen et al., 2007; Mohammed et al., 2007) formulations, and as a diluent in tablets (Rowe et al., 2006).

Maltotriose is a trisaccharide formed by two 1, 4 glycosidic linkage between three D-glucose molecules. It has a molecular formula of $C_{18}H_{32}O_{16}$ and weight of 504.44 g/mole.

Mannitol is a naturally occurring non-reducing sugar alcohol. The molecular formula and weight are $C_6H_{14}O_6$ and 182.17 g/mole, respectively. Mannitol is used extensively in tablet

formulation, due to its low hygroscopic profile, sweet taste and cooling sensation in the mouth (Rowe et al., 2006). It is, also, used as bulking agent in lyophilised preparations, as it readily crystallise and consequently improve the appearance and stability of the product (Pyne et al., 2003). Furthermore, mannitol is incorporated into ZYDIS® products to give crystallinity, hardness and elegance appearance (Seager, 1998).

2.3. Materials

Type B gelatin with 60 bloom strength (from calf skin) and 225 bloom strength (from bovine skin), xylitol, D-glucose, maltotriose, D-mannitol, and clonidine hydrochloride were purchased form Sigma-Aldrich Chemicals (Pool, UK). Trehalose (anhydrous) was supplied by Acros (New Jersey, USA). Triethylamine was supplied by Fisher Scientific (Loughborough, UK). All the chemicals used were of analytical grade.

2.4. Methods

2.4.1. Preparation of lyophilized tablets

2.4.1.1. Influence of gelatin bloom strength and concentration

Gelatin of different bloom strength (60 and 225) was dissolved in double distilled water at about 40 ºC to obtain a concentration of 2, 5, 7.5 and 10% w/w. 1.5 g of the solution was poured into a bijou tube, frozen at -80 °C for about 60 minutes and freeze-dried (ADVANTAGE Freeze-dryer, VIRTIS) according to an optimized regime (primary drying for 48 hours at shelf temperature of -40 °C and secondary drying for 10 hours at shelf temperature of 20 °C and vacuum of 50 m Torr. All the formulations were prepared in triplicate from three independent batches.

2.4.1.2. Influence of varying the concentration of different Saccharides

Xylitol, mannitol, glucose, trehalose and Maltotriose were added individually to 2 or 5 % (w/w) low bloom strength gelatin (60) stock solutions at concentration of 10, 20, 30, 40, 50, 60, 70, and 80 % of total solid material. 1.5 g of the solution was poured into a bijou tube, frozen at -

80 °C for about 60 minutes and freeze-dried (ADVANTAGE Freeze-dryer, VIRTIS) according to the optimized regime (primary drying for 48 hours at shelf temperature of -40 °C and secondary drying for 10 hours at shelf temperature of 20 °C and vacuum of 50 m Torr. All the formulations were prepared in triplicate from three independent batches.

2.4.1.3. Orally disintegration tablets containing clonidine HCl

17.120 mg clonidine HCl was solubilised in 256.745 g of a solution consisting of 237.500 g double distilled water, 12.500 g low bloom strength gelatin (60), 5.355 g mannitol and 1.390 g trehalose. This formulation resulted in a clonidine HCl dose of 100 µg per 1.500 g solution. 1.500 g of the solution was poured into a bijou tube, frozen at -80 °C for about 60 minutes and freeze-dried (ADVANTAGE Freeze-dryer, VIRTIS) according to the optimized regime (primary drying for 48 hours at shelf temperature of -40 °C and secondary drying for 10 hours at shelf temperature of 20°C and vacuum of 50 m Torr. The formulation was prepared in triplicate from three independent batches.

2.4.2. Sublimation rate and product temperature

Gelatin (60 bloom strength) was dissolved in double distilled water at about 40 °C to obtain a concentration of 2, 3.5, 5, 7.5 and 10% w/w. 1.5 g of the solution was poured into a PEG mould (13.80 mm diameter, 8.50 mm height), frozen at -80 °C for 60 minutes and freeze-dried at shelf temperature of -40 °C, condenser temperature of -80 °C and 55 mTorr vacuum. Samples were withdrawn from the freeze dryer at predetermined time intervals (2, 4, 10, 16, and 24 hours) and the amount of water sublimed was evaluated using weight difference method. The product temperature was automatically recorded using thermo couple that was inserted in the central bottom of the tablet.

2.4.3. Total porosity

The relative porosity was calculated from the apparent and true density of the tablet. Apparent density was found by dividing the mass of the tablet by the measured volume. The strut density was determined using helium pycnometry (Accupyc 1330, Micromeritics, UK)

with 3 cm^3 sample cup at 22 °C. Prior to analysis the helium pycnometry was calibrated against a standard steel ball. Each determination included 10 purges at 19.5 psi and 10 analytical runs at 19.5 psi with an equilibration rate of 0.0050 psi/min.

2.4.4. Differential scanning calorimetry studies

Differential scanning calorimetry (Pyris Diamond DSC and Intracooler 2P: Perkin Elmer, Wellessey, USA) was employed to determine glass transition temperatures (Tg) and crystallisation event of the formulation in their liquid state (before freeze drying). 10-15mg of the liquid samples were loaded into aluminium pans, cooled to -65 °C and then heated to 20 °C at 5 °C/min with a nitrogen purge of 20ml/min. An empty aluminium pan was used as reference for all measurements. The resulting graphs were analysed by Pyris manager software. Tg value was determined from the intersection of relative tangents to the baseline. All the measurements were done in triplicate of independently prepared samples.

The DSC was calibrated for temperature and heat flow using standard samples of indium (melting point: 156.6 °C, ΔHm: 28.42 J/g) and Zinc (melting point: 419.5 °C, ΔHm: 108.26 J/g).

2.4.5. Mechanical properties of the tablets

The mechanical properties of the tablets (hardness and fracturability) were investigated with a texture analyzer (QTS 25: Brookfield, Essex, UK) equipped with a 25 kg load cell. The instrument was calibrated by standard weight of 500 g and 5 kg. The tablet was placed in a holder with a cylindrical hole. The hardness was taken as the peak force after 1mm penetration of 5mm diameter probe at a speed of 6 mm/min. Fracturability was the peak force after 3mm penetration of 1mm diameter probe at a speed of 6 mm/min. The results were average of three measurements from independently prepared batches.

2.4.6. Disintegration time of the tablets

The disintegration time of the tablets was measured using a USP disintegration tester (Erweka ZT3, Erweka Apparatebau, Germany). Distilled water (800 ml) kept at 37 °C was used as a medium and the basket was raised and lowered at a fixed frequency of 30 cycles/min. At each

time, one tablet was placed in the basket rack assembly and covered by transparent plastic disk. The disintegration time was taken as the time required for ODTs to disintegrate completely without leaving any solid residue. All the formulations were evaluated in triplicate and standard deviation was calculated.

2.4.7. Morphological examination

The inner structural morphology and pore size of the freeze-dried tablets were examined by scanning electron microscopy (SEM, STEREOSCAN 90, Cambridge Instrument). A thin horizontal cross-section sample was prepared by cutting the tablet with a razor blade. The samples were placed onto double-sided adhesive strip on an aluminium stub. The specimen stub was coated with a thin layer of gold using a sputter coater (Polaron SC500, Polaron Equipment, Watford, UK) at 20 mA for three 3 minutes and then examined by the SEM. The acceleration voltage (kV) and the magnification can be seen on each micrograph.

2.4.8. HPLC analysis of clonidine HCl

HPLC analysis of clonidine HCl in the tablets was carried out using Reverse phase HPLC (Dionex AS 50 autosampler with GP50 gradient pump HPLC System: Dionex, UK) at room temperature using a 4.6 x 150 mm column (Phenomenex La Luna: Phenomenex, Torrance, USA) and 20 µL injection volume, with UV detection at 245 nm. The mobile phase consisted of methanol-water (60/40) and 0.5% Triethylamine (Sarisuta et al., 1999). The mobile phase flow rate was 1 ml/min. Under these HPLC conditions the retention time for clonidine HCl was about 3.35 minutes. Concentration of clonidine HCl in the tablets was determined by reference to a calibration curve prepared from dilutions of stock solution of clonidine HCl (5-100 µg/ml), using water as solvent. The calibration curve was performed in triplicate and resulted in a linear correlation in the concentration range studied (r^2=0.99).

2.4.9. Viscosity and pH measurements

The lyophilised tablet was dissolved in 2 ml water in order to measure the pH and viscosity. All the measurements were done in triplicate from independently prepared samples.

The pH was measured using a pH meter (MP230, Mettler Toledo) at room temperature. The pH meter was calibrated using standard solutions at pH 4 and 7.

The viscosity was measured using the automated micro-viscometer (Anton Parr, AMVn, Graz, Austria). Each sample (400 µL) was loaded into a glass capillary (diameter: 1.8 mm) using a 1ml syringe, and care was taken to ensure that no air bubbles were present in the loaded sample within the capillary. The glass capillary was loaded into the capillary block, where the temperature of the sample was equilibrated at 25 ˚C. Viscosity measurements were conducted by measuring the rolling ball time (ball diameter: 1.5 mm) four times through the capillary at an angle of 50˚.

2.4.10. Statistical analysis

The hardness, fracturability and disintegration of the lyophilised tablets produced from varied concentrations of low or high bloom strength gelatin were compared using one-way analysis of variance with Tukey-Kramer multiple comparison test. The hardness, Fracturability and disintegration of the lyophilised tablets after inclusion of saccharides were statistically compared to those of the control (composed of gelatin only) using one-way analysis of variance with Dunnett multiple comparison test. The significance level was 0.05.

2.5. Result and discussion

2.5.1. Studying the Influence of gelatin bloom strength and concentration on the hardness and disintegration time

2.5.1.1. Mechanical properties of the tablets

Generally, tablet dosage forms are exposed to various mechanical stresses during the manufacturing steps (e.g. packaging process), shipping and handling by patients. Therefore a successful tablet formulation must have an adequate mechanical strength.

In this study, the mechanical properties of the tablets were evaluated by applying combinations of compression and shear force. The hardness was measured using a 5 mm diameter probe, which provides more compression force. While the fracturability was measured using a 1 mm diameter probe, which provides more shear force. The results of the

hardness and fracturability are presented in **Figures 2.1** and **2.2**, respectively. The hardness of the tablets (**Figure 2.1**) increased significantly by increasing gelatin stock solution concentration (one way ANOVA/Tukey-Kramer: $\rho<0.05$). Surprisingly, the results showed that there was no impact of gelatin bloom strength on the hardness of the lyophilized tablets. Ciper and Bodmeier (2005) noticed that high bloom strength gelatin enhanced the mechanical properties of fast disintegration capsule. **Figure 2.2** illustrates the effect of gelatin bloom strength and stock solution concentration on the fracturability of the tablets. The test was not carried out for tablets that were fabricated from 2% gelatin stock solution. This was because the test's probe (1mm diameter) was unable to penetrate these tablets, due to the spongy nature of these tablets that tend to deform in response to the force applied by the probe. The results showed that increasing gelatin concentration in the stock solution significantly enhanced the fracturability of the tablets (one way ANOVA/Tukey-Kramer: $\rho<0.05$) with no significant difference between tablets based on low or high gelatin bloom strength at similar concentration (one way ANOVA/Tukey-Kramer: $\rho>0.05$). The results suggested that the fracturability of the tablet was influenced by the concentration of gelatin in the stock solution rather than the bloom strength.

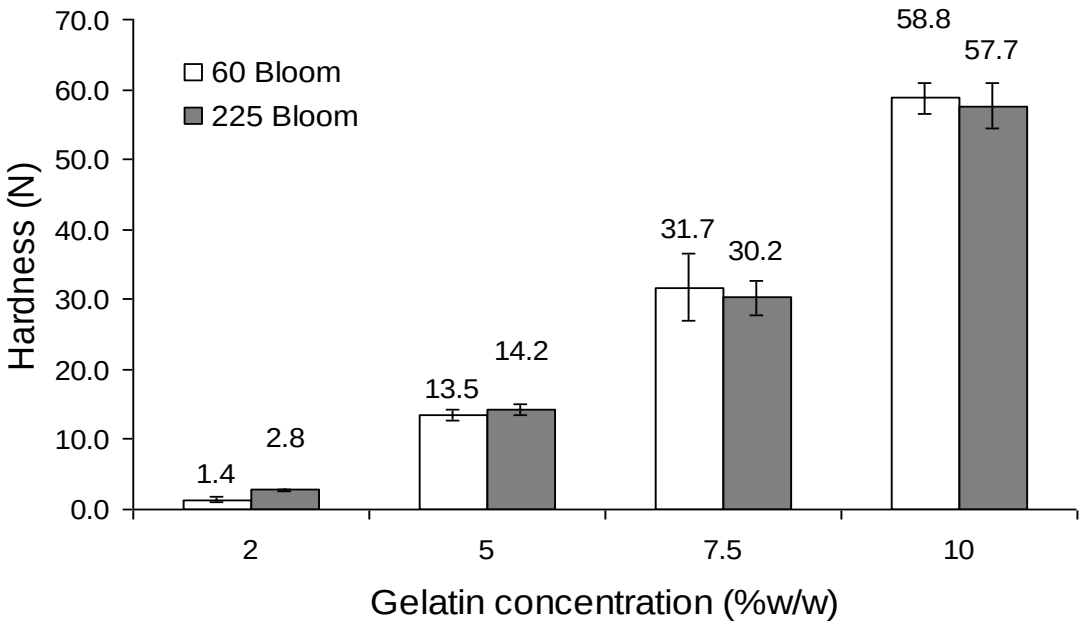


Figure 2.1 The effect of gelatin bloom strength and concentration in the stock solution on the hardness of the lyophilised tablets. (Mean ± SD, n=3).

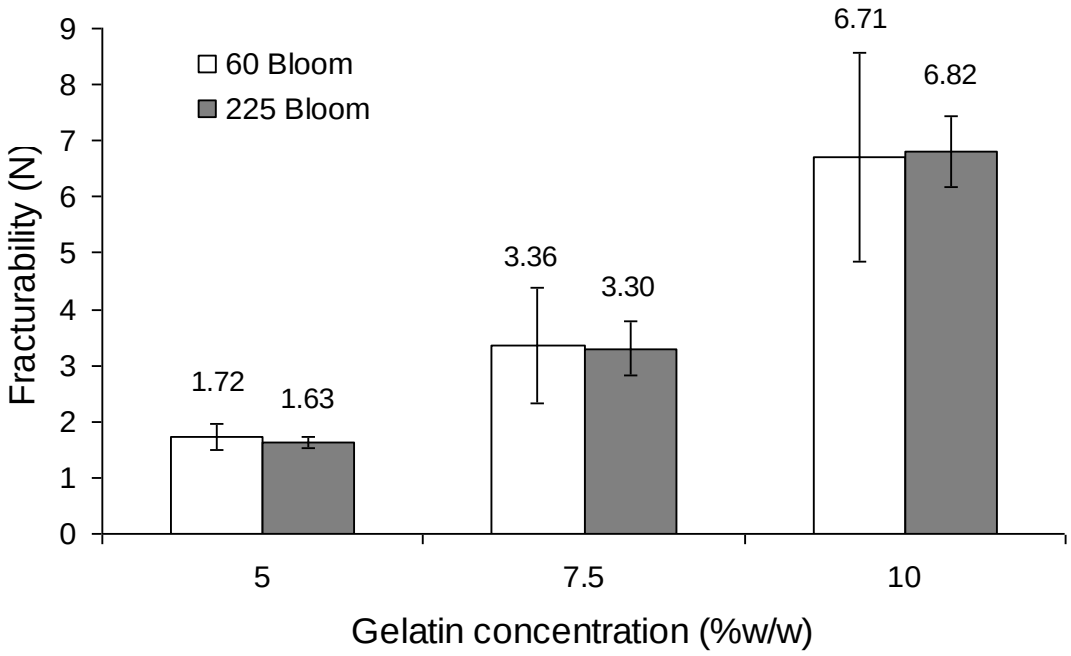


Figure2.2 The effect of gelatin bloom strength and concentration in the stock solution on the fracturability of the tablets. (Mean ± SD, n=3).

2.5.1.2. Disintegration time of the tablets

The assessment of the disintegration time is considered the fundamental issue in optimising and developing fast orally disintegrating tablets. According to the U.S. FDA specification, the disintegration time of such tablets should not exceed 30 seconds (US FDA, 2007). The effect of gelatin bloom strength and concentration in the stock solution on the disintegration time of the lyophilised tablets is presented in **Figure2.3**. The results showed that the disintegration time of the tablets decreased with decreasing gelatin bloom strength and stock solution concentration. The tablets produced from low bloom strength gelatin at 2, 5, 7.5 and 10 % (w/w) stock solution disintegrated in 3, 29, 189 and 360 seconds respectively, whilst high bloom strength gelatin (225) at similar concentrations disintegrated in 6, 38, 348 and 608 seconds, respectively. Also, the results indicated that the differences in the disintegration time between the tablets produced from high and low bloom strength gelatin increased with increasing gelatin stock solution concentration (3, 9, 159 and 448 seconds at 2, 5, 7.5 and 10% w/w stock solution, respectively).

Despite the fact that the USP disintegration test was able to detect the difference in the disintegration time between the formulations in the current study, concerns about the reliability, accuracy and suitability of the test to evaluate fast disintegrating tablets were experienced. The qualitative nature of the test that depends on the visual evaluation in addition to the fact that few seconds' inaccuracy in evaluating the disintegration time can cause huge error (as the disintegration time is very short) are the main problems associated with this test. Accordingly, new method for measuring the disintegration time is required for better evaluation and development of fast orally disintegrating tablets.

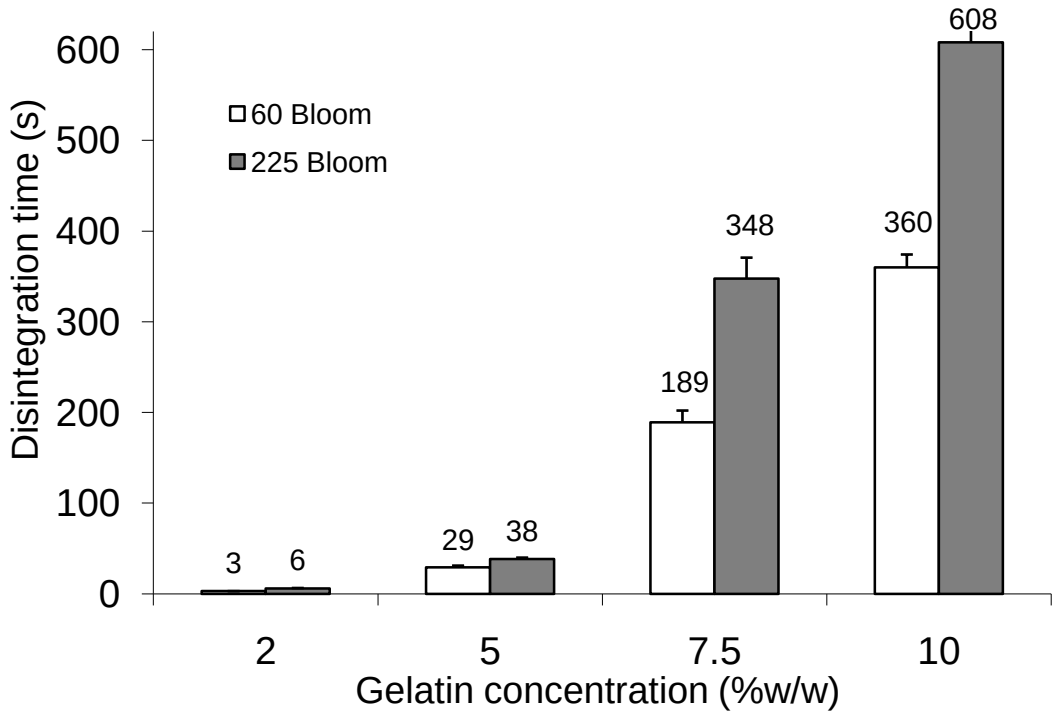


Figure 2.3 The effect of gelatin bloom strength and concentration in the stock solution on the USP disintegration time of thelyophilised tablets. (Mean ± SD, n=3).

2.5.1.3. Morphological examination

The inner structure of the lyophilised tablets was viewed by scanning electron microscopy. SEM micrograph offers a great opportunity for direct assessment of the size, shape and direction of the pores inside the tablets, thus aiding in understanding and explaining the differences in properties of the tablets. **Figure 2.4** shows SEM micrographs of lyophilised tablets produced from 2, 5, 7.5 and 10% gelatin stock solutions. The gelatin molecules in the tablets produced from freeze drying of a 2% low bloom strength (60 bloom) gelatin solution (**Figure 2.4 a**) seemed to be arranged in two dimensional ordered channel structure with an average channel height of about 35 µm. The walls of these channels were very thin (like a ribbon) and connected to each other along a length of 50 - 100 µm by very thin bridges (**Figure 2.4 a**). Partially, the same features were noticed in the case of 5% low bloom strength gelatin but with more partitioned channel and thicker wall (**Figure 2.4 b**). Higher concentration (10, 7.5 and partially 5%) of low bloom strength gelatin solution resulted in tablets composed of polygonal to spherical shaped pores arranged in three dimensional orders (**Figures 2.4 d, c**, and **b**, respectively). The pores were about 80, 40, and 70 µm in diameter, for the 5, 7.5, and 10% stock solution, respectively. Surprisingly, the 10% formulation had larger pores size compared to the 7.5% formulation, possibly due to fusion of the pores during the secondary drying phase. On the other hand, high bloom strength gelatin at the entire concentration range (2-10%) displayed three dimensional ordered pores which were polygons to spherical in shape (**Figures 2.4 e, f, g** and **h**). The pores were about 70, 40, 30, and 70 µm in diameter, for the 2, 5, 7.5, and 10% stock solution, respectively. However, all the high bloom strength formulation seemed to be more compacted than similar concentration of the low bloom strength. The formation of polygonal or spherical pores in gelatinous lyophilised tablets can be explained as a result of the film forming properties of gelatin molecules around water molecules in the gel state of the stock solution (Kaushik and Roos, 2006), as the freeze drying process is believed to retain the structure of the formulation (Abdelbary et al., 2004). The difference in the structural features between low and high bloom gelatin based tablets at low stock solution concentration (≤ 5%) can be explained in terms of their differences in gelling property. It is generally accepted that low bloom strength gelatin has weaker gelling capability (Segtnan et al., 2003); therefore it is incapable of forming film around the water at such low concentration and after freeze-drying channel like structure was formed instead of the spherical pores. This channel like structure seems to promote the entry of water and offers larger surface area for the water to interact and disrupt the intermolecular bond between

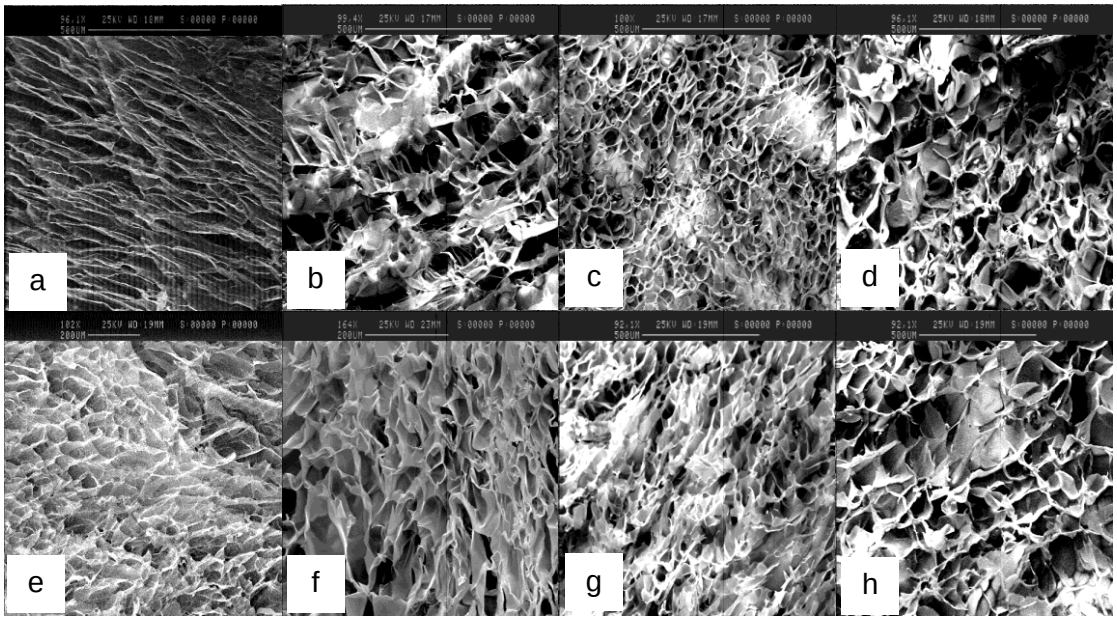


Figure 2.4 Scanning electron micrograph of the lyophilised tablets based on : a) 2% 60 bloom strength gelatin, b) 5% low bloom strength gelatin, c) 7.5% low bloom strength gelatin, d) 10% low bloom strength gelatin, e) 2% high bloom strength gelatin, f) 5% high bloom strength gelatin, g) 7.5% high bloom strength gelatin, and h) 10% high bloom strength gelatin.

gelatin molecules; thereby resulting in faster disintegration, as confirmed from the results (Figure 2.3).

2.5.1.4. Primary drying rate and product temperature during freeze drying

The effect of gelatin stock solution concentration on the primary drying rate is presented in **Figure 2.5**. The decrease in the sublimation rate with time is a result of increasing the thickness of the dried layer (Tang et al., 2006). The results clearly showed that increasing gelatin concentration in the formulation decreases the sublimation rate significantly, which means longer primary drying time is required to formulate ODT from high concentration of gelatin. The product temperatures during the primary drying (**Figure 2.6**) confirmed this observation as the fast sublimation rate of the formulation with low gelatin concentration maintained low product temperatures by increasing heat removal from the latent heat of sublimation (Patapoff and Overcashier, 2002).

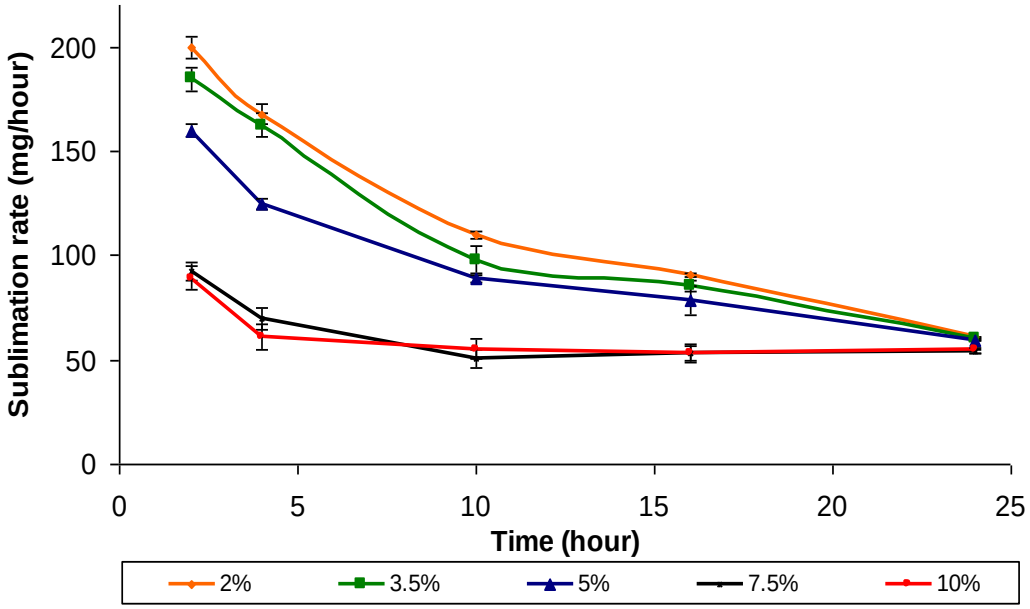


Figure 2.5 Effect of gelatin stock solution concentration on the sublimation rate of the ODT at shelf temperature of -40 °C vacuum of 50 m Torr. Results are mean ± SD, n=3.

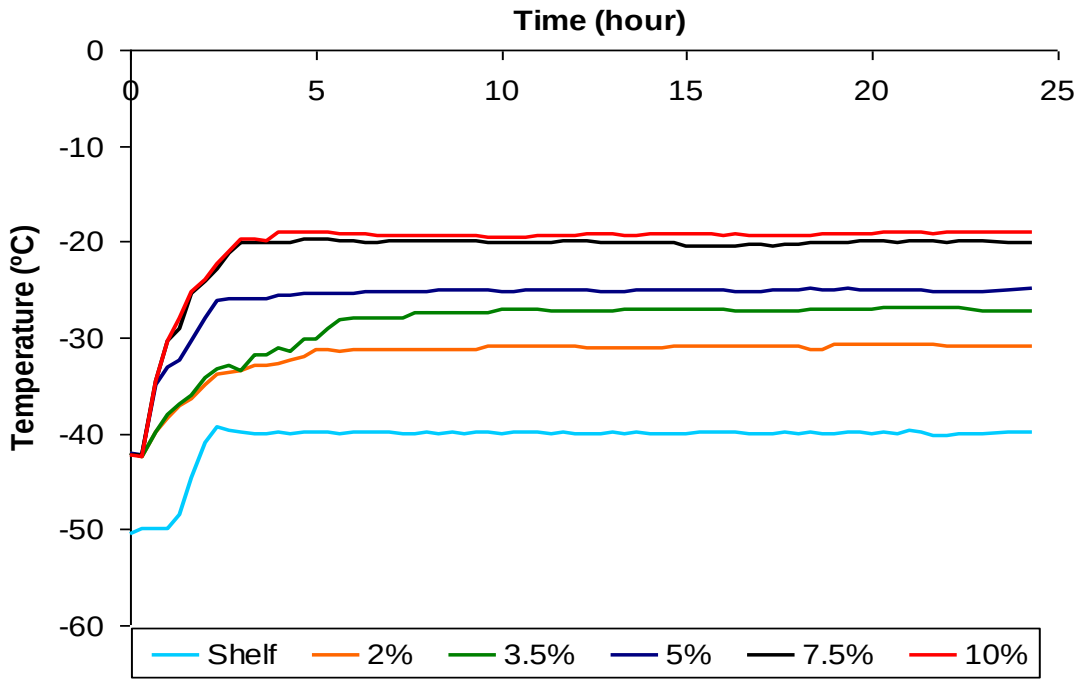


Figure 2.6 Effect of gelatin stock solution concentration on product temperature during primary drying at shelf temperature of -40 °C vacuum of 50 m Torr.

The results can be explained depending on the total porosity results of the lyophilized ODT (**Table 2.1**), where increasing gelatin concentration in the formulation decreases the total porosity and consequently increases the resistance of the dried layer to mass transfer of water vapor (MTR) (Patapoff and Overcashier, 2002).

Table 2.1 Total porosity of ODTs prepared from varied concentration of gelatin stock solution.

Gelatin concentration (%w/w)	Total porosity (%)
2.0	98.6 ± 0.1
3.5	97.6 ± 0.2
5.0	96.1 ± 0.2
7.5	90.3 ± 0.5
10.0	87.0 ± 0.4

2.5.2. Inclusion of varied concentration of saccharides

To the best of our knowledge, the effects of inclusion of saccharides on the properties of gelatin based lyophilised fast disintegrating tablets have not been documented. To investigate this, five saccharides, glucose, trehalose, maltotriose, xylitol and mannitol, were added to 2 and 5 % gelatin (60 bloom strength) stock solution with concentrations ranging from 10 to 80 % (w/w of solid material) and investigated for their disintegration time, mechanical and thermal properties.

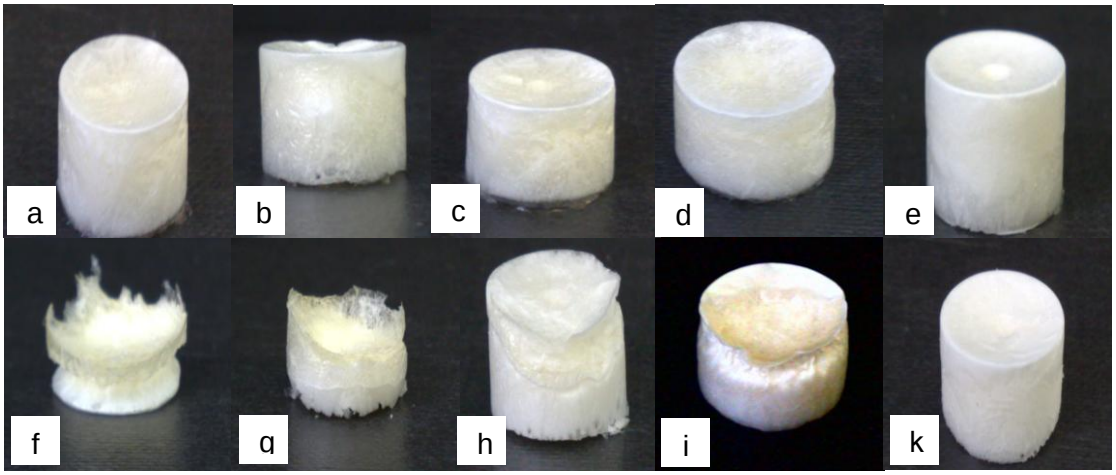
2.5.2.1. Impact of saccharides on the physical appearance of the tablets

Although all the formulations were freeze-dried according to the same cycle (primary drying for 48 hours, at a shelf temperature of -40 °C, secondary drying for 10 hours, at a shelf temperature of 20 °C and a constant vacuum of 50 m Torr) inclusion of saccharides showed different behavior in forming intact tablets depending on the type and concentration of saccharide used and on the concentration of gelatin stock solution. Addition of glucose or xylitol, up to 40 % (w/w of solid material), to both 2 and 5% gelatin stock solution resulted in

formation of intact tablets (**Figures 2.7a** and **b** respectively). However, higher concentration (50-80%) produced tablets with signs of deformation (**Figures 2.7f** and **g** respectively) as in the case of 5% gelatin stock solution, or with very weak mechanical properties as in the case of 2% gelatin stock solution. Trehalose and maltotriose provided intact tablets up to 50% (**Figures 2.7c** and **d**). On the other hand, addition of mannitol produced intact tablets throughout the entire concentration range (10-80%) (**Figures 2.7e** and **k**).

2.5.2.2. Differential scanning calorimetry investigation

Differential scanning calorimetry profiles of the liquid formulations were used to study the effect of saccharides at different concentration on the thermal properties (glass transition temperature and crystallisation event) of 2 and 5% gelatin stock solution. The glass transition temperatures of the frozen solution are summarised in **Table 2.2**. These values were based on 5%w/w gelatin solutions and have been confirmed for formulations based on 2%w/w gelatin (data not shown), where similar values were obtained. As expected, increasing the saccharides concentration in the formulation resulted in lowering their Tg, due to the plasticising effect of



Figures 2.7 Physical appearance of lyophilised tablets based on 5% gelatin stock solution after inclusion of: **a**) 30% xylitol w/w of total solid material, **b**) 30% glucose, **c**) 30% trehalose, **d**) 30% maltotriose, **e**) 30% mannitol, **f**) 60% xylitol, **g**) 60% glucose, **h**) 60% trehalose, **i**) 60% maltotriose, **k**) 60% mannitol.

Table 2.2 The glass transition temperature of 5% gelatin solution in water with 10, 30, 50, 70 % (of total solid material) of Xylitol, Glucose, Trehalose, Maltotriose and Mannitol.

Saccharide	Tg (˚C)			
	10%	30%	50%	70%
Xylitol	-17.12 ± 0.09	-31.21 ± 1.45	-40.56 ± 0.51	-44.40 ± 0.16
Glucose	-15.09± 0.11	-25.59 ± 0.51	-33.20 ± 0.18	-36.70 ± 0.23
Maltotriose	-12.89 ± 0.32	-15.63 ± 0.77	-18.10 ± 0.64	-20.25 ± 0.41
Mannitol	-17.05 ± 0.22	-27.45 ± 0.03	-33.32 ± 0.03	-29.31 ± 0.03
Trehalose	-14.91 ± 0.08	-18.31 ± 0.14	-21.49 ± 0.02	-24.97 ± 0.09

the saccharides. This was true for all the saccharides except in the case of 70% mannitol, where the Tg was higher than the Tg value for the 50% concentration. This was due to partial crystallisation of mannitol during the freezing step. Crystallisation of mannitol during the cooling step has been well studied in literature (Hawe and Friess, 2006a; Hawe and Friess, 2006b). Also, the results showed that xylitol had the highest plasticising effect on the gelatin solution when compared to the similar concentration of other saccharides, followed by mannitol and glucose (close Tg values) then trehalose, while maltotriose exhibiting the lowest effect (**Table 2.2**). It is interesting to note, that this order is directly related with the increase in the molecular weight of the saccharides, as xylitol has the lowest molecular weight (152.15), followed by mannitol, glucose, trehalose (182.17, 180.16 and 242.30respectively) and maltotriose (504.44). This is in agreement with literature, where low molecular weight compounds have lower Tg values when compared to the high molecular weight compounds (Roos, 1997). Glass transition temperature is an important parameter in understanding and developing the lyophilisation process, as it determines the mobility of the molecules inside the system at any temperature. Usually, lyophilisation of stock solutions at temperature 1 to 3 ˚C higher than their Tg results in the collapse of their structure. This temperature is known as the collapse temperature (Tc) (Pikal and Shah, 1990).

Given that the shelf temperature in the primary drying was -40 ˚C and the secondary drying was carried out at 20 ˚C, the anticipated Tc results (which are 1 to 3 ˚C higher than the Tg) suggested that the damage noticed in the lyophilised tablet at high concentration of saccharide might possibly occur during the primary drying step, as the Tc became closer to the

shelf temperature (-40 ˚C). Mannitol formulations did not show any damage or collapse during the lyophilisation; although it had close Tc values to glucose formulations. This might be due to the crystallisation behaviour of mannitol during the lyophilisation process.

On the other hand, DSC scans of the formulations showed that all the saccharides at the full concentration range studied (10-70 %) maintained an amorphous state during the heating step (from -65 to 20 ˚C), except in the case of 50 and 70% mannitol, where crystallisation exothermic peaks have been detected at about -24 ºC (onset) (**Figures 2.8**). This finding along with the increase in Tg at 70% mannitol mentioned above suggests that mannitol has higher tendency to crystallise than the other saccharides and increasing the concentration of mannitol in the gelatin stock solution promotes mannitol crystallisation. Although low concentration of mannitol in the stock solution (10-30 %) did not crystallise during the heating scan, crystallisation during the freeze drying step is highly expected (Pyne et al., 2003).

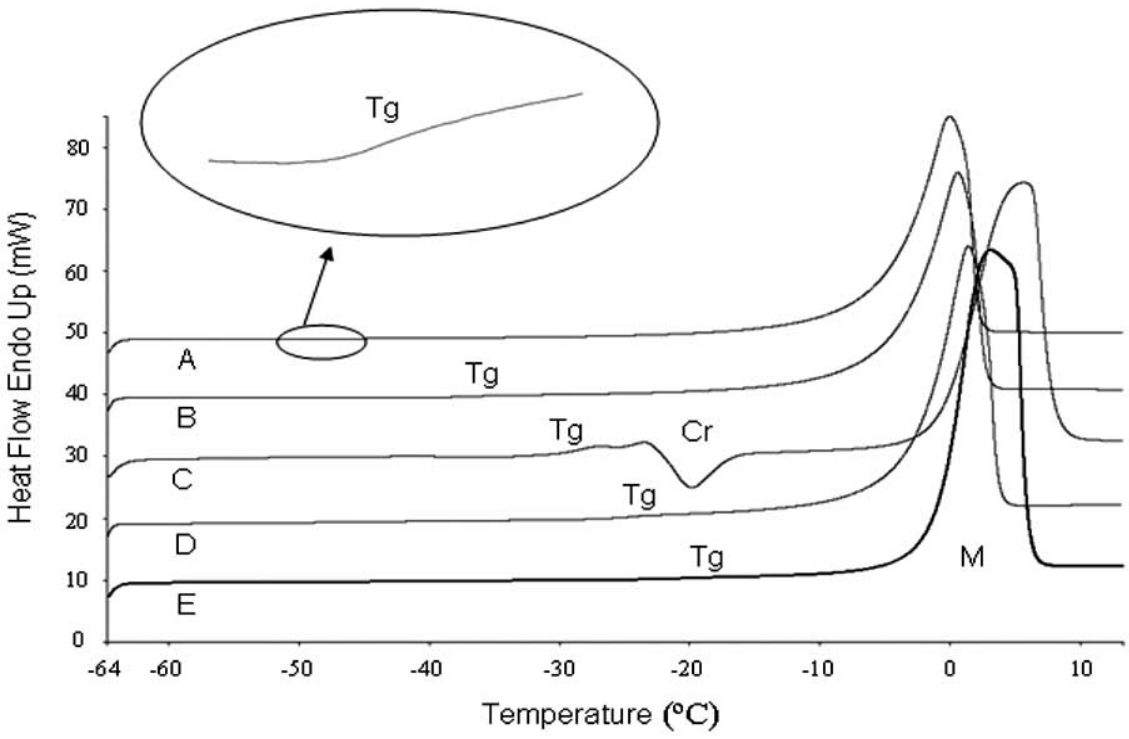


Figure2.8 Overlaid DSC heating curves of frozen gelatin stock solution (5%) with 70% (total solid materials) of the saccharides. The figure shows glass transition (Tg), crystallization (Cr) and ice melting (M) events as a function of temperature. **(A)** xylitol; **(B)** glucose; **(C)** mannitol; **(D)** trehalose; **(E)** maltotriose.

The DSC study along with the morphological evaluation of the lyophilised tablets (**Figures 2.7**) indicated that mannitol is the most suitable bulking agent among the other saccharides (xylitol, glucose, trehalose and maltotriose), as it readily crystallised during the lyophilisation process and produced elegant tablets. The other saccharides are more suitable as lyoprotectant agents, as they showed high tendencies to exist in the amorphous status (Crowe and Crowe, 2000). This is in agreement with literature, where mannitol was used for its crystalline bulking property combined with other materials such as trehalose and human serum albumin that maintain their amorphous nature throughout and after the lyophilisation process (Hawe and Friess, 2006a; Lu and Pikal, 2004; Izutsu and Kojima, 2002).

2.5.2.3 The influence of saccharide concentration on the mechanical properties

Enhancing the mechanical property of fast disintegration tablets and capsules by inclusion of saccharides has been applied in several studies (Seager, 1998; Ciper and Bodmeier, 2005). The effect of inclusion of varied concentration of the saccharides on the fracturability of the lyophilised tablets is presented in **Figure 2.9**. The test was done only for tablets that were fabricated based on 5%w/w gelatin stock solution. This was because the test's probe (1mm diameter) was unable to penetrate the tablets formulated based on 2%w/w gelatin solution due to the spongy nature of these tablets that tend to deform in response to the force applied by the probe. The results (**Figure 2.9**) showed that the fracturability of the tablets was improved by increasing the concentration of the saccharides in the stock solution. Statistically, maltotriose started to provide significantly higher fracturability at concentration of 30%, with fracturability of about 3.4 N, when compared to the reference tablets (made from 5 % gelatin solution alone), (one-way ANOVA/ Dunnett: $\rho < 0.05$), while the rest of the saccharides showed significant improvements at 40% (w/w), with fracturability of about 3.2, 2.8, 3.7 and 3.6 N for xylitol, glucose, mannitol and trehalose, respectively (one-way ANOVA/ Dunnett: $\rho < 0.05$). It is interesting to note that different saccharides at similar concentration provided tablets with no significant difference in the fracturability (one way ANOVA/Tukey-Kramer: $\rho>0.05$) suggesting that the fracturability of the lyophilised tablets is influenced by the concentration of the saccharides regardless of the type of the saccharide.

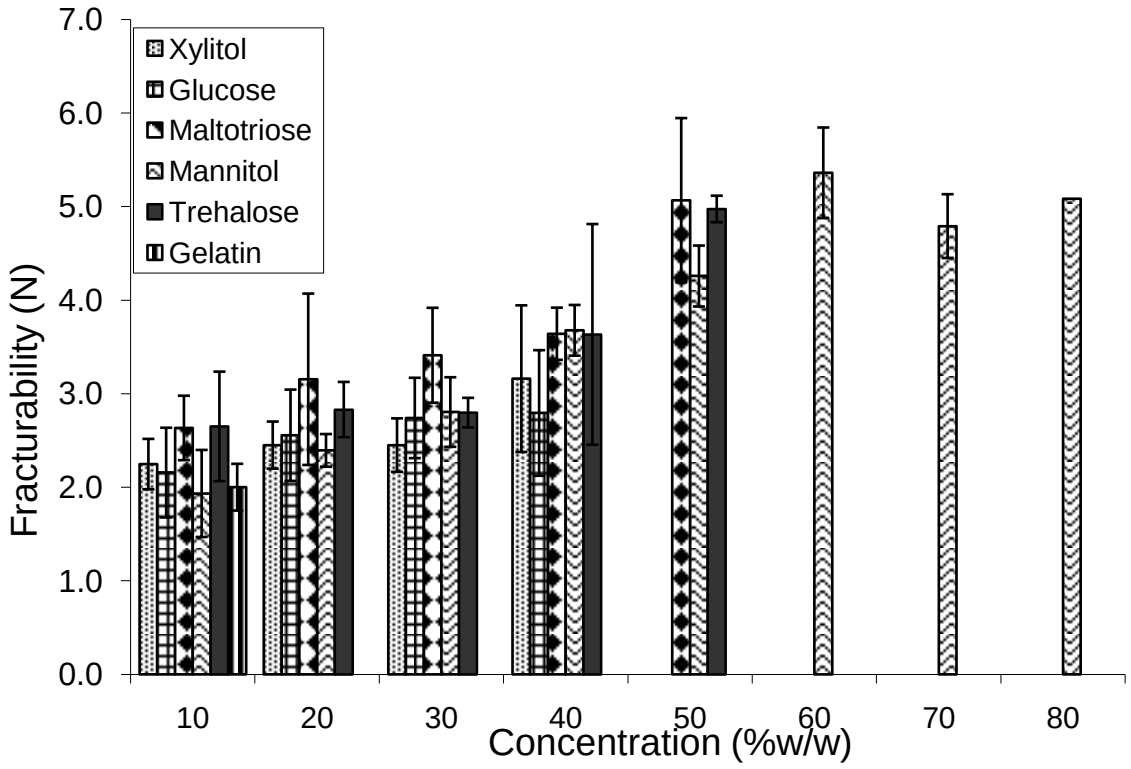


Figure 2.9 The effect of varying the concentration of xylitol, glucose, maltotriose, mannitol and trehalose on the fracturability of lyophilised tablets based on 5% Gelatin solution. Results are mean ± SD, n=3.

Figure 2.10 and 2.11 demonstrate the hardness of the tablets based on 2 and 5%w/w gelatin stock solution, respectively, after inclusion of varied concentration (10-80%) of the saccharides. The results showed that all the saccharides, at concentration of 10% w/w (solid material), significantly improved the hardness of the tablets when compared to tablets based 2% gelatin solution alone (one-way ANOVA/ Dunnett: $\rho < 0.05$). However, only trehalose, maltotriose and mannitol continued the trend at higher concentrations (**Figure 2.10**).

In the case of tablets formulated from 5% gelatin stock solution (**Figure 2.11**), xylitol and glucose did not show any significant improvement in the hardness for the entire concentration range (10-40 % w/w). Whilst trehalose, maltotriose and mannitol showed no significant differences at concentrations below 30%, higher concentrations resulted in significant increase in hardness (**Figure 2.11**). Although, at similar concentration, the improvement in the hardness was not significantly different between these three saccharides the hardness after inclusion of maltotriose was the highest, at any given concentration, followed by trehalose then mannitol,

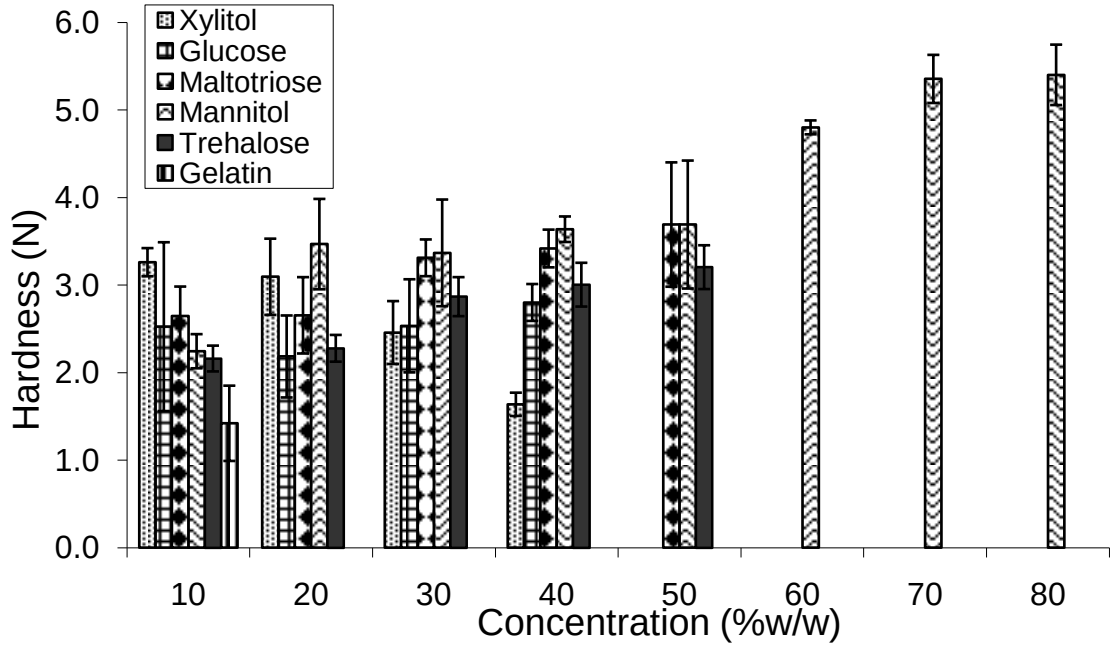


Figure 2.10 The effect of varying the concentration of xylitol, glucose, maltotriose, mannitol and trehalose on the hardness of lyophilised tablets based on 2% Gelatin solution. Results are mean ± SD, n=3.

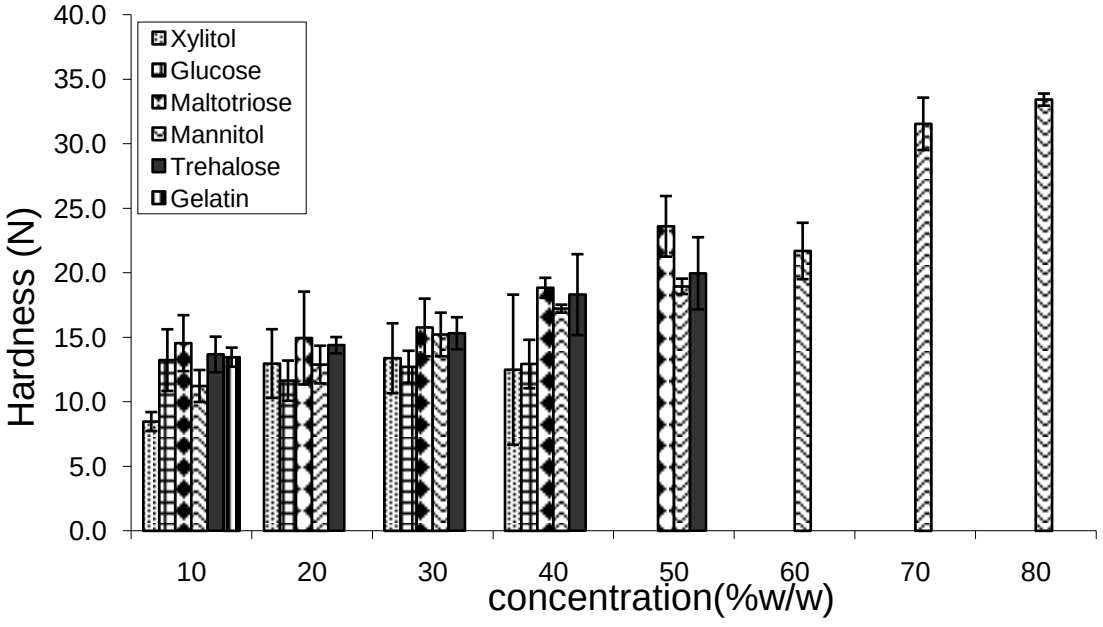


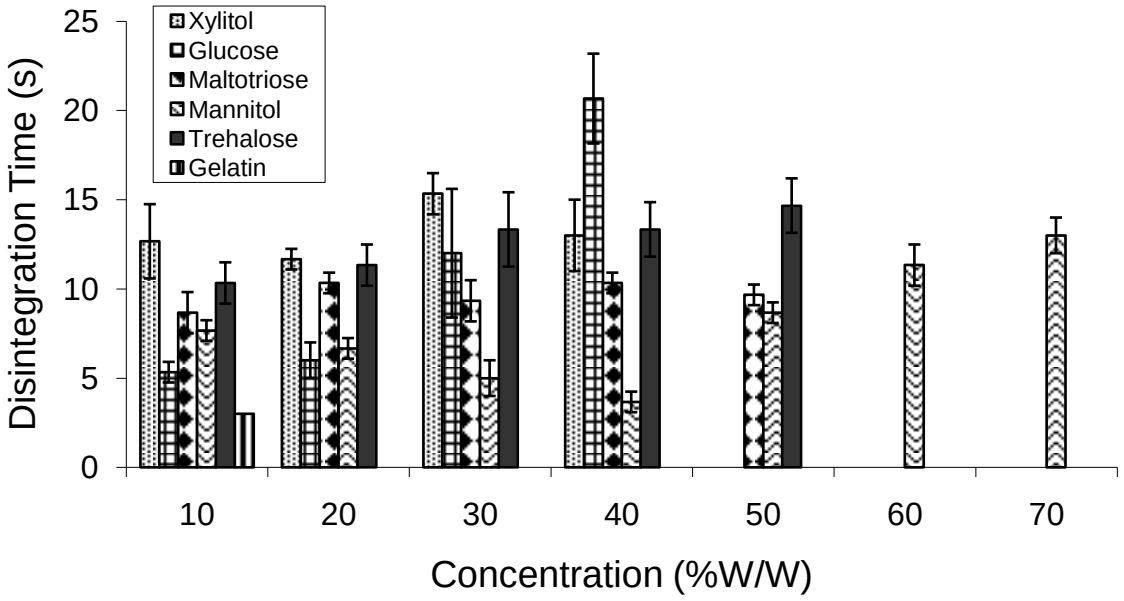
Figure 2.11 The effect of varying the concentration of xylitol, glucose, maltotriose, mannitol and trehalose on the hardness of lyophilised tablets based on 5% Gelatin solution. Results are mean ± SD, n=3.

which may suggest that increasing the molecular weight of the saccharide improves the hardness of the tablets. In conclusion, this study suggests that improving the mechanical properties of lyophilised tablets can be effectively achieved by inclusion of high concentration (equal or higher than 40% w/w) of trehalose, maltotriose or mannitol.

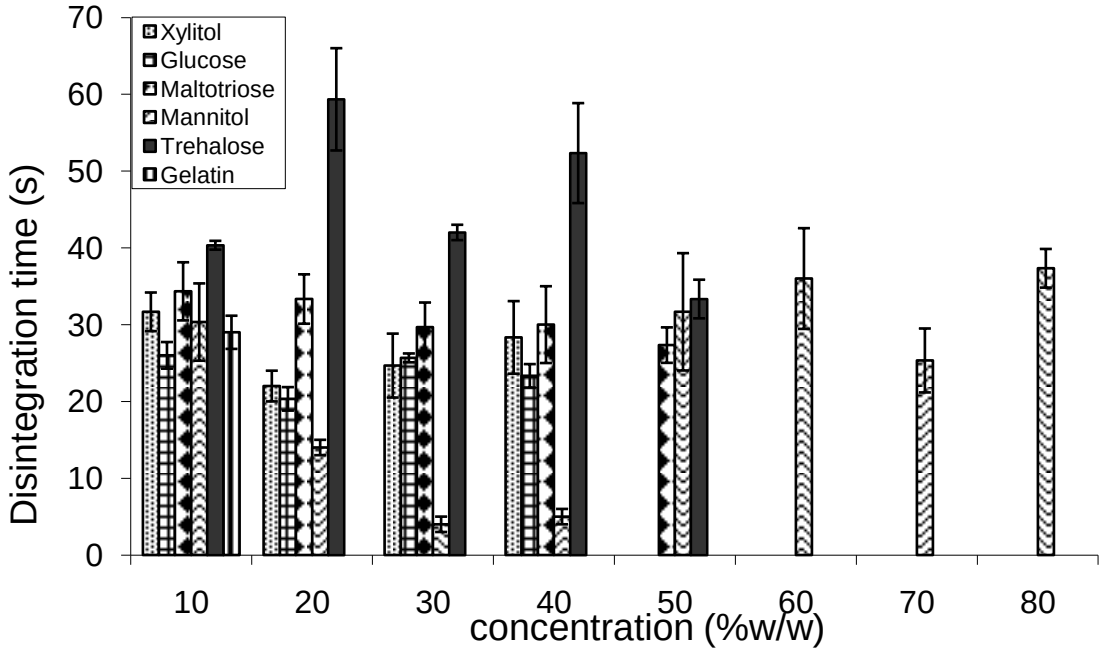
2.5.2.4. Investigation of disintegration time

The disintegration time of 2 and 5 % (w/w) tablets after incorporation of varied concentration of the saccharides are illustrated in **Figures 2.12** and **2.13**, respectively. The results indicated that the effect of the saccharides on the disintegration time was highly influenced by the concentration of gelatin in the stock solution, in addition to the type and concentration of saccharide used. The disintegration time of the tablets based on 2% gelatin stock solution seemed to be retarded (increased) by the saccharides in the entire concentration range. Statistical analysis of the data showed that all the formulations had significantly higher disintegration times when compared to the control, 2% gelatin alone ($P<0.05$), with the exception of low glucose concentration (10 and 20%) and moderate mannitol concentration (30 and 40), (which had disintegration times of 6 seconds or less (**Figures 2.12**). On the other hand, the effects of the saccharides on the disintegration time of tablets based on 5% gelatin stock solution when compared to the control, 5% gelatin alone, could be categorized statistically into three groups: i) significant increase in the disintegration time, which was noticed with trehalose at concentration range from 10 to 40% (w/w). ii) No significant effect, which was the case in high concentration of trehalose (50%), low mannitol concentration (10%), high mannitol concentration (40-80 %) and xylitol, glucose and maltotriose in the entire concentration range. iii) significant decrease, which was only achieved with moderate concentration of mannitol (20-40 %).

Interestingly, nearly all the disintegration time profiles (**Figures 2.12** and **2.13**) tended to form parabolic relationships with saccharide concentration with different dip values (shortest disintegration time) that were obtained at distinctive concentrations for each saccharide. For example, mannitol achieved the shortest disintegration time of 4 and 5 seconds at concentration of 30% (in 2 and 5% gelatin formulations, respectively), whilst the shortest disintegration time for glucose formulations was 5 and 20 seconds, for 2 and 5% gelatin based tablets, respectively, and occurred at concentration of 20% in both cases. This parabolic



Figures 2.12 The disintegration time of tablets based on 2% gelatin stock solution after inclusion of varied concentration of xylitol, glucose, maltotriose, mannitol and trehalose. Results are mean ± SD, n=3.



Figures 2.13 The disintegration time of tablets based on 5% gelatin stock solution after inclusion of varied concentration of xylitol, glucose, maltotriose, mannitol and trehalose. Results are mean ± SD, n=3.

relationship could be explained by the disintegration mechanism of the lyophilised tablets. The porous structure of the tablets allows fast diffusion of water (disintegrating medium) through hydrophilic matrixes that disintegrate/dissolve rapidly with water. Accordingly, the tablet's porosity and hydrophilicity play a major role in determining the disintegration time (Sunada and Bi, 2002). Addition of saccharide to the formulation increases the hydrophilicity of the matrix but, at the same time, decreases the porosity, as a result of decreasing the water concentration in the stock solution (because water is the porogen element in the formulation). Therefore, each saccharide has an optimal concentration where an optimal balance between the porosity and hydrophilicity is created and consequently gives the shortest disintegration time.

2.5.2.5. The lyophilised tablet index

Successful development of fast disintegrating tablets by lyophilisation technique requires careful optimization of formulation parameters in order to obtain an optimal balance between the tablet properties, namely: mechanical properties and disintegration time. Different saccharides at varied concentration range were included in the formulation to enhance the mechanical properties and disintegration time in parallel. However, the results (see above) showed that the disintegration time and mechanical properties of the tablets were improved in different ways and to different extent (see above discussion). Therefore, a value that assesses the improvement in both parameters together was identified as following:

$LTI = (H/DT) \div (H^{\circ}/DT^{\circ})$, Where:

LTI: lyophilized tablets index

H: hardness of the tested tablet

DT: disintegration time of the tested tablet

H°: hardness of the control tablets

DT°: disintegration time of the control tablet

The index was formulated by using the hardness and disintegration time only, as the fracturability was simply being influenced by the concentration of material in the stock

solution. The concept of LTI ensures higher value for the better improvement in both parameters. The lyophilized tablet index values of the 2 and 5% gelatin based formulation are presented in **Tables 2.3** and **2.4**, respectively. Although different values have been obtained from 2 and 5% gelatin formulation for the same saccharide the two sets of values are in agreement about the best formulation (highest LTI value). For example, mannitol at concentration of 30 and 40 % has the highest values in both Tables (**2.3 and 2.4)**.

Table 2.3 The lyophilised tablet index values of tablets based on 2% gelatin stock solution and varied concentration of xylitol, glucose, maltotriose, mannitol and trehalose.

Saccharide	**LTI**							
	10%	20%	30%	40%	50%	60%	70%	80%
Xylitol	0.54	0.56	0.34	0.27	-	-	-	-
Glucose	1.00	0.49	0.28	0.18	-	-	-	-
Trehalose	0.44	0.42	0.45	0.48	0.46	-	-	-
Maltotriose	0.65	0.45	0.75	0.70	0.81	-	-	-
Mannitol	0.62	1.10	1.42	2.10	0.90	0.90	0.87	0.65

- No intact lyophilised tablets were formed

Table 2.4 The lyophilised tablet index of tablets based on 5% gelatin stock solution and varied concentration of xylitol, glucose, maltotriose, mannitol and trehalose.

Saccharide	**LTI**							
	10%	20%	30%	40%	50%	60%	70%	80%
Xylitol	0.57	1.25	1.15	0.94	-	-	-	-
Glucose	1.08	1.22	1.05	1.18	-	-	-	-
Trehalose	0.72	0.52	0.78	0.74	1.27	-	-	-
Maltotriose	0.90	0.95	1.13	1.34	1.84		-	-
Mannitol	0.79	1.96	8.10	7.33	1.27	1.28	2.65	1.90

- No intact lyophilised tablets were formed

2.5.3. Fast disintegration tablets of clonidine HCl

Clonidine HCl (as model drug) was formulated as a lyophilized fast disintegrating tablet based on the formulation that had achieved the highest LTI value, 30% mannitol (w/w of total solid material) in 5% gelatin stock solution (**Tables 2.4**). Trehalose was added to the formulation in low concentration to act as lyoprotectant, as it well known for its efficient lyoprotectant activity in protein formulation (Richards et al., 2002; Elbein et al., 2003). The low dose of clonidine HCl (100 µg/tablet) was not expected to affect the formulation properties. The composition resulted in successfully freeze dried and elegant tablets that were strong enough to be easily handled. The tablets disintegrated in 6.3 ± 0.6 seconds and had a hardness of 17.3 ± 0.7 N and fracturability of 3.6 ± 0.3 N. The results suggested that the tablet properties (mechanical properties and disintegration time) were not significantly different when compared to formulations containing only 30% mannitol. The mean drug content in one tablet analysed by HPLC was 92.5 µg with standard deviation of 2.0. Reconstitution of one tablet in 2 ml water resulted in solution with viscosity of 3.1 ± 0.1 m.pas/s and pH value of 5.2 ± 0.1. The results suggest the ability of such system, to deliver a clonidine HCl dose in efficient and convenient way.

2.6. Conclusion

The disintegration time of the tablets dramatically decreased by decreasing the concentration and bloom strength of gelatin in the stock solution, whereas the mechanical properties of the tablets were influenced by the concentration of gelatin rather than the bloom strength. Enhancing the mechanical properties of the freeze-dried tablets by increasing gelatin concentration inversely influences their disintegration time. Low bloom strength gelatin with stock solution concentration between 2-5% (w/w) is most suitable for developing rapid disintegrating lyophilised tablets. Mannitol crystallises during the freeze drying process and consequently produces elegant tablets. Xylitol, glucose, trehalose and maltotriose are more resistant to crystallisation, which proposes their lyoprotection role in the formulation. The disintegration time profiles of the gelatin/saccharide systems are parabolic with different dip values (shortest disintegration time) at distinctive concentrations for each saccharide. High concentration of trehalose, maltotriose and mannitol (equal or higher than 40% w/w) significantly enhances the mechanical properties of the tablets. Mannitol at concentrations

between 30 to 40 % w/w (of total solid material) achieved the greatest balance between the disintegration time and hardness as demonstrated by the LTI value. The optimised rapid disintegrating tablet in this study is able to efficiently deliver clonidine HCl.

Chapter Three: Formulation and Characterisation of Lyophilised ODTs Using Amino Acids as Matrix Forming Agents

Papers relating to this chapter

AlHusban, F., Perrie, Y., **Mohammed, A.** (2010) Formulation and characterisation of lyophilised rapid disintegrating tablets using amino acids as matrix forming agents. *European Journal of Pharmaceutics and Biopharmaceutics,* 75, 2, 254-262.

AlHusban, F., Mohammed, A. (2010) Novel zero saccharide orally disintegrating tablets. *Industrial Pharmacy*, 26, 16-18.

Alhusban FA, Perrie Y, Mohammed A - Saccharide free lyophilised orally disintegrating tablets (ODTs) using novel combination of amino acids UKICRS Symposium April 2010, London, UK.

Alhusban FA, Perrie Y, Mohammed A - A novel application for amino acids as matrix forming agents in lyophilised rapid disintegrating tablets. British Pharmaceutical Conference: September 2009, Manchester, UK.

Alhusban FA, Perrie Y, Mohammed A - Novel-Saccharide Free Rapid Disintegrating Tablets Containing Amino Acids. *36th Annual Meeting of the Controlled Release Society*, July 2009, Copenhagen, Denmark.

Formulation and Characterisation of Lyophilised ODTs Using Amino Acids as Matrix Forming Agents

3.1. Introduction and Aims

The fabrication of lyophilised ODTs is based on creating a porous matrix by subliming the water from pre-frozen aqueous formulation of the drug containing matrix forming agents and other excipients such as lyoprotectants, preservatives and flavours (Seager, 1998). The matrix of the lyophilised ODT consists of two components that work together to ensure the development of a successful formulation. The first component is water soluble polymers such as gelatin, dextran, alginate (Seager, 1998), maltodextrin (Corveleyn and Remon, 1998). This component maintains the shape and provides mechanical strength to the tablets (binder). The second constituent is matrix supporting/ disintegration enhancing agents such as sucrose and mannitol, which acts by cementing the porous framework provided by the water soluble polymer and accelerates the disintegration of the ODT (Chandrasekhar et al., 2009). Although there is wide availability of literature describing the preparation of ODTs by lyophilisation, the number of matrix supporting/ disintegration enhancing agents used has been limited to saccharides and polyols with majority of the work dedicated to the inclusion of mannitol (Seager, 1998; Chandrasekhar et al., 2009). This is primarily because the incorporation of these matrix forming agents requires fulfilment of stringent characteristics such as reasonable drying time, stability during freeze-drying process, as well as formation of elegant tablets with short disintegration time and adequate mechanical properties (see chapter two). However, high concentration of saccharides and polyols is required to achieve these quality features (Seager, 1998; Chandrasekhar et al., 2009), thus restrains their application in delivering drugs for the treatment of long term chronic conditions especially for children, diabetic and obese patients, due to limited intake requirement. Therefore this chapter aims to explore alternative novel excipients by investigating the feasibility of using amino acids as matrix supporting agents (second component) in the fabrication of rapid disintegrating tablets prepared by freeze drying in order to produce tablets with enhanced properties and wider application to pediatric and geriatric patient population.

Amino acids are the basic structural units (monomer) of proteins. An alpha amino acid consists of an amino group, a carboxyl group, a hydrogen atom, and a distinctive side chain bonded to a

carbon atom (alpha carbon). The side chains of amino acids are responsible for the variation in their physicochemical properties. Naturally occurring amino acids can exist in both the L (laevo) and the D (dextro) forms, which are mirror images of each other. However incorporation of the D form of the amino acid has been limited for pharmaceutical applications due to their potential pharmacological activity, microbiological concerns and toxicity (Tsai et al., 1998; Williams et al., 2005; Friedman, 1999). On the other hand, the L form of the amino acids has been used extensively in pharmaceutical and cosmetic formulations such as pH sensitive drug carrier (Oh et al., 2008), cicatrisation topical dermatological preparations (Marrubini et al., 2008), salt conjugate of poorly soluble drug (Anacardio et al., 2003), oral tablets, as lubricant (Rotthauser et al 1997) and disintegration enhancer (Fukami et al., 2006), inhalable delivery systems (Alhusban and Seville, 2009) and freeze dried product, as cryoprotectants (Mohammed et al., 2007) and bulking agent (Akers et al., 1995).

In this study, L-amino acids with adequate aqueous solubility, which allow their inclusion at varied concentration, were chosen (alanine, arginine, threonine, glycine, cysteine, serine, histidine, lysine, valine, asparagine, glutamine and proline) and their potential as matrix supporting/ disintegration enhancing agents were investigated individually at concentration of 10, 30, 50 and 70 % w/w (total solid) using 5% aqueous solution of low bloom strength gelatin (60 bloom strength) as a binder. The formulations were examined for their thermal properties in their frozen state in order to explain their behaviour during the freeze drying process. The freeze dried tablets were evaluated for their disintegration time and mechanical properties. In addition, the porosity of the ODTs and the wettability profile of the amino acids were investigated to explain the disintegration time and mechanism.

3.2. Materials

Gelatin of bloom strength 60 (from calf skin), L-alanine, L-arginine, L-threonine, glycine, L-cysteine, L-serine, L-histidine, L-lysine, L-valine, L-asparagine, L-glutamine and L-proline were purchased form Sigma-Aldrich Chemicals (Pool, UK). All the chemicals were of analytical grade.

3.3. Methods

3.3.1. Preparation of lyophilized tablets

The amino acids were added individually to 5 % (w/w) gelatin (60 bloom strength) stock solutions at concentrations of 10, 30, 50 and 70% of total solid material. 1.5 g of the solution was poured into the tablet mould (13.80 mm diameter, 8.50 mm height), frozen at -80 °C for about 60 minutes and freeze-dried (ADVANTAGE Freeze-dryer, VIRTIS) according to an optimized regime (primary drying for 48 hours at shelf temperature of -40 °C and secondary drying for 10 hours at shelf temperature of 20 °C and vacuum of 50 m Torr). All formulations were prepared in triplicate from three independent batches.

3.3.2. Differential scanning calorimetry studies

Differential scanning calorimetry (Pyris Diamond DSC and Intracooler 2P: Perkin Elmer, Wellessey, USA) was used to determine the glass transition temperature (Tg) and crystallisation event of the formulation in its frozen state (before freeze drying). 10-15mg of the liquid formulation were loaded into aluminium pans, cooled to -65 ˚C and then heated to 20 ºC at 5 ºC/min with a nitrogen purge of 20ml/min. To determine the glass transition temperature of the maximally freeze concentrate sample (Tg'), after initial cooling to -65 ºC, annealing for 10 min at -15 ºC was added before carrying out the above method. An empty aluminium pan was used as reference for all measurements.

The resulting plots were analysed by Pyris manager software. Tg and Tg' values were determined from the intersection of relative tangents to the baseline. All the measurements were done in triplicate from independently prepared samples.

The DSC was calibrated for temperature and heat flow using standard samples of indium (melting point: 156.6 ºC, ΔHm: 28.42 J/g) and Zinc (melting point: 419.5 ºC, ΔHm: 108.26 J/g).

3.3.3. Mechanical properties of the tablets

The mechanical properties of the tablets (hardness) were investigated with a texture analyzer (QTS 25: Brookfield, Essex, UK) equipped with a 25 kg load cell. The instrument was calibrated with standard weight of 500 g and 5 kg. The tablet was placed in a holder with a cylindrical

hole. The hardness was taken as the peak force after 1mm penetration of 5mm diameter probe at a speed of 6 mm/min. The results were average of three measurements from independently prepared batches.

3.3.4. Disintegration time of the tablets

The disintegration time of the tablets was measured using a USP disintegration tester (Erweka, ZT3). Distilled water (800 ml) kept at 37 ºC was used as a medium and the basket was raised and lowered at a fixed frequency of 30 cycles/min. One tablet was tested at a time. All the formulations were evaluated in triplicate and standard deviation was calculated.

3.3.5. Porosity

The relative porosity was calculated from the apparent and strut density of the tablet. Apparent density was found by dividing the mass of the tablet by the measured volume. The strut density was determined using helium pycnometry (Accupyc 1330, Micromeritics, UK) with 3 cm^3 sample cup at 22 ºC. Prior to analysis the helium pycnometry was calibrated against a standard steel ball. Each determination included 10 purges at 19.5 psi and 10 analytical runs at 19.5 psi with an equilibration rate of 0.0050 psi/min.

3.3.6. Wetting profile

The wetting profile of the amino acids was analysed by measuring their contact angle using Wilhelmy method. The amino acids were analysed in their powder form after brief milling using mortar and pestle. Cover slides (24*24 mm) were covered by double sided tape (Scotich 12*1 mm) and dipped into a container of the milled amino acid to create a uniform coating. Excess powder was removed by tapping the cover slide. After measuring the perimeter (width and thickness), using a micrometer, the coated cover slide was attached to the balance loop of microbalance in the tensiometer (QCT-100 Interfacial Tensiometer, Camtel Ltd, UK). The beaker under the sample was filled with 75 ml double distilled water at temperature of 25 ºC (liquid medium).

The computer was programmed to lower the sample to a distance of 10 mm after contact with the liquid medium at a constant speed of 0.20 mm/s. The contact angle was calculated automatically (using Wilhelmy equation) at regular interval and recorded as a function of time.

3.3.7. Morphological examination

The inner structural morphology and pore size of the freeze-dried tablets were examined by scanning electron microscopy (SEM, STEREOSCAN 90, Cambridge Instrument). Thin horizontal cross-section sample was prepared by cutting the tablet with a razor blade. The samples were placed onto double-sided adhesive strip on an aluminium stub. The specimen stub was coated with a thin layer of gold using a sputter coater (Polaron SC500, Polaron Equipment, Watford, UK) at 20 mA for three 3 minutes and then examined by SEM. The acceleration voltage (KV) and the magnification can be seen on each micrograph.

3.3.8. Statistical analysis

The effect of inclusion amino acids on the glass transition temperature of the formulation in the frozen state was compared to those of the control (composed of gelatin only) and against each other using one-way analysis of variance with Dunnett multiple comparison test and one-way analysis of variance with Tukey-Kramer multiple comparison test, respectively. The hardness, fracturability and disintegration of the lyophilised tablets after inclusion of the amino acids were statistically compared to those of the control (composed of gelatin only) using one-way analysis of variance with Dunnett multiple comparison test. The total porosity of the tablets and the wetting parameters of the amino acids were compared against each other using one-way analysis of variance with Tukey-Kramer multiple comparison test. The significant level was 0.05.

3.4. Result and discussion

3.4.1. Thermal analysis

Thermal analysis of the frozen formulations is crucial in the development of lyophilised tablets to ensure the formation of intact tablets with minimal morphological defects and also to determine the molecular state of the excipients (amorphous or crystalline). Measurement of glass transition temperature of maximally freeze concentrated (Tg') solution reflects the molecular mobility of the excipients as a function of temperature within the frozen matrix which in turn dictates the stability of the formulation during the lyophilisation process. Freeze-drying of formulations at temperatures 1-3 ˚C above their Tg' (collapse temperature, Tc) usually induces physical collapse due to the increase in the mobility of the frozen solution (Pikal and Shah, 1990). Accordingly, to protect the formulation matrix from possible collapse, the temperature of the freeze dried product should not exceed the collapse temperature and a safety margin is required between the two temperatures (2-5 ˚C) to ensure the reproducibility of the process (Tang and Pikal, 2004). This has a direct impact on the freeze drying regime, as lower shelf temperature is required to successfully freeze dry formulations comprising of low Tg', which in turn prolongs the primary drying time significantly (Pikal, 1990). In addition, crystallisation during the freeze drying stages (freezing, annealing or primary drying) is believed to give more stability to the formulation, protect against possible collapse and produce elegant lyophilised product (Seager, 1998; Chandrasekhar et al., 2009). Therefore, excipients that crystallise during the freeze drying process are more suitable as bulking agents (Lu and Pikal, 2004). However amorphous materials are also required in the lyophilized formulation to replace the sublimed water molecules and consequently protect against any structural changes or aggregation in the final product (lyoprotectant) (Crowe and Crowe, 2000).

The thermal properties of frozen aqueous solutions containing 5% gelatin and various concentrations of amino acids are summarized in **Table 3.1**. Limitations in the aqueous solubility of some amino acids prevented them from undergoing thermal analysis at higher concentration. At concentration of 10% w/w (total solid) of amino acids, the tested formulations showed thermal step in the baseline, glass transition of maximally freeze concentrated sample (Tg'), of the heating scan, indicating that the formulations remained in amorphous state during the freezing, annealing and heating processes. Given that the Tg' of the control (5% gelatin without amino acid) was -11.72 ± 0.72 ˚C (n=3), addition of 10% w/w of

the different amino acids significantly lowered the Tg' of the formulation (one-way ANOVA/ Dunnett: $\rho < 0.05$). The lowest Tg' was recorded for alanine and proline. The decrease in the glass transition of the formulations was possibly due to the plasticizing effect of the amino acids. This is in line with previously reported research, which has shown that freeze dried systems upon inclusion of solutes within the formulation results in lowering of the glass transition temperature and is dependent on the interactions between the added excipient and unfrozen water (Nesarikar and Nassar, 2007). Addition of plasticizing agents potentially reduces the intermolecular forces between binder molecules and increases polymer chain mobility thereby providing a cushioning effect. However, the degree of plasticizing varied between the amino acids, which can be attributed to the differences in their physicochemical properties (Kagimoto et al., 2006) and total number of moles added.

In order to further understand the differences, a plot between the molecular weight and plasticising effect of amino acids on gelatin solution was plotted (**Figure 3.1**). The low correlation coefficient (R^2= 0.695) was probably due to the role of other physicochemical properties such as solubility and viscosity (Kagimoto et al., 2006). However a general trend which showed that low molecular weight amino acids had a higher plasticising effect was observed (**Figure 3.1**). This could be a consequence of the higher number of amino acid moles provided by the low molecular weight amino acid in the formulation, as all the amino acids were added to the formulation mixture as a weight per weight percent. The presence of larger number of particles within the formulation may eventually have a higher cushioning effect resulting in greater decrease of intermolecular forces between the gelatin as well as gelatin-water molecules.

Upon increasing of concentration to 30 % w/w, all the tested amino acids showed significant reduction in their Tg' values when compared to their 10% formulation (one-way ANOVA/ Dunnett: $\rho < 0.05$) except glycine and valine, where partial crystallization was observed (**Table 3.1**). At this concentration, the amino acids that showed lower Tg' values appeared to retain their amorphous state throughout the heating range (-65 to 20 ˚C) except glycine, cysteine and valine, where partial crystallization was observed. However at a concentration of 50% w/w alanine, serine, glycine, cysteine and valine exhibited crystallisation, whereas the rest of the amino acids retained their amorphous state in the formulation during the cooling, annealing and heating processes as demonstrated by their Tg' values (**Table 3.1**). At the highest studied concentration (70% w/w), arginine, threonine, lysine and proline retained their amorphous

Table 3.1 The glass transition temperature of maximally freeze concentrated (Tg') and crystallisation event of 5% gelatin solution in water with 10, 30, 50 and 70 % (of total solid material) of amino acids (mean ± SD, n=3).

Amino Acid	10 %		30%		50%		70%	
	Tg' (˚C)	Cr (˚C)	Tg' (˚C)	Cr (˚C)	Tg' (˚C)	Cr (˚C)	Tg' (˚C)	Cr (˚C)
Alanine	-21.55 ± 0.50	*	-36.68 ± 0.15	*	-12.85 ± 0.22	-32.77 ± 0.43	*	-40.11 ± 0.80
Arginine	-14.46 ± 0.27	*	-21.36 ± 0.13	*	-27.32 ± 0.21	*	-32.60 ± 0.09	*
Threonine	-18.51 ± 0.11	*	-30.21 ± 0.45	*	-35.41 ± 0.37	*	-38.61 ± 0.49	*
Glycine	-20.46 ± 0.17	*	-12.51 ± 0.82	-28.81 ± 0.85	*	-45.53 ± 0.52	*	-32.32 ± 1.00
Cysteine	-17.22 ± 0.69	*	-25.01 ± 0.39	-10.33 ± 0.40	-13.14 ± 0.29	-23.01 ± 0.40	*	*
Serine	-18.75 ± 0.22	*	-25.70 ±0.58	*	-12.56 ±0.18	-16.98 ± 0.33	*	-24.00 ± 0.53
Histidine	-16.25 ± 0.41	*	-21.34 ± 0.13	*	-24.59 ± 0.30	*	-	-
Lysine	-20.34 ± 0.20	*	-34.63 ± 0.63	*	-39.08 ± 0.21	*	-46.84 ± 0.22	*
Valine	-19.09 ± 0.17	*	-12.02 ± 0.26	-24.25 ±0.44	*	*	-	-
Asparagine	-16.82 ± 0.28	*	-21.90± 0.16	*	-	-	-	-
Glutamine	-17.57 ± 0.60	*	-24.84 ± 0.14	*	-	-	-	-
Proline	-21.47 ± 0.51	*	-37.05 ± 0.86	*	-50.43 ± 0.30	*	> -65	*

(Tg') Glass transition temperature of the maximally freeze concentrate sample
(Cr) Crystallisation
(*) No event detected
(-) Not soluble

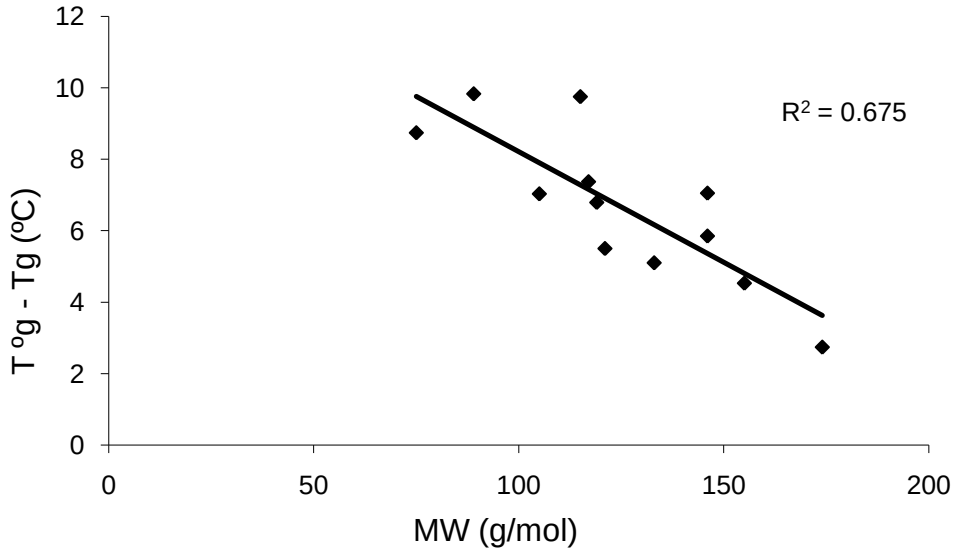


Figure 3.1 The effect of molecular weight of amino acids on the glass transition temperature of frozen solutions containing 5% aqueous gelatin solution at concentration of 10% w/w (total solids) of the tested amino acids. Tg': glass transition temperature of maximally freeze concentrated sample of 5% gelatin with 10% amino acid, T˚g': glass transition of maximally freeze concentrated sample of 5% gelatin solution.

state. The ability of arginine to preserve the amorphous behavior in the freeze concentrated solution has previously been documented by Izutsu et al. (2005), studying the effect of counterions on the physical properties of arginine in frozen solutions and freeze-dried solids. Although there was no event detected in 70 % proline formulations it can be anticipated that the glass transition was below the heating range employed (-65 to 20 ˚C), based on the data recorded for lower concentrations where lowering of the glass transition was noted upon increase of proline concentration. On the other hand, the crystallisation behavior of alanine, glycine, serine and cysteine prohibited the formulations from undergoing any glass transition event at this high concentration (**Table 3.1**).

Freeze drying of the formulations in this study using the applied regime (primary drying for 48 hours at shelf temperature of -40 °C and secondary drying for 10 hours at shelf temperature of 20 °C and vacuum of 50 mTorr) revealed that the formation of intact tablets (with no signs of morphological defect) was crucially influenced by the above thermal properties of the formulation. All the formulations that showed tendency to crystallise formed elegant tablets

with no signs of morphological defect regardless of their Tg' temperatures, which confirms the role of readily crystalline excipient in the formulation of lyophilised ODTs as discussed above. For amorphous formulations, the formation of intact tablet was dependant on Tg'. Formulations with Tg' lower than -40 °C showed major structural collapse after freeze drying, while intact tablets were formed from higher Tg'. In the case of 30% proline formulation, partial collapse was noticed possibly due to the narrow safety margin between the shelf temperature and Tg', therefore these tablets were excluded from further characterisation.

3.4.2. Porosity

The porosity of the ODTs at amino acid concentrations of 10, 30, 50 and 70% (w/w) is summarised in **Table 3.2**. The results suggested that each increment in the concentration of the amino acid in the ODTs was associated with a significant decrease in the total porosity ($\rho < 0.05$), possibly due to a decrease in the water concentration in the stock solution (because water is the porogen element in the formulation). The results also showed that inclusion of different amino acids at concentration of 10% (w/w) produced tablets with insignificant differences in their total porosity ($\rho > 0.05$). However, at higher concentrations (30, 50 and 70% w/w) of amino acids some variations in the total porosity were noticed. As all tablets in this study were produced using the same procedure and the same binder stock solution, any differences in their porosity were attributed to the inclusion of amino acids and their concentration. Tablets based on the same concentration of alanine, arginine, threonine, serine, cysteine, histidine and asparagine had very close total porosity values (less than 2% variation), whereas tablets fabricated from glycine and lysine at similar concentration produced tablets with slightly lower total porosity ($\rho < 0.05$) and even much lower porosity was displayed by valine and glutamine formulations ($\rho < 0.001$) when compared to the rest of the amino acids. Further discussion about the impact of porosity on ODT characteristics is described in the sections of mechanical properties and mechanism of disintegration (below).

Table 3.2 The total porosity of ODTs based on 10, 30, 50 and 70% (w/w) amino acids.

Amino acid	Porosity (%)			
	10%	30%	50%	70%
Alanine	96.01 ± 0.32	94.12 ± 0.13	91.37 ± 0.15	86.12 ± 0.41
Arginine	95.84 ± 0.41	94.09 ± 0.27	90.70 ± 0.22	85.31 ± 0.52
Threonine	95.92 ± 0.22	94.36 ± 0.24	92.76 ± 0.23	86.61 ± 0.54
Glycine	95.43 ± 0.35	92.42 ± 0.23	88.14 ± 0.21	82.03 ± 0.40
Cysteine	96.12 ± 0.45	94.67 ± 0.27	92.71 ± 0.31	86.31 ± 0.35
Serine	96.47 ± 0.30	95.00 ± 0.24	93.14 ± 0.32	87.83 ± 0.29
Histidine	95.79 ± 0.43	94.14 ± 0.20	92.64 ± 0.30	-
Lysine	95.21 ± 0.27	92.45 ± 0.31	88.21 ± 0.15	*
Valine	95.12 ± 0.25	88.49 ± 0.27	74.94 ± 0.34	-
Asparagine	96.35 ± 0.12	94.94 ± 0.35	-	-
Glutamine	95.17 ± 0.50	87.21 ± 0.62	-	-
Proline	96.10 ± 0.18	*	*	*

(*) No intact lyophilised tablets were formed
(-) Not soluble

3.4.3. Mechanical properties

One of the inherent issues associated with the formulation of lyophilized orally disintegrating tablets is their weak mechanical properties (Fukami et al., 2006; Kuno et al., 2005; Narazaki et al., 2004) with the consequence that additional protection in the form of specialized packaging is required for the tablet to withstand mechanical stresses during shipping, storage and handling by patients. The poor mechanical properties are as a result of the porous anatomical architecture of the lyophilized ODT consisting of a three dimensional network of binder molecules (see **Figure 3.2**). Our previous research (chapter 2) has shown that the two common methods to enhance the mechanical strength of the lyophilized ODTs is the inclusion of higher concentration of the binder or addition of excipients such as matrix supporting agents (saccharides and polyols). However increase of binder concentration has a detrimental effect on the disintegration time of the tablets due to increase in intermolecular attraction between the binder molecules resulting in retardation in disintegration time profile leaving the incorporation of matrix supporting agents as a more pragmatic method.

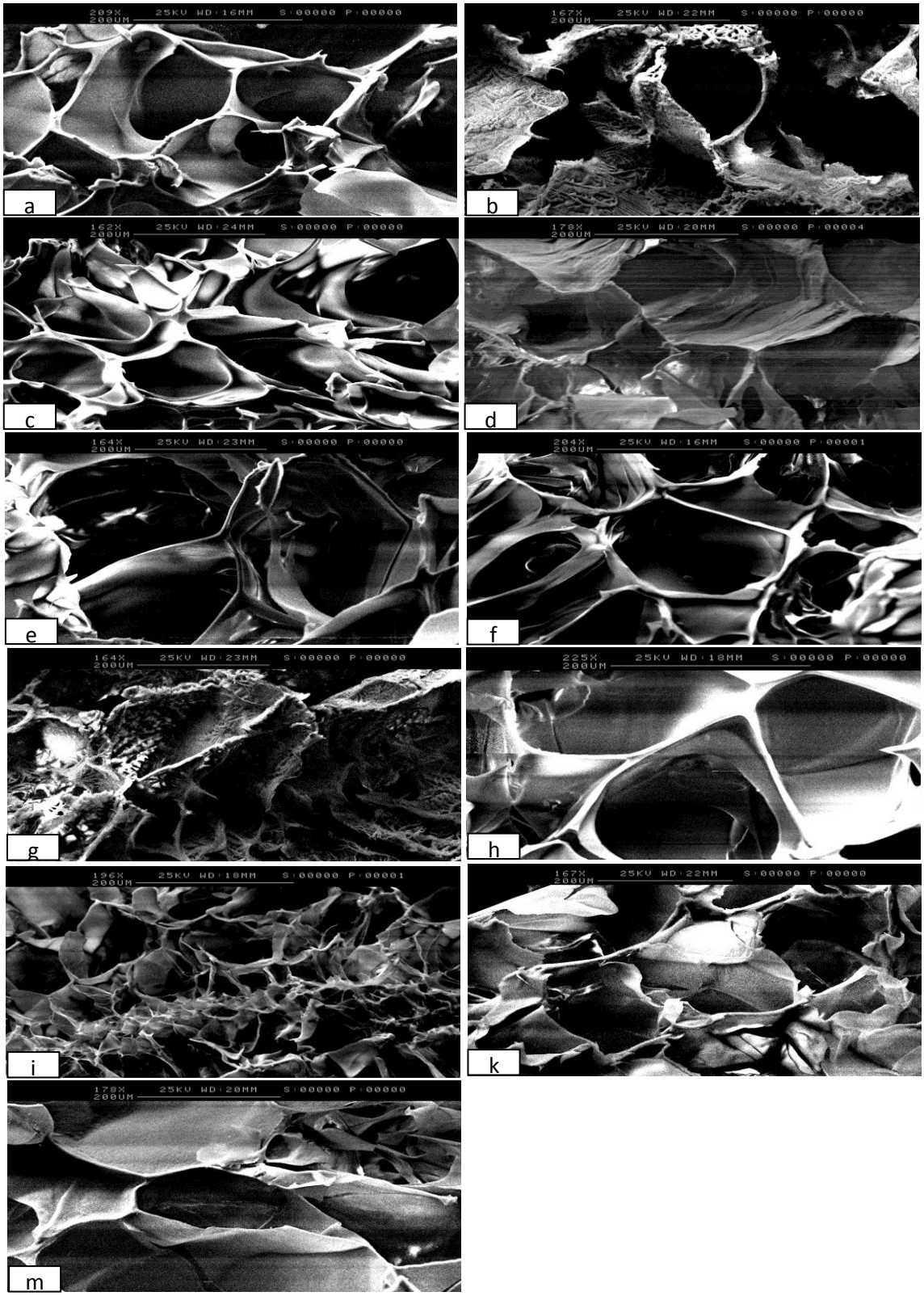


Figure 3.2 Scanning electron micrographs of ODTs based on: (a) 50% arginine, (b) 50% valine, (c) 50% lysine, (d) 50% alanine, (e) 50% threonine, (f) 50% serine, (g) 30% glutamine, (h) 50% histidine, (i) 50 % cysteine, (k) 30% asparagine, (m) 50% glycine.

In this study the use of 5% (w/w) gelatin stock solution as a binder proved to give the ODTs high resistant to friability, less the 0.15% (data not shown). However, due to the highly porous structure, the ODTs have a spongy nature, which is easy to deform in response to external forces. Therefore, the effect of inclusion of varied concentration of amino acids on the mechanical properties of the tablets was evaluated by applying a compression force through a 5 mm diameter probe, and the peak force after 1mm compression was taken as the hardness.

The hardness of the ODTs after inclusion of varied concentration of amino acids is presented in **Figure 3.3**. The results showed that inclusion of amino acids at low concentration of 10 and 30 % w/w (total solid) did not improve the hardness of the tablets significantly when compared to gelatin only formulation (one way ANOVA/Tukey-Kramer: $\rho > 0.05$). However upon increase of concentration to 50%, alanine ($\rho < 0.01$), arginine ($\rho < 0.05$), threonine ($\rho < 0.05$), glycine ($\rho < 0.05$) and serine ($\rho < 0.01$) significantly (one way ANOVA/Tukey-Kramer) improved the hardness of the tablets from 13.5 ± 0.7N for gelatin only tablet (control) to 18.3 ± 1.0N, 17.5 ± 1.8N, 20.3 ± 1.2N, 18.1 ± 0.9N, 19.7 ± 1.5N and 22.2 ± 1.7N, respectively. At the highest studied concentration (70% w/w) only tablets based on arginine, glycine and serine achieved progressive enhancement in hardness over their 50% formulation, with the highest hardness recoded by the serine formulation (37.0 ± 4.5N).

Generally, the mechanical properties of tablets are mainly influenced by the intermolecular bonding force and contact points between the excipients (Bi et al., 1996). The extent of contact between the matrix forming agents within the lyophilised ODTs is influenced by the total porosity of the tablets, decreasing the porosity increases the contact points between the matrix forming agents within the ODT. Accordingly, the improvement in the mechanical properties of the ODTs upon increasing the concentration of amino acids in the formulation was a result of decreasing the porosity (see porosity results). However, the degree of improvement was varied between the amino acids as a consequence of their variation in the molecular interaction with the binder (gelatin). For instance, although valine and glutamine formulation had the lowest porosity values (higher contact points) no improvement in the hardness was achieved and even significant deteriorations were noticed in the 10% glutamine and 50% valine formulations when compared to the control, which suggests weak bonding

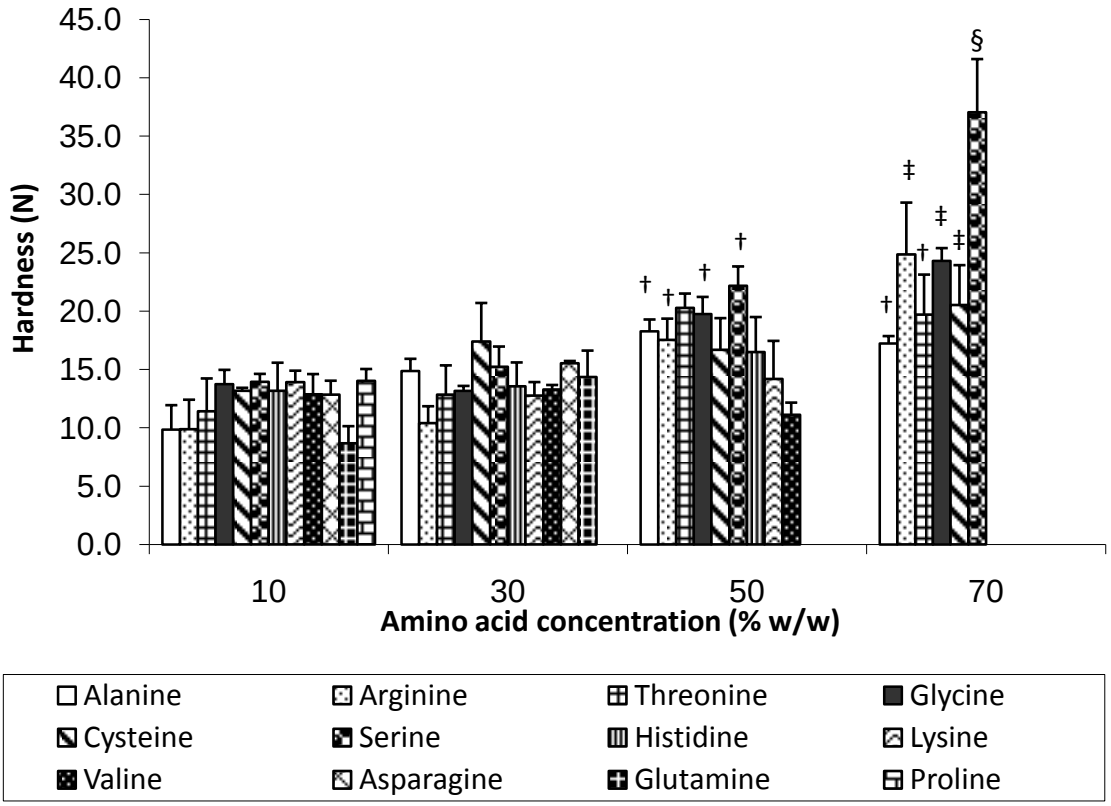


Figure 3.3 The effect of varied concentration of amino acids on the hardness of lyophilised tablets based on 5% Gelatin solution. Results are mean ± SD, n=3. Statistical difference (one way ANOVA/Tukey-Kramer) from control: † $\rho < 0.05$, ‡ $\rho < 0.01$, § $\rho < 0.001$.

interaction of these amino acids with gelatin fibers. These data appear to be supported by scanning electron microscopy (SEM) images (**Figure 3.2**) of the inner structure of the ODTs, which show that valine (**Figure 3.2b**) and glutamine (**Figure 3.2g**) molecules deposited at the surface of gelatin fibres instead of integrating within the fibre suggesting incompatibility of these amino acids with gelatin. On the other hand, SEM images of tablets based on amino acids that improved the hardness show homogenous network of fibres without any segregation/deposition of particles on the surface suggesting that these amino acids integrated completely with gelatin fibre and consequently added extra support to the tablet structure (**Figure 3.2**).

3.4.4. Disintegration time

The results from the disintegration study are summarised in **Figure 3.4**. As expected, the disintegration profile of the ODTs was distinctive for each amino acid (**Figure 3.4**), possibly due to differences in their physicochemical characteristics. At concentration of 10% (w/w), all of the tested amino acids showed no improvements on the disintegration time when compared to 5% gelatin formulation except alanine and glycine, which decreased the disintegration significantly (one way ANOVA/Tukey-Kramer: ρ <0.05), from 29 ± 2s for the 5% gelatin formulation to 17 ± 3s and 16 ± 4s, respectively. By increasing the concentration to 30% (w/w), alanine progressively promoted the disintegration profile to 6 ± 1s, which was the shortest disintegration time in the current study, whereas glycine showed a significant deterioration when compared to its 10% formulation (ρ <0.05). Interestingly, tablets based on 30% histidine

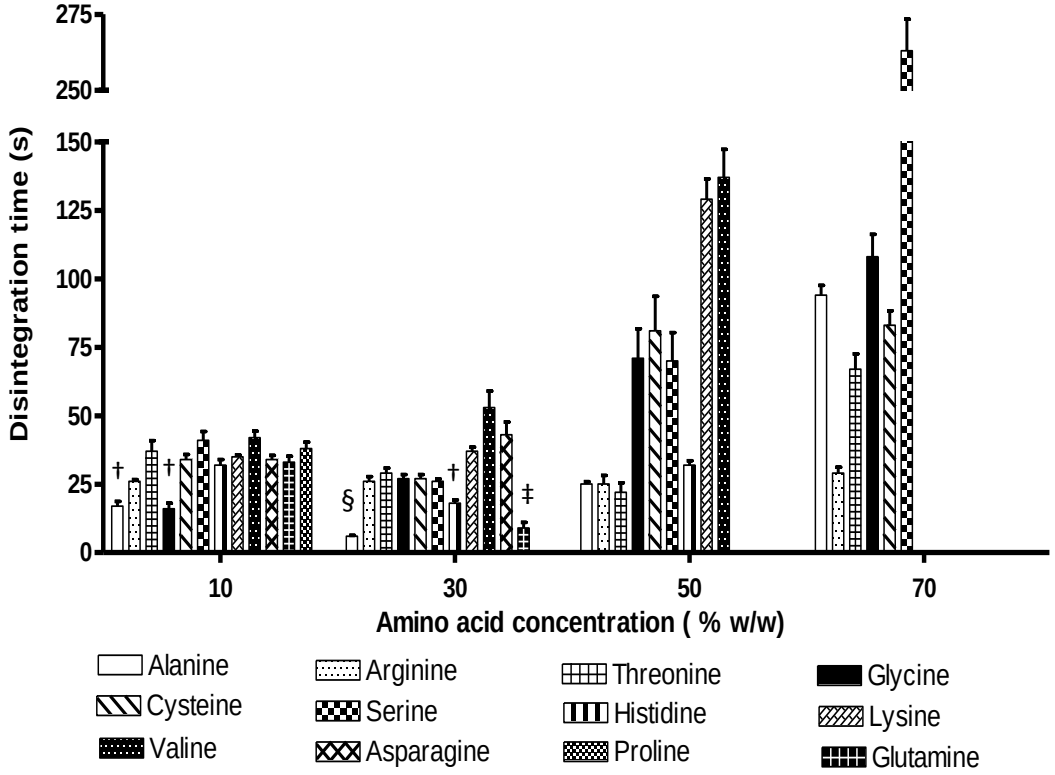


Figure 3.4 The disintegration time of tablets based on 5% gelatin stock solution after inclusion of varied concentration of amino acids. Results are mean ± SD, n=3. Statistical difference (one way ANOVA/Tukey-Kramer) from control (shorter): † ρ <0.05, ‡ ρ <0.01, § ρ <0.001.

and glutamine achieved significantly shorter disintegration times compared with their 10% counterparts and control (5% gelatin) and recorded disintegration times of 18 ± 2s ($\rho < 0.05$) and 9 ± 4s ($\rho < 0.01$), respectively. The rest of the tested amino acids continued their trends by not offering any improvement over the disintegration time of the control (**Figure 3.4**). Inclusion of higher concentration of amino acids: (50 and 70% (w/w)) seemed to have negative effect on the disintegration profile of the tablets, except in case of arginine, where the disintegration profile seemed to be independent of concentration (one way ANOVA/Tukey-Kramer: $\rho > 0.05$).

3.4.5. Wettability and wetting time

The wettability of compressed ODT formulations has been investigated and correlated to the disintegration profile in previous research (Bi et al., 1996; He et al., 2008). However, in the case of lyophilised ODT, measurement of the wetting properties of the whole tablet is extremely difficult due to the very short disintegration time of the tablets.

In the current study, all the ODTs were formulated by adding amino acids individually at varied concentration to a fixed concentration of gelatin stock solution (5% w/w). Therefore, the disintegration time of the ODTs is believed to be influenced by both the concentration and wetting properties of the amino acid. Accordingly, the wetting profiles of the tested amino acids in the powder form were investigated and correlated to the disintegration time of the ODTs.

Measuring the wettability (expressed as contact angle) of pharmaceutical powder requires precision in sample preparation and is associated with extreme experimental care (Kwok and Wilhelm Neumann, 2003). Among the different techniques available, the Wilhelmy method which uses powder coated glass slides as a measurement plate has been shown to demonstrate superior reproducibility and accurate measurement of contact angle (Dove et al., 1996).

The contact angle (θ) profiles of the tested amino acids are presented in **Figures 3.5** and **3.6**. Valine displayed the highest contact angle which increased steadily with time until it was stabilized on an average of 147 ± 5 (n=5), indicating that valine is not wettable in water ($\theta > 90^{\circ}$). Serine, lysine, glutamine and histidine showed partial wetting profile ($90^{\circ} < \theta < 0^{\circ}$) with

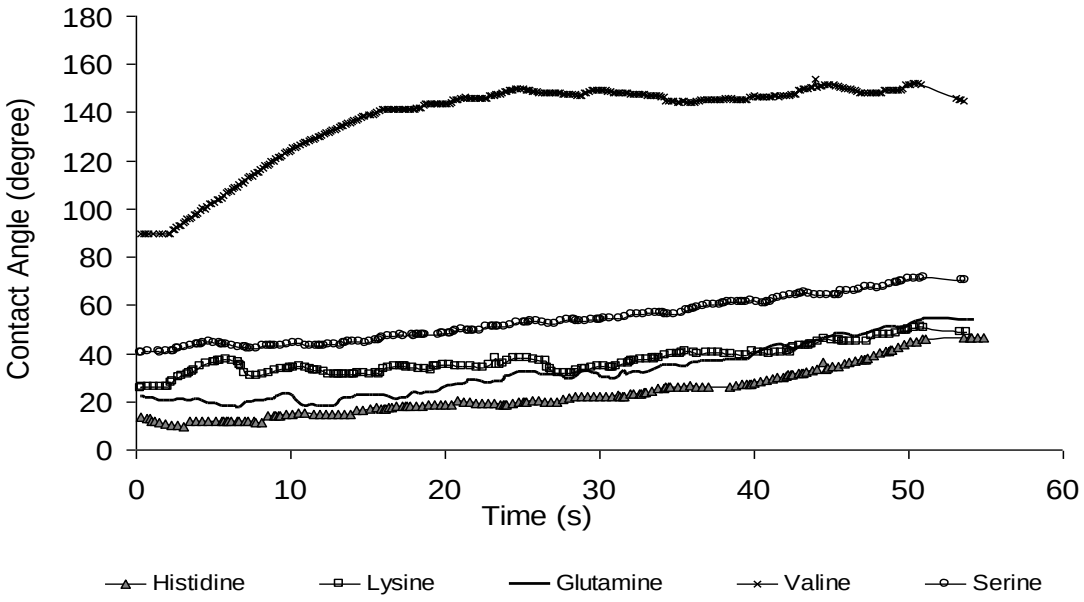


Figure 3.5 Representative profiles of contact angles of water on poorly and partially wettable amino acids as a function of time.

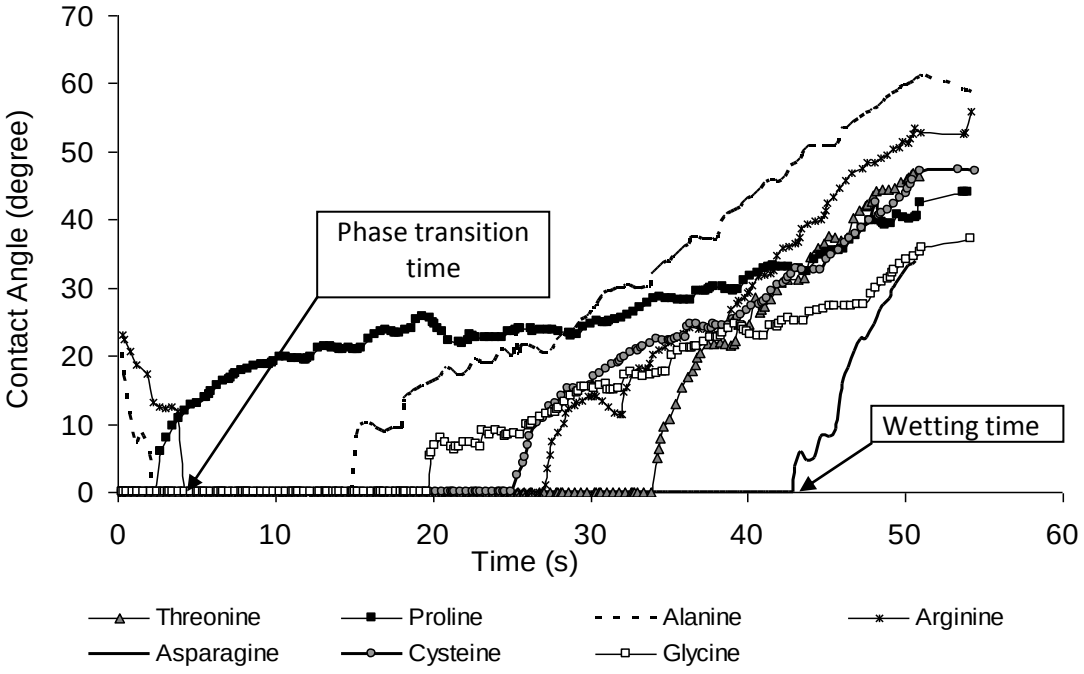


Figure 3.6 Representative profiles of contact angles of water on highly wettable amino acids as a function of time. Phase transition time: is the time required for phase transition from partial (90˚< θ <0˚) to complete wetting (θ=0˚). Wetting time: is the time taken for the complete wetting phase to finish.

average contact angle values of 50 ± 3˚, 39 ± 2˚, 27 ± 4˚ and 23 ± 3˚ (n=5), respectively (**Figure 3.5**). The rest of the tested amino acids (alanine, arginine, threonine, glycine, cysteine, asparagine and proline) displayed complete wetting profile (zero contact angle) (**Figure 3.6**). To differentiate between the wettability profiles of these amino acids, two parameter were identified, the phase transition time, which is the time required for phase transition from partial (90˚< θ <0˚) to complete wetting (θ=0˚), and wetting time, the time taken for the complete wetting phase to finish, which appears in the wettability profile as sudden increase in the contact angle (**Figure 3.6**). This increase in the contact angle is caused by the exposure of the adhesive layer to the water (wetting medium) as the tested powder starts to depart the plate into the liquid medium (Dove et al., 1996). The summary of the two parameters is presented in **Table 3.3**. The results revealed that proline, threonine, glycine, cysteine and asparagine showed complete wetting without delay (phase transition time = 0s), whilst alanine and arginine required 1.0 ± 0.7s and 4.9 ± 2.1s, respectively, to display complete wetting. On other hand, proline displayed the shortest wetting time of 1.3 ± 0.6 s, followed by alanine, glycine, cysteine, arginine, threonine and asparagine (**Table 3.3**). Interestingly, alanine, which is classified as hydrophobic amino acid, had shorter wetting time than arginine, threonine and asparagine, which are known to be more hydrophilic. The shorter wetting time of alanine compared to higher hydrophilic amino acids has been previously reported (Fukami et al., 2005).

Table 3.3 The wetting properties of the amino acids that showed complete wetting. Results are mean ± SD, n=5.

Amino acid	Phase transition time (s)	Wetting time (s)
Proline	0	1.3 ± 0.6
Alanine	1.0 ± 0.7	15.8 ± 3.7
Glycine	0	20.0 ± 3.2
Arginine	4.9 ± 2.1	27.5 ± 1.2
Threonine	0	34.3 ± 1.5
Cysteine	0	25.5 ± 2.2
Asparagine	0	42.9 ± 0.3

3.4.6. Mechanism of disintegration

In this study, the disintegration time profiles of the ODTs as a function of amino acid concentration (**Figure 3.4**) was analysed depending on the wetting profile of the incorporated amino acids in order to determine the factors that influence the disintegration of the ODTs and consequently understand the mechanism of disintegration. In the case of poorly wettable amino acid (valine), the inclusion of higher concentration of valine in the formulation deteriorated the disintegration time possibly due to the decrease in the total porosity and creation of matrix that interacts less favorably with water (low wettability). For highly wettable amino acids (alanine, arginine, threonine, glycine, cysteine and asparagine), parabolic relationships between the disintegration time and the concentration of amino acid were seen, but with different dip values (shortest disintegration time) that were obtained at distinct concentrations for each amino acids (**Figure 3.4**). This parabolic relationship may be due to the inclusion of highly wettable amino acid within the formulation of ODTs which enhances the interaction of tablet's matrix with water (disintegrating medium) but, at the same time, decreases the porosity which inhibits water penetration into the tablet. Therefore, each amino acid exhibited a decrease in disintegration time at an optimal concentration where a balance between porosity and high wettability was created and consequently achieved the shortest disintegration time. **Figure3.7** represents a correlation between the wetting time of these highly wettable amino acids and average disintegration time of ODTs. The linearity of the correlation observed suggested that the measured wetting time of the amino acid plays an important role in determining the disintegration time of ODTs. However, this role is seemed to be highly affected by the porosity of the ODTs. For instance, the correlation between the wetting time and disintegration time for ODTs based on 50% amino acids was poor, due to different porosity of the ODTs at this concentration (**Table 3.2**). Accordingly, the total porosity of the tablet and wetting time of the amino acid play a major role in determining the disintegration time. This mechanism of disintegration, usually referred as wicking, is due to weakening of the intermolecular bonds upon penetration of the disintegration medium between the tablet's excipients and consequently resulting in complete disintegration of the tablets.

On the other hand, partially wettable amino acids (serine, lysine, glutamine and histidine) showed a mix of the two previous profiles. The amino acid with lower contact angle (higher wettability) such as glutamine and histidine, mimicked the highly wettable amino acid profiles

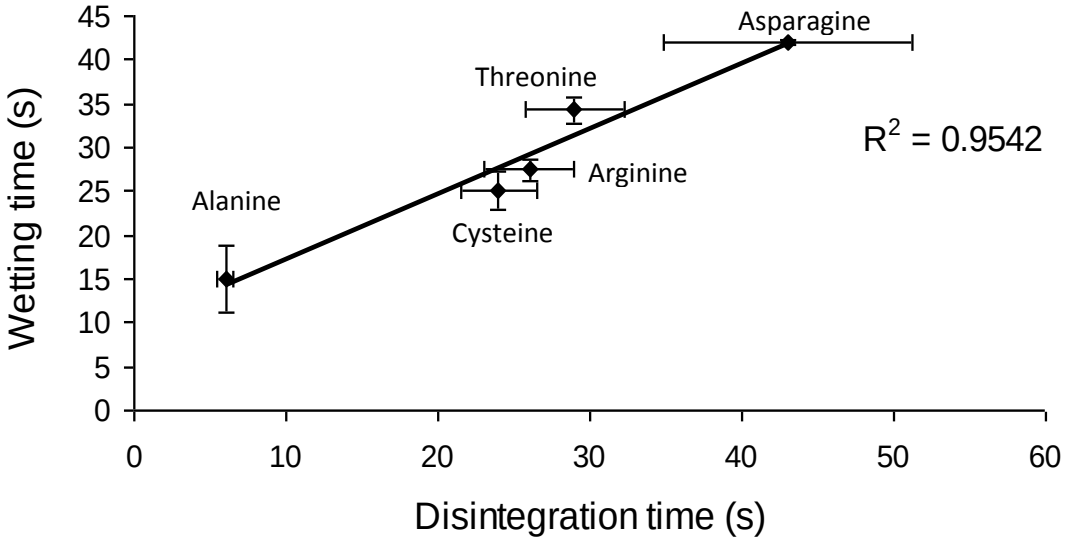


Figure 3.7 Relationship between wetting time of the amino acids and disintegration time of the ODTs at amino acids concentration of 30%.

and a parabolic relationship with the concentration was noticed, whereas amino acids with higher contact angle such as serine and lysine, followed the trend of poorly wettable amino acids as increasing their concentration in the ODT profoundly increased the disintegration time.

3.4.7. The lyophilised tablet index

In order to evaluate the effect of inclusion of amino acids on the hardness and disintegration at the same time and compare it to the gelatin only formulation (control), lyophilised tablets index (LTI) values were calculated according to the following equation:

$$LTI = (H/DT) \div (H^{\circ}/DT^{\circ})$$

Where H: hardness of the tested tablet, DT: disintegration time of the tested tablet, H°: hardness of the control tablets, DT°: disintegration time of the control tablet.

The LTI value provided a ratio indicative of whether the prepared amino acid formulation was better than the gelatin only formulation (chapter 2). Values greater than 1 indicate

improvements over the gelatin formulation, whereas lower values suggest retardation in the overall tablet properties (disintegration time and hardness). In addition, LTI values can be used to rank the improvements in tablet properties among various formulations. The results (**Table 3.4**) revealed that alanine, glutamine, glycine, arginine, histidine, serine and threonine were able to improve the overall tablets properties to different extent at different concentration. Alanine achieved the highest value at concentration of 30% (w/w) with LTI value of 4.99, followed by the 30% glutamine formulation (LTI= 3.39) and then the 10% glycine (LTI= 3.39). Our data in chapter 2 showed that the inclusion of saccharides and polyols in formulation of lyophilised ODTs based on 5% gelatin stock solution (similar conditions to the current study) enhanced the overall tablet properties by recording LTI values ranged between 0.52 - 8.10, which are comparable to the LTI values from this current study demonstrating the suitability of the amino acids in the formulation of ODTs.

Table3.4 The lyophilised tablet index of tablets based on 5% gelatin stock solution and varied concentration of amino acids.

Amino Acid	**LTI**			
	10 %	30%	50%	70%
Alanine	1.26	4.99	1.58	0.39
Arginine	0.82	0.85	1.47	1.84
Threonine	0.66	0.95	1.96	0.63
Glycine	2.54	1.01	0.59	0.48
Cysteine	0.82	1.35	0.44	0.53
Serine	0.72	1.26	0.67	0.30
Histidine	0.87	1.63	1.10	1.73
Lysine	0.84	0.73	0.23	-
Valine	0.65	0.54	*	*
Asparagine	0.79	0.77	*	*
Glutamine	0.55	3.39	*	*
Proline	0.78	-	-	-

(*) No intact lyophilised tablets were formed
(-) Not soluble

3.5. Conclusion

The current study suggests that successful formulation of saccharides free lyophilised ODTs requires amino acids that crystallise in the frozen state or display relatively high Tg' in the formulation, interact and integrate completely with the binder and, also, display short wetting time with the disintegrating medium. The tested amino acids have showed varied capability to fulfil all the required characteristics for the formulation of lyophilised ODTs. However, inclusion of an optimised concentration of alanine achieved the best balance and therefore produced ODTs with superior characteristics.

Chapter Four: Investigation of Formulation and Process of Lyophilised ODTs Using Novel Amino Acid Combinations

Papers relating to this chapter

Alhusban FA, Perrie Y, Mohammed A - Investigation of formulation factors on the sublimation rate of orally disintegrating tablets. *37th Annual Meeting of the Controlled Release Society*, July 2010, Portland, USA.

AlHusban, F., ElShaer, A., Kansara, J., Smith, A., Grover, L., Perrie, Y., Mohammed, A. (2010) Investigation of formulation and process of lyophilised orally disintegrating tablet (ODT) using novel amino acid combination. *Pharmaceutics,* 2 (1), 1-17.

Investigation of Formulation and Process of Lyophilised ODTs Using Novel Amino Acid Combinations

4.1. Introduction and Aims

Investigating the feasibility of using individual amino acids as matrix supporting/ disintegration enhancer agents in the formulation of lyophilised orally disintegrating tablets (chapter 3) showed varied capability of the amino acids to fulfil all the required characteristics for the formulation of lyophilised ODTs. For instance, proline showed complete wettability in water (disintegrating medium) with short wetting time, which is expected to improve the disintegration of ODTs; however, its inclusion in freeze dried formulations was limited due to the extremely low glass transition temperatures and consequently resulting in the collapse of the prepared formulations. On the other hand, serine based formulations displayed higher collapse temperature and produced elegant tablets even at high concentration, due to its tendency to crystallise in the frozen state, but was characterised by long disintegration time, which was explained by serine's partial wetting property, as the measured contact angle (θ) with water was $0^{\circ} < \theta < 90^{\circ}$.

The main aim of the this chapter was to combine the benefits of proline and serine in the formulation of ODT with the aim to achieve a tablet with shorter disintegrating time (mainly due to the presence of highly wettable proline) and enhanced stability during freeze drying (due to the high glass transition and crystallisation capacity of serine). The study investigated the influence of inclusion of various ratios of proline and serine at different total concentrations on the thermal properties of the frozen formulations, formation of intact tablets after freeze drying and ODT characteristics in terms of disintegration time and mechanical properties. Furthermore, the optimised formulation was then used to investigate the effect of freezing drying conditions on the sublimation rate, disintegration time and mechanical properties of ODTs.

Typical freeze drying cycle consists of three main stages; freezing, primary drying and secondary drying. Primary drying is the longest stage in the freeze drying cycle and takes several hours to few days to complete. The rate of primary drying is governed by factors related to the process conditions, including: shelf temperature, vacuum pressure and heat transfer process from the shelf fluid to the frozen formulation, and factors related to the

product, which determine the mass transfer resistance (MTR) to sublimation (Kuu et al., 2006). Non optimum freeze drying conditions and/or formulation factors may result in longer cycle and consequently higher cost than is necessary.

In the freeze-drying process, the freezing step is one of the most important steps as it determines the size and morphology of the ice crystals within the frozen material and, consequently, the final inner-structural feature of the freeze-dried material (Hottot et al., 2004). Accordingly, the freezing protocol can influence the primary drying process by affecting the mass transfer resistance (MTR) to sublimation, as the sublimed water vapour should flow through the formed pores to the condenser (Hottot et al., 2007). Moreover, in lyophilised tablets, the freezing protocol is expected to influence the ODT characteristics after freeze-drying (the disintegration time and mechanical properties), due to its effects on the total porosity and pore size.

In this chapter, three freezing protocols; freezing at -80 °C using pre-cooled shelves with or without annealing at -20 °C for 12 hours and flash freezing using liquid nitrogen, were investigated for their effects on the sublimation rate, inner-structural features of the freeze dried tablets and tablets characteristics.

4.2. Materials

Gelatin from bovine skin, type B (Bloom strength ~ 75), L-Proline (C5H9NO2, Reagent plusTM ≥ 99%), L-Serine (C3H7NO3, Reagent plusTM ≥ 99%), were all purchased from SIGMA®, USA. All the materials were used as received.

4.3. Methods

4.3.1. Formulation of ODTs to investigate the effect of L-proline and L-serine combination on the tablets characteristics

Various ratios (100:0, 85:15, 70:30, 45:55, 30:70, 15:85, 0:100) of L-proline and L-serine at total concentrations of 10%, 30%, 50%, and 70% w/w (total solid) were added to 5% (w/w) gelatine stock solution. 1.5 g of the prepared solution was transferred to a PEG mould, frozen at -80 °C for 2 hours and then freeze-dried (ADVANTAGE Freeze-dryer, VIRTIS) according to an

optimized regime (primary drying for 48 hours at a shelf temperature of -40 °C and secondary drying for 10 hours at a shelf temperature of 20 °C and vacuum of 50 mTorr. All the formulations (28 different formulations) were prepared in triplicate from three independent batches. From each batch 3 tablets were freeze dried and characterised for disintegration time, hardness and fracturability. In total 252 tablets were prepared (28 X 3 X 3).

4.3.2. The influence of freezing protocol on the primary drying rate and ODTs characteristics

The formulation with the best performance in terms of disintegration time and mechanical properties from the previous study (2.2.1) was used to investigate effects of freezing protocols on the sublimation rate and tablets characteristics. The following three freezing protocols were applied:

Protocol 1: the formulation was frozen in -80 °C freezer.

Protocol 2 (flash freezing): The formulation was immersed in liquid nitrogen for 40 seconds then kept at -80 °C freezer.

Protocol 3 (annealing): the formulation was frozen at -80 °C pre-cooled freezer for 2 hours, annealed at -20 °C pre-cooled freezer for 12 hours and then transferred back to -80 °C freezer.

The sublimation rate was studied by freeze drying samples (from each protocol) at shelf temperature of -40 °C, condenser temperature of -80 °C and 55 mTorr vacuum. Samples were withdrawn from the freeze dryer at predetermined time intervals (2, 6, 12, 24, 36 and 48 hours) and the amount of water sublimed was evaluated using weight difference method. All the measurements were done in triplicate of independently prepared samples.

In order to study the effect of freezing protocol on tablet characteristics, nine samples from each protocol entered a complete freeze drying cycle using similar regime used in section (2.2.1).

4.3.3. Differential scanning calorimetry

Differential scanning calorimeter (Pyris Diamond DSC) was used to investigate the glass transition temperatures (Tg) and the crystallization events of the frozen formulations. 10–15 mg of the liquid formulation was transferred into an aluminium pan (50 µL capacity) and then sealed with an aluminium top. The sample was cooled to -65 ºC and then heated to 20 ºC at 5 ºC/min. To determine the glass transition temperature of the maximally freeze concentrate sample (Tg'), after initial cooling to -65 ºC, annealing for 10 min at temperature of 2 ºC higher than the relevant glass transition temperature (Tg) was added before carrying out the above method. Nitrogen was used as a purge gas at a flow rate of 20 mL/min. Indium and zinc were used to calibrate the heat flow and melting point onset (melting point: 156.6 °C, ΔHm: 28.42 J/g for Indium and melting point: 419.47 °C ΔHm: 108.26 J/g for Zinc). The obtained thermograms were analysed using Pyris Manager Software (version 5.00.02) where Tg and Tg' values were determined from the intersection of relative tangents to the baseline. The experiment was performed in triplicate and an empty aluminium pan was used as a reference cell for all the measurements.

4.3.4. Texture analysis

In order to investigate the fracturability and hardness of the prepared tablets, QTS 25 texture analyser (CNS Farnell, Hertfordshire, UK) was used. Fracturability was studied by using 1 mm diameter penetration probe which penetrates 4 mm of the tablet at a speed of 6 mm/min and the peak force was measured in Newton (N) after 3 mm of penetration. The tablet hardness was measured using a 5 mm diameter compression probe which compresses the tablets to 2 mm depth at a speed of 6 mm/min and the peak force is measured in Newtons after 1 mm compression. The obtained data was analysed by TexturePro software. All fracturability and hardness measurements were performed in triplicate for each formulation and the data is presented as mean ± standard deviation.

4.3.5. In vitro disintegration study of the tablets

Disintegration time is the time required for ODTs to disintegrate completely without leaving any solid residue. In vitro disintegration time for lyophilised ODTs was evaluated using US

pharmacopoeia monograph (<701> disintegration). Erweka (ZT3, Appartebau, GMBH) was used in this study as a disintegration apparatus and distilled water (800 mL) as disintegration medium; the disintegration medium temperature was maintained at 37 °C by thermostat. At each time, one tablet was placed in the basket rack assembly and covered by transparent plastic disk. The disintegration time was taken as the time required for ODTs to disintegrate completely without leaving any solid residue. All the measurements are carried out six times and presented as (mean ± standard deviation).

4.3.6. Mercury porosimetry

Mercury porosimetry was used to evaluate the influence of the freezing protocol on the pore size distribution of the resulting tablets. Measurements were made using an Autopore IV 9500 mercury porosimeter (Micromeritics, UK). Samples were stored overnight in a vacuum to remove moisture and were then weighed and loaded into a 5 cc bulb 1.190 ml stem, penetrometer (Micromeritics, UK). Measurements of pore size distribution were made in the low and high pressure chambers of the porosimeter to provide the pore size distribution in the range 6 nm to 360 µm. The resulting measurements of intrusion volume (ml/g/nm) were used to calculate pore size distribution.

4.3.7. Statistical analysis

Graph Pad Instat® software was used for the statistical analysis study. Data groups were compared using one way analysis of variance (ANOVA) and pair-wise multiple comparisons method (Tukey-Kramer multiple comparison test). Standard deviation (SD) was used to report the error in the figures and texts. Probability values of 95% ($P < 0.05$) were used to determine the significant difference.

4.4. Result and discussion

4.4.1. Thermal analysis and formation of intact tablets

The successful production of intact lyophilised tablets is totally dependent on the thermal profile of the frozen formulation and freeze drying conditions. The maximum tolerable product temperature during primary drying which ensures the formation of intact tablets, known as collapse temperature, can be estimated from the DSC profile of the frozen formulation. For amorphous formulations, the collapse temperature is usually 1 to 3 ˚C higher than the glass transition (Pikal and Shah, 1990).

At total amino acids concentration of 10% and 30% w/w (serine:proline combinations), all the studied combinations of serine and proline showed glass transition step (Tg') in their heating scans at different temperatures depending on the total concentration and ratio of both amino acids (**Table 4.1**). The inclusion of these two amino acids in the formulation had a plasticising effect in the formulation as increasing the total concentration of the amino acids significantly lowered the Tg' temperature. However, proline had a higher plasticising effect on the system than serine since a gradual increase in proline ratio within the formulations was associated with a steady decrease in Tg' values. For example, at a total concentration of 30% (w/w), increasing proline ratio from 0 to 45 to 100 decreased the Tg' from -25.66 ± 0.01 to -32.26 ± 0.1 to -37.65 ± 0.24 ˚C, respectively (**Table 4.1**). Estimation of the collapse temperatures for 10% w/w amorphous formulations suggested the presence of a high safety margin between the shelf and collapse temperature which resulted in the formation of intact and smooth tablets. On the other hand, formulations at 30% w/w total concentration of the combined amino acids did not reveal any morphological deterioration despite the small difference between the glass transition and shelf temperature. The possibility of any micro collapse for these formulations cannot be ruled out.

Table 4.1 Glass transition temperatures (˚C) of maximally freeze concentrate solutions of 5% gelatin after inclusion combinations of proline and serine at total concentration of 10% and 30% w/w. Values are represented as mean ± standard deviation (n = 3).

Combination (proline:serine)	Total concentration (w/w)	
	10%	30%
0:100	-18.63 ± 0.05	-25.66 ± 0.01
15:85	-19.12 ± 0.11	-27.97 ± 0.12
30:70	-19.71 ± 0.09	-29.52 ± 0.42
45:55	-20.35 ± 0.21	-32.26 ± 0.10
70:30	-20.87 ± 0.16	-34.24 ± 0.10
85:15	-21.31 ± 0.08	-35.57 ± 0.07
100:0	-21.47 ± 0.12	-37.65 ± 0.24

DSC analysis of formulations with total concentration of 50% and 70% w/w (total solid) of combinations of proline and serine are presented in **Figure 4.1** and **Table 4.2**, respectively. At these high concentrations, formulations containing serine only displayed crystallisation event during their heating scans. Inclusion of small amount of proline, 15:85 (proline:serine), seemed to drift the crystallisation temperature of serine to a higher temperature when compared to serine alone formulation (**Figure 4.1A**). Further increase in proline ratio within the formulation inhibited serine crystallisation completely and the trend continued as observed in 10% and 30% w/w formulations, which was evident by lowering of the glass transition temperature (**Figure 4.1B** and **Table 4.2**). Freeze drying of these formulations was less efficient when compared to 10% and 30% w/w formulations which can be explained by higher concentration of proline that decreases the glass transition and inhibits serine crystallisation. As a result, all the formulations with Tg' less than -40 ˚C collapsed and therefore no tablet was formed. Freeze drying of such formulations is possible by decreasing the shelf temperature of the cycle, but it is associated with significant increase in the primary drying time. It has been shown previously that lowering the shelf temperature by 5 ˚C may result in increase in the primary drying time of about 15 hours (Rambhatla et al., 2006).

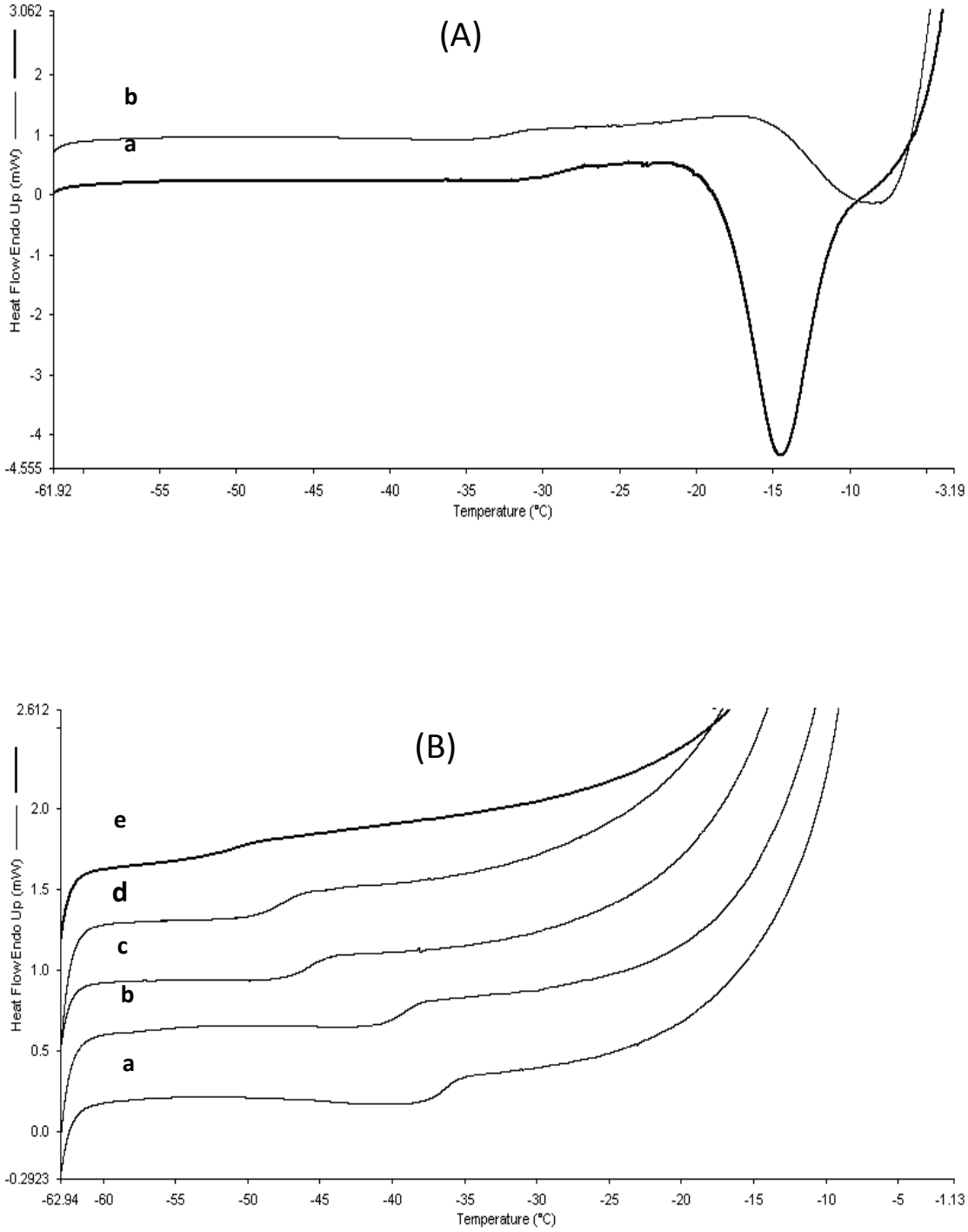


Figure 4.1 Overlaid DSC heating curves of frozen formulations: (**A**) that exhibited serine crystallisation at total concentration of 50% amino acid. (a) Serine to proline ratio of 100:0. (b) Serine to proline ratio of 85:15. (**B**). that did not show tendency to crystallize at total concentration of 50% amino acids. (a) 70:30 (serine: proline); (b) 55:45 (serine: proline); (c) 70:30 (serine: proline); (d) 85:15 (serine: proline); (e) 0:100 (serine: proline).

Table 4.2 Glass transition temperatures of maximally freeze concentrate solutions (Tg') and crystallisation temperatures of frozen solutions of 5% gelatin after inclusion of combinations of proline and serine at total concentration of 70%. Values are represented as mean ± standard deviation (n = 3).

Proline/Serine ratio	Tg' (˚C)	Crystallization temperature (˚C)
0:100	*	-23.99 ± 0.53
15:85	-33.13 ± 0.43	-16.62 ± 0.95
30:70	-39.54 ± 0.32	-14.02 ±1.08
45:55	-44.91 ± 0.64	*
70:30	-51.44 ± 2.27	*
85:15	-57.63 ± 0.97	*
100:0	>65	*

(*) No events were detected

4.4.2. Characterisation of ODTs

4.4.2.1. Mechanical properties

All the successfully freeze dried formulations were characterised in terms of mechanical properties by measuring their resistance to compression by a 5 mm diameter probe (hardness) and penetration by a 1 mm diameter probe (fracturability). The influence of the total amino acids concentration and proline to serine ratio within the formulation on the hardness and fracturability of ODTs are presented in **Figures 4.2** and **4.3**, respectively. The results showed that the hardness of the ODTs was significantly improved by inclusion of a higher total concentration of both amino acids (one way ANOVA/Tukey- Kramer: $\rho < 0.05$). For instance, each increment in the total concentration of 15:85 of proline:serine formulation was associated with a significant increase in the hardness, from 14.46 ± 1.33 N at concentration of 10% to 17.24 ± 0.92 N at 30% w/w, to 21.29 ± 2.26 N at 50% and then to 37.96 ± 0.68 N at concentration of 70% (one way ANOVA/Tukey-Kramer: $\rho < 0.05$). However, at the same total concentration, combinations with higher serine ratio provided stronger tablets compared to tablets with high proline ratio suggesting better capability of serine to enhance the hardness of lyophilised ODTs (**Figure 4.2**). For example, at total concentration of 10%, increasing serine ratio from zero to 55% resulted in significant improvement in the ODTs hardness from 9.85 ±

0.41 N to 12.47 ± 0.5 N (one way ANOVA/Tukey-Kramer: $\rho < 0.05$) and then to 14.47 ± 1.3 N(one way ANOVA/Tukey-Kramer: $\rho < 0.01$) by further increase serine ratio to 85% w/w of the total amino acids.

The ODTs fracturability results are presented in **Figure 4.3**. Statistical analysis of the data showed that increasing the total amino acids concentration from 10% to 30% did not improve the fracturability. However, significant improvements were achieved by increasing the total concentration to 50% (one way ANOVA/Tukey-Kramer: $\rho < 0.01$) or 70% (one way ANOVA/Tukey-Kramer: $\rho < 0.001$). Also, the results showed no particular influence of changing the ratio of proline and serine within the formulation. Accordingly, the results suggested that the fracturability was mainly influenced by the total concentration of the amino acids rather than the ratio of proline to serine within the formulation.

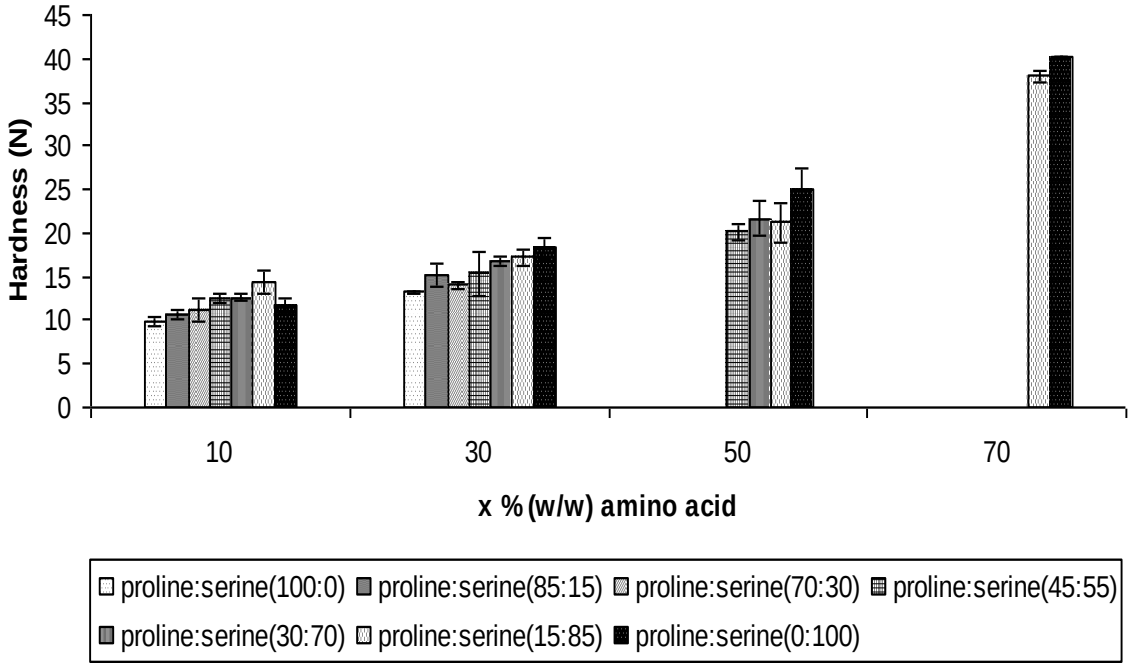


Figure 4.2 The hardness (Newton) of the ODTs after inclusion combinations of proline and serine at total concentrations of 10%, 30%, 50%, and 70% w/w. Values are represented as mean ± standard deviation (n = 3).

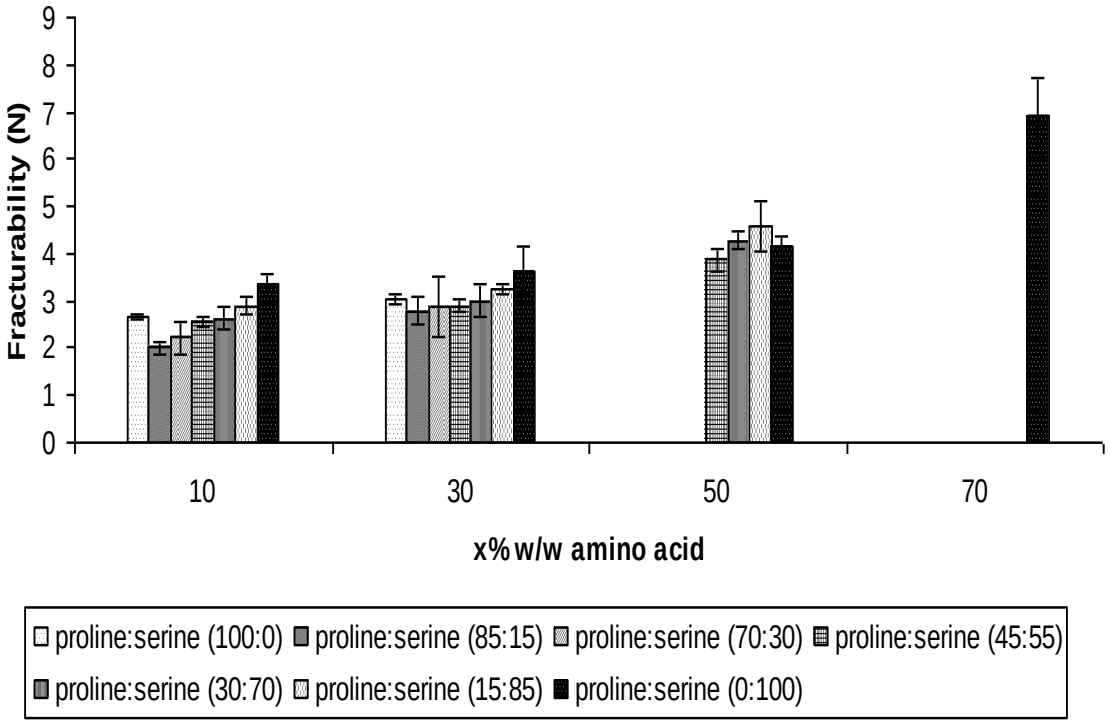


Figure 4.3 The fracturability (Newton) of the ODTs after inclusion combinations of proline and serine at total concentrations of 10%, 30%, 50%, and 70% w/w. Values are represented as mean ± standard deviation (n = 3).

4.4.2.2. Disintegration time of the ODTs

The disintegration time profile of the tablets is presented in **Figure 4.4**. At a total concentration of 10%, tablets containing proline only achieved the shortest disintegration time of 21.0 ± 2.1 s (n =3). Upon gradual increment in serine ratio, the disintegration times increased steadily to 29.0 ± 2.2 s for the 45:55 of proline:serine formulation and then to 33.0 ± 1.0 s for tablets with serine only. At a total amino acids concentration of 30% w/w, the shortest disintegration time was 17.3 ± 0.6 s for the 45:55 of proline:serine combination. It was anticipated that formulations with higher proline ratio (higher than 45%) would achieve the shortest disintegration time but because of their narrow freeze drying safety margin, invisible partial micro collapse (as discussed in the section on thermal properties) might have deteriorated their disintegration profile. Formulations with high freeze drying safety margin, which contained proline ratio less than 45%, confirmed this theory by following the expected trend where longer disintegration time was associated with any increase in serine ratio (**Figure 4.4**).

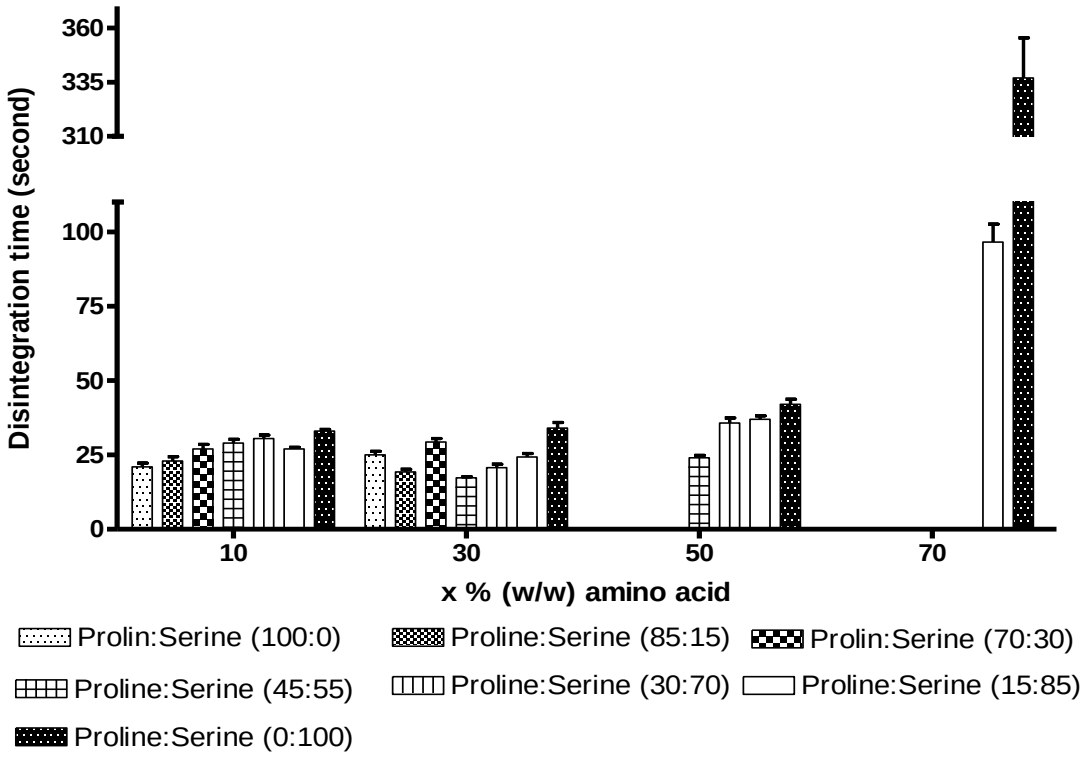


Figure 4.4 The disintegration time (seconds) of the ODTs after inclusion combinations of proline and serine at total concentrations of 10%, 30%, 50%, and 70% w/w. Values are represented as mean ± standard deviation (n = 3).

The successfully freeze dried tablets based on total concentrations of amino acids of 50 and 70% followed the expected trend and the shortest disintegrations at both concentrations were recorded by formulations with the highest ratio of proline (**Figure 4.4**). These results can be explained depending on the mechanism of disintegration of ODTs. Generally, the fast disintegration profile of lyophilised ODTs is attributed to the highly porous structure that allows fast diffusion of water (disintegrating medium) through highly wettable matrixes, which disintegrate/dissolve rapidly upon contact with water (Sunada and Bi, 2002). In the current formulations, inclusion of higher concentration of proline is expected to increase the wettability of the matrix while increasing total concentration of the amino acids reduces the total porosity of the tablets. Accordingly, a balance between the wettability and porosity is required to achieve short disintegration time. The current results (**Figure 4.4**) suggest that

45:55 combination of proline:serine at a total concentration of 30% achieved best balance between wettability (containing 13.50% proline) and porosity with total amino acid concentration of 30% w/w (intermediate concentration) which consequently achieved the shortest disintegration time in the study (17.3 ± 0.6 s). It is interesting to note that that even small intervention in this balance can lead to significant deterioration in the disintegration time. For example, formulations with higher porosity (lower total concentration of amino acids) but slightly lower wettability (lower concentration of proline), as in tablets based on proline only at total concentration of 10% (of total tablet weight), displayed significantly longer disintegration time. Similarly, formulations with higher wettability but smaller porosity, as in tablets based on 45:55 of proline:serine at total concentration of 50%, did not achieve shorter disintegration time (**Figure 4.4**). Similar trend was observed from previous chapters that investigated the influence of saccharides (chapter 2) and amino acids (chapter 3) on disintegration time, where parabolic relationships were noticed within optimal concentration (30–40% w/w) of matrix supporting/disintegration enhancing agents.

4.4.2.3. Lyophilised tablet index

The tablet characterisation results (hardness and disintegration time) have shown that serine to proline ratio and their total concentration in the formulation have contrasting influence on the mechanical properties and disintegration time of the lyophilised ODTs. Therefore, the overall tablets properties were evaluated depending on a single parameter that integrates the hardness and disintegration time of ODTs, called lyophilised tablet index (LTI) (chapter 2 and 3). LTI is calculated by dividing the measured hardness by the disintegration time of certain ODT formulation which means the higher value the better the overall properties (high hardness and low disintegration time). The results (**Table 4.3**) proved that combining proline and serine as matrix supporting /disintegration enhancing agents in the formulation creates ODTs with superior overall properties (disintegration time and harness) than using proline or serine individually. The highest LTI value was 0.88 for the formulation with 45:55 of proline:serine at total concentration of 30% (LTI= 0.88), followed by the same combination of proline and serine but at concentration of 50% with LTI value of 0.82, suggesting that the deterioration in the disintegration, caused by increasing the total concentration from 30% to 50%, was more profound than the improvement in the hardness.

Table 4.3 The lyophilised tablet index values of ODTs ODTs that are produced from varied concentration of proline and serine combinations.

Combination (prolin:serine)	Total concentration (w/w)			
	10%	30%	50%	30%
100:0	0.47	0.51	-	-
85:15	0.46	0.78	-	-
70:30	0.41	0.48	-	-
45:55	0.43	0.88	0.84	-
30:70	0.41	0.82	0.61	-
15:85	0.54	0.71	0.58	0.39
0:100	0.44	0.54	0.59	0.12

4.4.3. The influence of freezing protocol on the primary drying rate and ODTs characteristics

In the freeze-drying process, the freezing step is one of the most important steps as it determines the size and morphology of the ice crystals within the frozen material and, consequently, the final inner-structural feature of the freeze-dried material (Hottot et al., 2004). Thus, in lyophilised tablets, the freezing protocol is expected to influence not only the freeze drying process (sublimation rate and primary drying time) (Hottot et al., 2007) but also tablet characteristics after freeze-drying (the disintegration time and mechanical properties). In this study, three freezing protocols; freezing at -80 °C using pre-cooled shelves with or without annealing at -20 °C for 12 hours and flash freezing using liquid nitrogen, were investigated for their effects on the sublimation rate, inner-structural features of the freeze dried tablets and tablets characteristics of the formulation with the highest LTI value (45:55 of proline: serine at total concentration of 30% w/w).

Mercury porosimetry was used to investigate the structure of the freeze dried tablets, because it preserves the internal morphology of the sample during the measurement, does not require cutting the tablets which may alter the cake structure and also gives consistent estimation of the pore size and pore size distribution for the whole tablets.

4.4.3.1. Influence on primary drying rate

Figure 4.5 shows primary drying rates of tablets based on 45:55 of proline: serine at total concentration of 30% w/w after applying different freezing methods. At all time points the average drying rates of the annealed tablets were significantly higher than tablet frozen using -80 °C precooled shelves or liquid nitrogen (flash freezing), both without annealing (one way ANOVA/Tukey-Kramer: $\rho < 0.01$). Moreover, the decrease in drying rate with time due to increasing the thickness of the dried layer seemed to be steady and consistent for the annealed tablets compared to the tablets without annealing. Given that all the tablets were of a similar formulation and freeze dried under the same conditions, all the differences in the primary drying profiles are attributed to the inner morphology of the tablets imposed by the freezing regime.

The mercury porosimetry data (pore size distribution) are presented in **Figure 4.6**. The results showed that flash freezing produced tablets with the smallest modal pore diameter (6 µm), but with a broad pore size distribution between 284 nm to 30 µm. When the formulation froze at -80 °C, the pores exhibited a larger modal diameter (30 µm) with pores in the range 1 to 60 µm. On the other hand, annealed tablets exhibited the largest pores with a modal diameter of 60 µm distributed from 13 to 370 µm. In case of flash freezing and pre-cooled shelves at -80 °C, freezing at lower temperature and faster rate resulted in a larger number of dispersed minute ice crystals, and consequently smaller pores after freeze drying. These small pores create narrow and complex channels for water vapor removal during the sublimation and therefore higher mass transfer resistance (MTR), which in turn decreases the sublimation rate (Searles et al., 2001). Moreover, the lack of direct control over ice nucleation temperature using these freezing methods resulted in wide pore size distributions (**Figure 4.6**) and hence heterogeneous MTR values (Patapoff and Overcashier, 2002), which is translated as inconsistent decrease in the average primary drying rate over time (**Figure 4.5**).

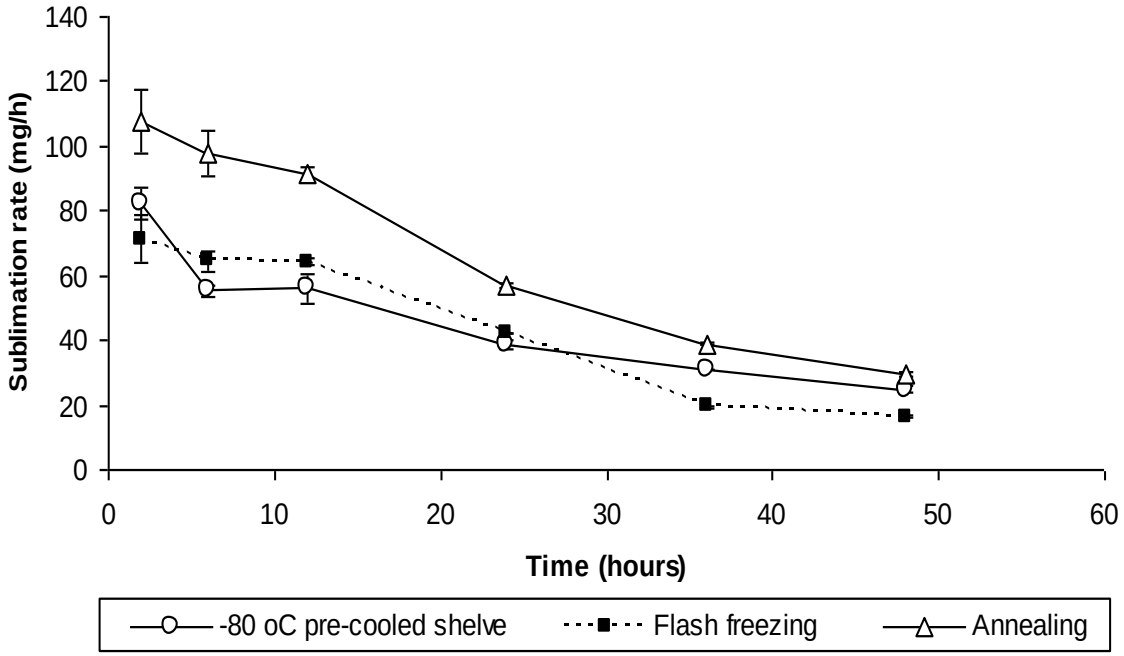


Figure 4.5 Sublimation rate (mg/h) as a function of time for the ODT formulation with 45:55 of proline: serine at total concentration of 30% w/w frozen by various methods. Values are represented as mean ± standard deviation.

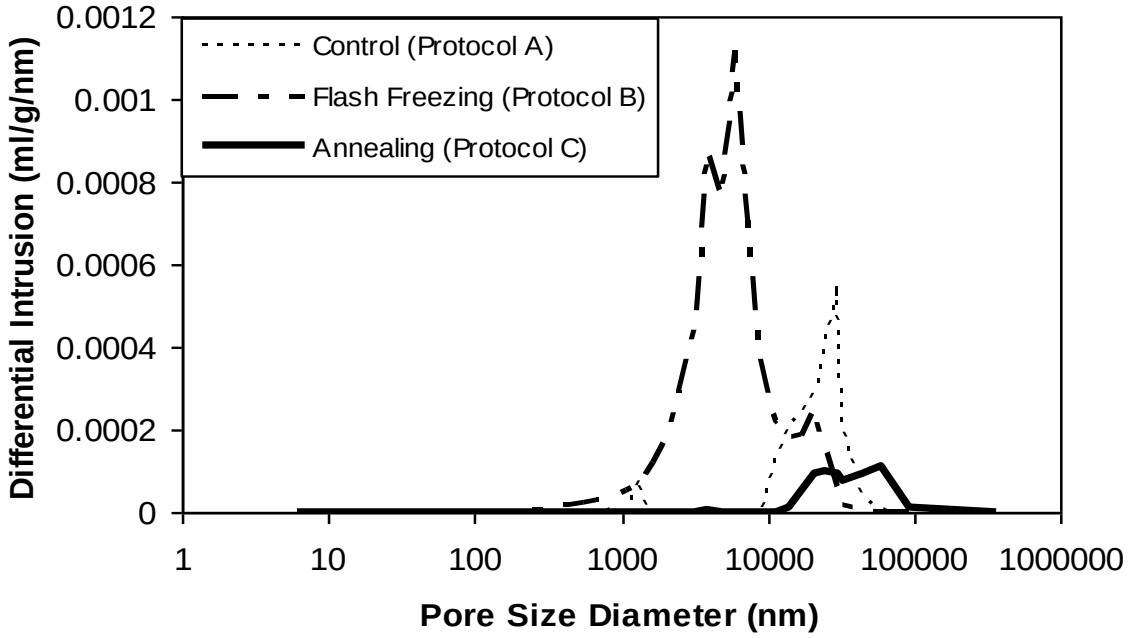


Figure 4.6 Pore size distributions of ODTs prepared using different drying protocols, including: freeze drying, flash freezing and annealing.

Annealing, on the other hand, is a known technique to enhance the growth of ice crystals and eliminate the initial variation in crystal size distribution by a phenomena known as Ostwald ripening, where smaller ice crystals melt quickly and then merge with larger crystals (not completely melted) as a result of raising the temperature above Tg', whereas re cooling the sample fixes the structure of the large crystals (Dawson and Hockley, 1992; Kang et al., 1999; Searles et al., 2001a). Thus, the annealed tablets in this study exhibited larger mean pore diameter with narrower size distribution compared to the tablets without annealing (**Figure 4.6**). These structural features facilitated and homogenised water-vapor transmission (low and constant MTR) and therefore high and consistent primary drying rates were achieved. The current results are consistent with previous studies (Hottot et al., 2004; Hottot et al., 2007; Searles et al., 2001b; Abdelwahed et al., 2006), where adding annealing to the freezing regime has enhanced ice crystals growth and, consequently, increasing the sublimation. Other researchers have employed different physical approaches to enhance and control ice crystals growth, with the aim of reducing primary drying times, including ultrasounds (Morris et al., 2004), vacuum induced surface freezing (Kramer et al., 2002) and high electrical field (Petersen et al., 2006).

4.4.3.2. Influence on ODT characteristics

We have demonstrated above that the structure of the freeze dried cake had changed significantly when applying different freezing protocols. These morphological changes can directly influence the basic properties of the lyophilised formulation. For ODTs, the disintegration time and mechanical properties are the key aspects to investigate.

The effect of different freezing protocols on the ODTs disintegration time is presented in **Figure 4.7**. The results revealed that the annealed tablets had significantly shorter average disintegration time of 8.6 ± 0.6 s when compared to 17.5 ± 0.5 s and 17.3 ± 0.6 s for tablets frozen using liquid nitrogen and at -80 °C Pre-cooled shelve, respectively, (one way ANOVA/Tukey-Kramer: $\rho < 0.001$). The fast disintegration of the annealed tablets can be attributed to their large pores (**Figure 4.6**) that facilitate rapid diffusion of water (the disintegrating medium).

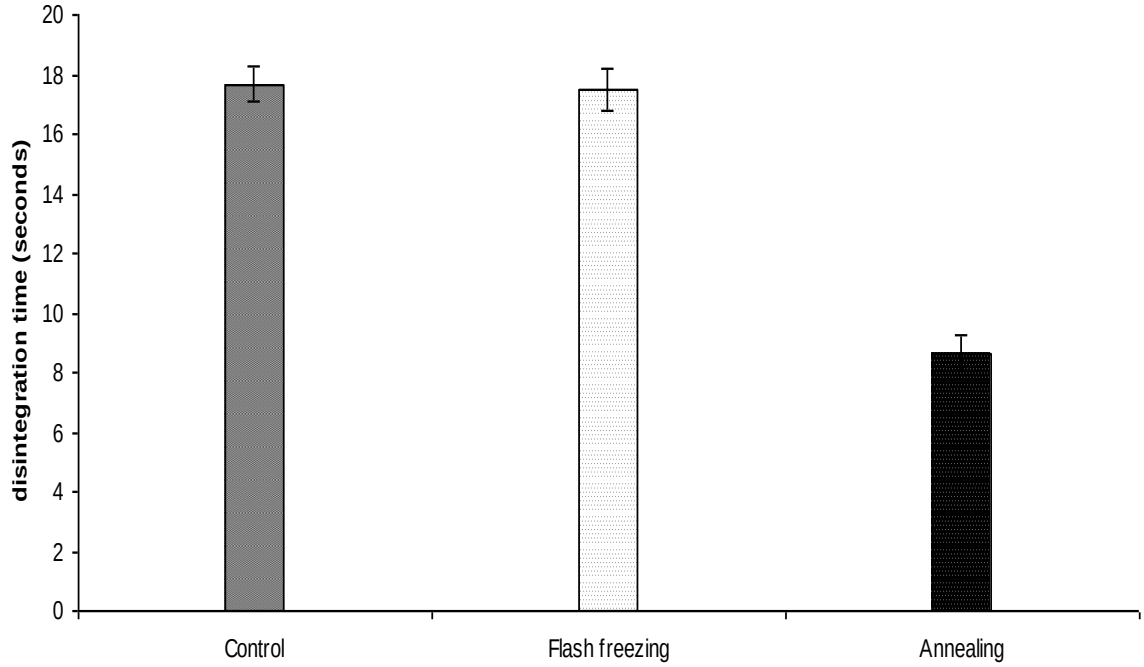


Figure 4.7 Disintegration time of ODTs based on 45:55 of proline: serine at total concentration of 30% w/w after applying different freezing protocols. Values are represented as mean ± standard deviation (n = 3).

The hardness and fracturability of the ODTs are presented in **Figure 4.8**. The effect of annealing on the mechanical properties of the ODTs was not significant when compared to ODTs frozen at -80 °C pre-cooled shelve without annealing, statistically, there was no difference in terms of their hardness nor fracturability ($\rho > 0.05$). However, the results showed that flash freezing of the formulation using liquid nitrogen significantly modified the mechanical properties of the tablets, as lower hardness of 12.7 ± 0.3 N was recorded compared to 16.0 ± 1.3 N for the annealed ODTs ($\rho < 0.05$) but with significantly higher fracturability (4.4 ± 0.1 N compared to 2.8 ± 0.1 N for the annealed, $P < 0.05$). Thus, the organised larger pores structure of the annealed tablets seems to have stronger resistance for the compression by the hardness probe (5 mm diameter) but weaker resistance toward penetration of the thin probe (1 mm diameter) that measures the fracturability, compared to tortuous and smaller pores structure of the flash frozen ODTs (**Figure 4.9**).

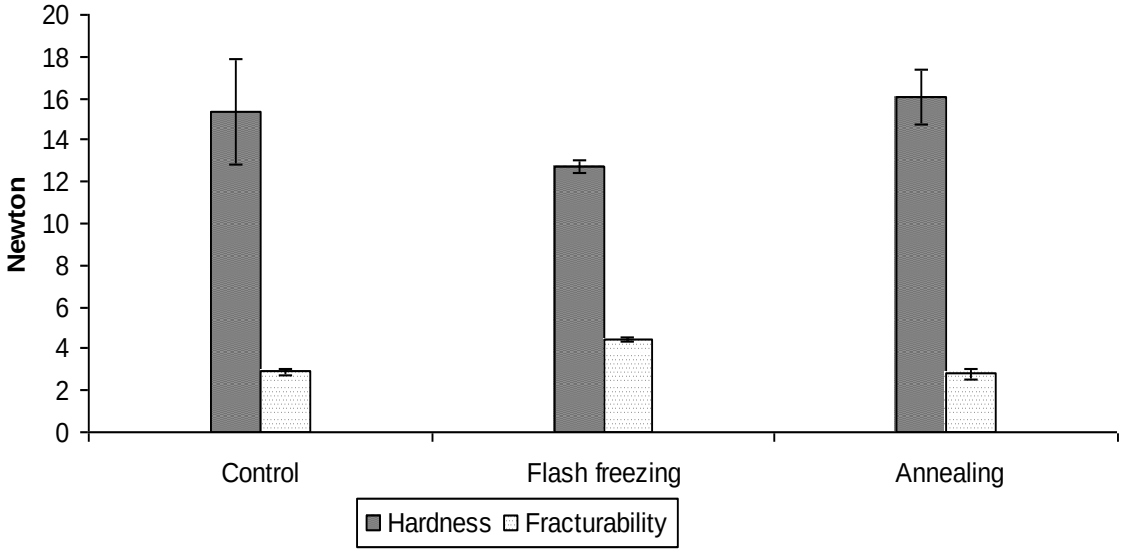


Figure 4.8 Hardness and fracturability of ODTs based on 45:55 of proline:serine at total concentration of 30% w/w after applying different freezing protocols. Values are represented as mean ± standard deviation (n = 3).

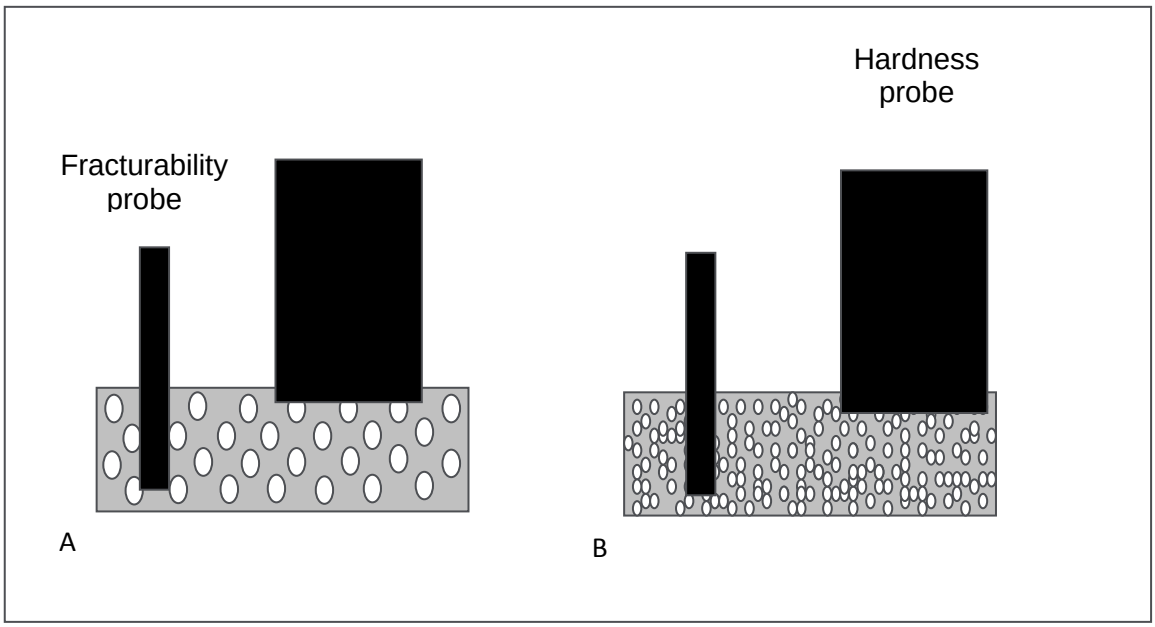


Figure 4.9 Schematic representations of the effect of freezing protocol on hardness and fracturability of ODTs. The organised larger porous structure of the annealed tablets (A) seems to have stronger resistance for the compression by the hardness probe (5 mm diameter) but weaker resistance toward penetration of the thin probe (1 mm diameter) that measures the fracturability, compared to tortuous and smaller pores structure of the flash frozen ODTs (B).

4.5. Conclusion

This study has demonstrated that inclusion of optimised combinations of serine and proline in the formulation of lyophilised orally disintegrating tablets can combine the benefits of high wettability and stability resulting in the formation of tablets with superior properties over that of individual amino acids. The inclusion of serine in the formulation at high concentration enhances the mechanical properties of the ODTs without compromising the formation of intact tablets. On the other hand, proline promotes the disintegration by enhancing the wettability of the ODTs. Annealing induces morphological changes in the ODTs that not only allow faster sublimation rate but also shorter disintegration time.

Chapter Five: Formulation of Multiparticulate Systems as Lyophilised ODTs

Patents relating to this chapter

Alhusban, F., Perrie, Y., Mohammed, A. Freeze-dried Tablets. Filed 9th April 2010. SCB/HMC/P111450GB00.

Formulation of Multiparticulate Systems as Lyophilised ODTs

5.1. Introduction and Aims

Orally disintegrating (dissolving) tablets (ODTs) are solid dosage forms that are placed in the mouth, rapidly disintegrate/dissolve when in contact with the saliva and then easily swallowed without the need for water (European pharmacopoeia, 2002). The fast disintegrating behaviour of the ODT in the mouth limits the active ingredients that can be incorporated to drugs that exhibit good taste, stability in gastric conditions and long half life. Bitter tasting drugs can cause discomfort to patients and consequently reduce their compliance, whereas incorporating drugs that suffer from instability in gastric fluids reduce the efficiency of the dosage form (bioavailability). On the other hand, delivering active drugs that have short half life in ODTs compromise the practicality of the dosage form as more frequent administration is required. To address these issues, a great deal of interest has been directed towards incorporating multiparticulate drug delivery system in ODT formulations (chapter 1).

The multiparticulate drug delivery system comprises of drug particles encapsulated or coated by one or more layers of polymers that control the release of the drug. The polymer can be selected to provide extended, delayed or pulsed drug delivery, allowing the rate of release of the drug to be tailored as required. Therefore, multiparticulate drug delivery systems can mask the unpleasant taste of active drugs, protect acid-labile drugs from possible degradation in the stomach, and extend the drug release over several hours. Moreover, they provide many advantages over single-unit dosage forms because of their multiplicity nature and small sizes such as reduced risk of systemic toxicity, enhanced bioavailability, reduced risk of local irritation and reduced patient to patient variability as a result of their more predictable gastric emptying (Dey et al., 2008). Accordingly the formulation of multiparticulate into ODTs can extend their application to more challenging drugs (eg. acid sensitive) by overcoming some restrictions imposed by the nature of these drugs and combine the benefits of ODTs and multiparticulate drug delivery system (chapter 1).

The compression of multiparticulate into ODT formulations has attracted substantial attention in both academia and industry and resulted in many scientific publications (Beckert et al.,

1996) and patent applications (chapter 1). However, to produce a tablet with good structural integrity, relatively high compression pressures are required. These high pressures can cause damage to the polymer layers of the multiparticulate system, and, as a result, compromise their release controlling properties (Bodmeier, 1997).

Freeze drying is an alternative technique to produce ODTs without applying any compaction force, which could be useful in the formulation of multiparticulate into ODTs. However, three major requirements need to be addressed in order to ensure successful formulations. Firstly, the need for high viscous liquid formulation that is able to suspend the multiparticulate long enough to complete formulation and freezing without compromising the disintegration performance. Secondly, minimum interaction between the liquid formulation and the multiparticulate that may lead to unwanted changes in the original properties of the multiparticulate such as early drug leakage. For example, for multiparticulate coated with hydrophobic polymers, the use of thick hydrophilic environment in the formulation reduces the chance of premature drug release, whereas for enteric coated multiparticulate, the use of acidic formulation ensures multiparticulate integrity. Thirdly, physical protection against possible damage during freezing and annealing step as a result of ice crystal growth.

The current study aimed to optimise ODT formulations suitable for multiparticulate delivery based on gelatin, carrageenan and alanine. The selection of these excipients can potentially benefit the formulation in many ways. From one side, the selection can exploit the electrostatic associative interaction between the anionic sulphate groups of carrageenan polymer and the positive net charge of gelatin (below its pI) to produce highly viscous solution at relatively low concentration of both polymer (Michon et al., 2000), which ensures fast disintegration property and shorter freeze drying cycle (chapter 2). Also, carrageenan has cryoprotectant activity which might be useful to protect the multiparticulate integrity during freezing and annealing steps (Choi et al., 2007). Additionally, gelatin and alanine showed superior properties as matrix supporting agents in ODT formulations (chapter 3).

Successful development of new pharmaceutical formulations requires extensive and comprehensive research to determine the significant factors that influence formulation, understand their effects (individually and collectively), and optimise them to obtain high quality products. For lyophilised ODTs, traditional experimentation approach can be time and material consuming and consequently is associated with high cost, due to the existence of multiple factors that influence the formulation performance and manufacturing process.

Recently, design of experiment (DoE) supported by statistical software has been reported as an efficient and powerful tool in the development and optimization of pharmaceutical dosage forms (Nagarwal et al., 2009). The design evaluates the influence of various formulation parameters and their interaction with the lowest number of experiments, hence reducing the cost and time of the work (Bhavsar et al., 2006). Moreover, design of experiment is considered an essential part of quality by design paradigm (QbD) which is recommended by the FDA as a new regulatory requirement for approval of generic drugs (Yu, 2008).

Response surface modelling (RSM) was applied in this study to evaluate the influence of varying the concentration of the selected excipients (independent variables), gelatin, carrageenan and alanine, on four crucial responses, disintegration time, hardness, viscosity and pH. Quantitative estimation of the significant model terms (linear, polynomial and interactive) was used to build statistical model for each response that can describe the relationship between the dependant and independent variables. These models were used to optimise the concentration of the excipients that maximize the quality of the formulation. Further, ODTs containing therapeutic dose of enteric coated pellets of omeprazole were prepared based on the optimised formulations and fully characterised to evaluate their feasibility as drug delivery system.

5.2. Materials

Gelatin from bovine skin, type B (Bloom strength ~ 75), lambda carrageenan and L-alanine ($C_3H_7NO_2$, Reagent plusTM ≥ 99%) were all purchased from Sigma-Aldrich Chemicals (Pool, UK). Enteric coated pellets of omeprazole (8.5% omeprazole, batch number: OME-020907) were supplied by MKPPL (Pune, India). All the materials were used as received.

5.3. Methods

5.3.1. Design of experiment

The statistical experimental design in this study was performed using MODDE software version 8 (Umetrics Inc., NJ, USA). The top RSM (response surface modeling) design choice suggested by the software was a central composite face centered (CCF) that composed of 34 experiments in total, 15 fractional factorial runs in duplicate (15x 2) and four replicated center points. The concentration of gelatine (X_1), carrageenan (X_2) and alanine (X_3) were selected as independent variables at three levels. The three factorial levels for each independent factors, low, medium and high, were coded as -1, 0 and 1, respectively. The disintegration time (Y_1), hardness (Y_2), viscosity (Y_3) and pH (Y_4) were investigated as dependant variables (responses).

5.3.2. Preparation of ODTs for RSM experiments

A required amount of gelatine was solubilised in 100 ml double distilled water at about 40 °C to obtain a concentration of 3, 4 and 5% (w/v). Carrageenan was added to the solution at concentration of 0.2, 0.5 and 0.8% (w/v) and after the formation of clear solution, alanine was added at concentration of 2, 3.5 and 5% (w/v). A constant mass of 1.50 g of the formulation was poured in a tablet mould with internal diameter of 13.50 mm, frozen at -80 °C for about 60 minutes, annealed in -20 °C a pre-cooled freezer for 12 hours and then transferred back to the -80 °C freezer. The frozen formulation was freeze-dried (ADVANTAGE Freeze-dryer, VIRTIS) according to an optimized regime (primary drying for 48 hours at a shelf temperature of -40 °C and secondary drying for 10 hours at a shelf temperature of 20 °C and vacuum of 50 mTorr). The optimised formulation was prepared by the same method and the observed (experimental) and the predicted (from the model) values for the responses were compared to evaluate the validity of the model.

5.3.3. Viscosity and pH measurements

The viscosity of the formulation was measured using a rotational viscometer (Brookfield LVT , Stoughton, MA, USA) with its spindle number 3 rotating at speed of 20 rpm at room temperature in a 100-mL beaker with the spindle guard.

The pH was measured using pH meter (MP230, Mettler Toledo). The pH meter was calibrated using standard solutions at pH 4 and 7.

5.3.4. Disintegration time

Disintegration time is the time required for ODTs to disintegrate completely without leaving any solid residue. In vitro disintegration time for lyophilised ODTs was evaluated using US pharmacopoeia monograph (<701> disintegration). A dissolution tester (Erweka ZT3) was used in this study as a disintegration apparatus and distilled water (800 mL) as disintegration medium; the disintegration medium temperature was maintained at 37 °C by a thermostat. At each time, one tablet was placed in the basket rack assembly and covered by transparent plastic disk. The disintegration time was taken as the time required for ODTs to disintegrate completely without leaving any solid residue. The results were mean of three measurements.

5.3.5. Mechanical properties

The mechanical properties of the freeze dried tablet (hardness) were investigated using a texture analyzer (QTS 25: Brookfield, Essex, UK) equipped with a 25 kg load cell. The instrument was calibrated by standard weight of 500 g and 5 kg. The tablet was placed in a holder with a cylindrical hole. The hardness was taken as the peak force after 1mm penetration of 5mm diameter probe at a speed of 6 mm/min. The results were average of three measurements.

5.3.6. Density and diameter of the enteric coated pellets

The density of the pellets was determined on 2g of the pellets using Multipycnometry (MVP-D160-6, Quantachrome, UK) with 4.25 cm^3 sample cup at 22 ˚C. Prior to analysis the helium pycnometry was calibrated against a standard steel ball. Each determination included 10 purges at 19.5 psi and 10 analytical runs at 19.5 psi with an equilibration rate of 0.0050 psi/min. The results were average of three measurements.

The diameter of 50 randomly chosen pellets were measured using a digital calibre (Whiteworth, CA, USA).

5.3.7. Drug content and HPLC analysis

50 mg of omeprazole pellets was dissolved in 50ml of a mixture of acetonitrile:PBS mobile phase (28:72) and transferred immediately to an amber container. After good shaking, the solution was filtered through a 0.45 µm nylon filter (CHROMACOL LTD, Herts, UK) in autosampler vials for HPLC assay.

The HPLC analysis was carried out using Reverse phase HPLC (Dionex AS 50 autosampler with GP50 gradient pump HPLC System: Dionex, UK) at room temperature using a Gemini 5 µm, 4.6 x 150 mm, column (Phenomenex La Luna: Phenomenex, Torrance, USA). The mobile phase was a mixture of USP phosphate buffer: acetonitrile (72:28). The mobile phase flow rate was 1 ml/min, the injection volume was 20 µl and the UV absorbance was at 280 nm (Türkoğlu et al 2004). Under these conditions, the retention time was 3.31 min. The concentration of omeprazole was determined by reference to a calibration curve constructed from dilutions of a stock solution (1mg/mL), using the mobile phases, in a concentration range between 5 to 200 µg/mL. The calibration curve was performed in triplicate and resulted in a linear correlation in the studied concentration range (r^2=0.99).

5.3.8. Dissolution studies

The dissolution profiles of approximately 120.5 mg unprocessed pellets (containing 10 mg omeprazole) and prepared ODTs that contained therapeutic doses of omeprazole pellets(10 mg omeprazole) were evaluated using the USP type 2 dissolution apparatus (Erweka DT 600, Heusenstamm, Germany) with baskets at a rotational speed of 50 rpm, in 900 mL dissolution medium at 37 ˚C. Acidic dissolution medium (0.1 N HCl was used during the first 2 hours, followed by 1 hour in phosphate buffer saline (pH 6.8). At fixed time intervals, 5 ml samples were withdrawn and immediately 1 mL of 0.25 N NaOH was added. The samples were replaced with fresh medium (37 ˚C). The samples were filtered through a 0.45 µm nylon filter (CHROMACOL LTD, Herts, UK) in autosampler vials for HPLC assay.

5.4. Results and discussion

5.4.1. Design of experiment

The aim of this work was to optimise formulation parameters for incorporation of enteric coated multiparticulate (pellets) of omeprazole in lyophilised ODTs. In theory, a successful formulation should keep the pellets stable and suspended throughout and after the formulation process, exhibit adequate mechanical strength in the dry state and disintegrate quickly upon hydration. Suspending the pellets in the binder solution for enough time can be controlled by the viscosity of the solution, whereas the stability of the pellets is linked with the pH of the surrounding environment, due to the presence of enteric coating around the pellets. Therefore, the crucial responses that were selected as dependant variables were disintegration time (Y_1), hardness (Y_2), viscosity (Y_3) and pH (Y_4).

Gelatin, carrageenan and alanine were selected as main excipients. Gelatin was used as matrix forming agent which gives shape and provides mechanical strength to the tablets (chapter 2). Moreover, it forms thermo-reversible gels upon hydration with melting points around 35-37 °C (just below body temperature), which provides smooth feeling in the mouth after disintegration. Our previous study (chapter 2) suggested that gelatin at stock solution concentration between 2-5% (w/v) is most suitable for developing lyophilised orally disintegrating tablets. Carrageenan was added as viscosity modifying agent that drastically increases the viscosity of gelatin stock solution, due to the formation of complex coacervates (associative interaction) between the two polymers (Michon et al., 1996). Preliminary studies (see appendix) showed that concentrations from 0.2 to 0.8% (w/v) of carrageenan were capable of increasing the viscosity of gelatin stock solutions (2-5% w/v) efficiently. Alanine was used as a matrix supporting/disintegration enhancing agent. Chapter 3 suggested that inclusion of 2- 5 % (w/v) of alanine in ODT formulations based on gelatin as a binder were able to cement the porous structure of the lyophilised tablets and accelerate the disintegration at the same time. Moreover, alanine showed tendency to crystallise in the frozen formulation and consequently stabilise the formulation against possible collapse (chapter 3).

Accordingly, the influence of varying these three formulation (independent) variables, at three concentration levels within their pre-optimised ranges (see above), on the selected responses was studied using response surface modelling (RSM). The top RSM design choice suggested by the software was a central composite face centered (CCF) which was composed of 34

experiments in total, 15 fractional factorial runs in duplicate (15x 2) and four replicated center points. The full worksheet is presented **Table 5.1**.

Table 5.1 The CCF design worksheet

Exp Name	Run Order	Gelatin %(w/v)	Carrageenan %(w/v)	Alanine %(w/v)	Disintegration time (s)	Hardness (N)	Viscosity (mPa.s)	pH
N1	32	3	0.2	2	15	6.36	98.8	5.6
N2	5	5	0.2	2	12	15.45	159.4	5.5
N3	14	3	0.8	2	23	5.42	153.3	5.8
N4	17	5	0.8	2	286	16.23	391.9	5.7
N5	10	3	0.2	5	27	8.44	113.9	5.6
N6	18	5	0.2	5	24	21.38	156.4	5.5
N7	20	3	0.8	5	63	10.5	134.1	5.8
N8	21	5	0.8	5	339	19.53	448.5	5.7
N9	22	3	0.5	3.5	22	8.84	92.3	5.7
N10	1	5	0.5	3.5	44	16.91	400.3	5.6
N11	19	4	0.2	3.5	32	12.06	211.9	5.6
N12	6	4	0.8	3.5	229	15.26	239.1	5.7
N13	34	4	0.5	2	28	11.27	210.3	5.6
N14	26	4	0.5	5	91	15.36	213.4	5.6
N15	23	4	0.5	3.5	93	17.36	216.9	5.6
N16	11	4	0.5	3.5	100	17.84	270	5.6
N17	16	4	0.5	3.5	97	17.74	237.3	5.6
N18	9	3	0.2	2	14	6.63	105.8	5.6
N19	31	5	0.2	2	8	15.94	170.5	5.5
N20	24	3	0.8	2	23	5.56	142.3	5.8
N21	30	5	0.8	2	81	16.71	381	5.7
N22	27	3	0.2	5	28	8.79	97.5	5.6
N23	15	5	0.2	5	36	19.14	181	5.6
N24	12	3	0.8	5	66	11.16	144.8	5.8
N25	28	5	0.8	5	341	20.5	453.1	5.7
N26	25	3	0.5	3.5	26	7.85	108.1	5.7
N27	3	5	0.5	3.5	43	16.63	420.1	5.6
N28	2	4	0.2	3.5	31	9.67	142	5.5
N29	4	4	0.8	3.5	232	16.25	236.7	5.7
N30	33	4	0.5	2	28	10.56	190.3	5.6
N31	7	4	0.5	5	93	15.09	203.4	5.6
N32	29	4	0.5	3.5	93	16.56	237.1	5.6
N33	13	4	0.5	3.5	100	16.64	224.2	5.6
N34	8	4	0.5	3.5	102	18.02	206.9	5.6

The results (**Table 5.1**) showed that the disintegration time of the tablets varied from 8 to 341 s, the hardness from 5.42 to 21.38 N, viscosity from 92.3 to 453.1 % and pH from 5.5 to 5.8. The wide variation in the disintegration time, hardness and viscosity values for different formulations and the high degree of repeatability (**Figure 5.1**) suggested that these responses are strongly dependent on the selected independent factors. In case of pH, although small variations were noticed between different formulations, the results seemed to be systematic and repeatable, which may suggest dependency on the studied factors.

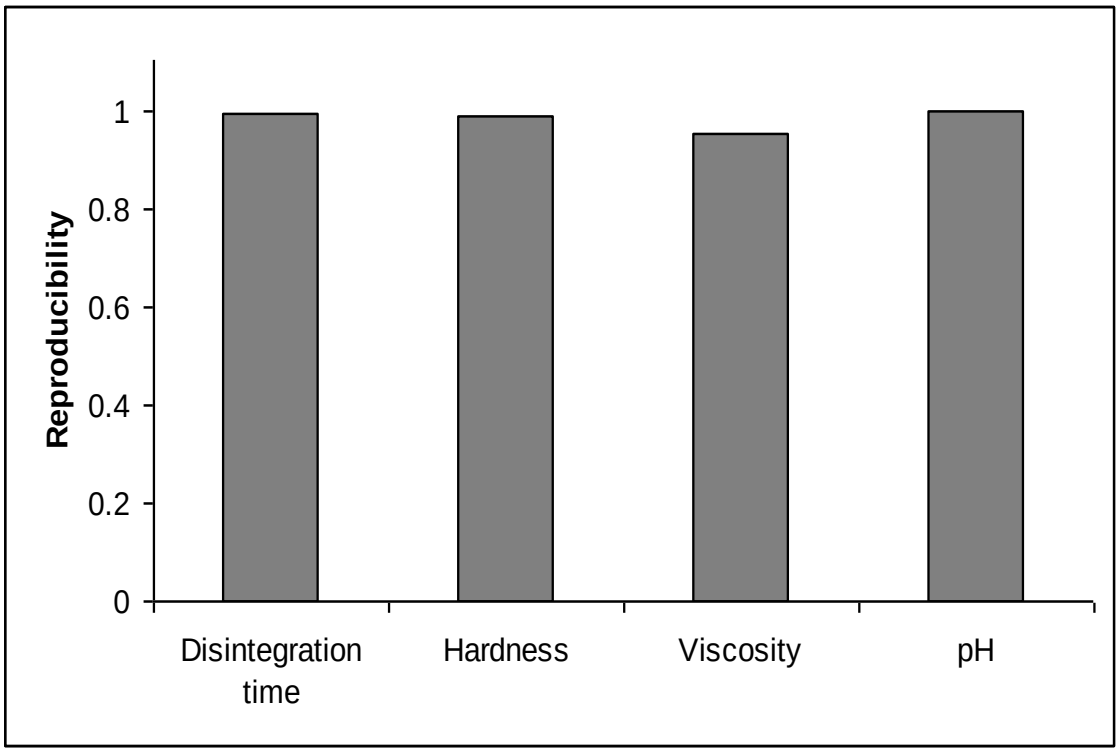


Figure 5.1 Reproducibility of the results for all four responses. Reproducibility: is the variation of the response under the same conditions (pure error) compared to the total variation of the response. Reproducibility = 1 - (MS(Pure error)/MS(total SS corrected)). A reproducibility value of 1 represents perfect reproducibility.

5.4.2. Analysis of variance (ANOVA)

A quadratic statistical model incorporating interactive and polynomial terms was used to evaluate the influence of the studied factors (independent factors) on the responses (dependent variables).

$$Y_i = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_{12} X_1X_2 + b_{13} X_1X_3 + b_{23} X_2X_3 + b_{11} X_1^2 + b_{22} X_2^2 + b_{33} X_3^2 + b_{123} X_1X_2 X_3$$

Where Y_i is the response (dependent variable), b_0 is the arithmetic mean response of the 34 trials, b_i is the estimated coefficient for the relevant model terms, X_1 is gelatin concentration, X_2 is carrageenan concentration, and X_3 is alanine concentration. The main effects (X_1, X_2 and X_3**)** represent the average result of changing one factor at a time from its low to high value while keeping the other factors at their canter point. The interaction terms (X_1X_2, X_1X_3, X_2X_3 and X_1X_2 X_3) show the change in the response when factors are varied simultaneously. The polynomial terms (X_1^2, X_2^2 and X_3^2) express non linear correlations with the response.

Analysis of variance (ANOVA) was performed to evaluate the significance of the quadratic models (linear, interactive and polynomial) on the responses and to estimate their quantitative effects. **Table 5.2** summarises the effects of the model terms and associated p values for all four responses. At a 95% confidence level, a model was considered significant if the p value <0.05. The sign and value of the quantitative effect indicate trend and magnitude of the term's influence on the response, respectively. Positive signs indicate an increase in the response value, while negative signs demonstrate a decrease in the response value. The results indicate that the disintegration time of the tablets was significantly influenced by the linear models of gelatin (X_1), carrageenan (X_2) and alanine (X_3), in addition to the interactive model of gelatin-carrageenan (X_1X_2) and carrageenan-alanine (X_2X_3).

Quantitative estimation of the significant models indicated that carrageenan and gelatin had the prime influence on the disintegration time linearly and interactively, suggesting that increasing carrageenan and/or gelatin concentration in the formulation increases the disintegration time drastically. The deteriorating effect of X_1 and X_2 on the disintegration could be explained by the associative interaction between gelatin and carrageenan upon hydration which forms a strong complex and consequently more resistant to disintegration in aqueous medium. Similar behaviour was reported by Bonferoni et al (2004) where muchoadhesive systems based on carrageenan and gelatine showed high resistance to erosion in an aqueous environment (lachrymal fluid) as a result of their associative interaction.

Table 5.2 The quantitative factor effects and associated p value for the responses.

	Disintegration time		Hardness		Viscosity		pH	
Term	Effect	P value	Effect	P value	Effect	P value	Effect	P value
X_1	33.9755	<0.0001	3.8395	<0.0001	77.3120	<0.0001	-0.0348	<0.0001
X_2	55.9070	<0.0001	0.5089	0.1318	50.9857	<0.0001	0.0707	<0.0001
X_3	26.9802	0.0008	1.5766	<0.0001	3.3790	0.5917	0.0028	0.5007
X_1^2	-16.8769	0.1220	-0.7425	0.1431	7.2172	0.4477	0.0201	0.0032
X_2^2	20.8862	0.0590	-0.4343	0.3844	-11.5445	0.2291	0.0097	0.1273
X_3^2	-4.2117	0.6923	-0.5014	0.3166	-9.7039	0.3098	0.0021	0.7388
X_1X_2	34.5981	<0.0001	-0.0416	0.8845	31.4688	<0.0001	-0.0041	0.2580
X_1X_3	9.9296	0.1162	0.0541	0.8504	5.2006	0.3461	0.0036	0.3149
X_2X_3	14.5584	0.0252	0.1815	0.5283	2.5914	0.6363	-0.0044	0.2303
$X_1X_2X_3$	6.9345	0.1566	-0.2460	0.2763	3.9755	0.3547	-0.0031	0.2727

Moreover, the formation of viscous solution upon hydration as a consequence of this interaction might limit the movement of water (Michon et al., 2001) inside the tablet and consequently reduce rate of penetration of the disintegration medium and therefore result in longer disintegration time. The large positive coefficient (34.5981) of the interactive term (X_1X_2) suggested that detrimental effect of gelatine and carrageenan on the disintegration of the tablet is synergised by increasing the concentration of both polymers simultaneously, which might be explained by the existence of more polymer chains available for complexation and consequently stronger interaction resulting in viscous environment upon hydration. On the other hand, increasing alanine concentration showed significant increase in disintegration, linearly (X_3) and interactively with carrageenan (X_2X_3) but to a lower degree when compared to gelatin and carrageenan. The inclusion of high concentration of alanine decreases the porosity of the tablets (chapter 3) and increases the probability of forming complex with carrageenan due to the presence of positive amino group on alanine structure that can form a complex with the negative sulphate group of carrageenan.

For hardness (Y_2), ANOVA results (**Table 5.2**) suggested that gelatin concentration (X_1) and alanine concentration (X_3) were the only significant terms with a p value <0.00001. Increasing gelatin concentration was the most effective way to improve the hardness as indicated by its

large positive coefficient (3.8395), possibly due to the formation of more extensive 3D networks of gelatin fibres (chapter 2). Increasing alanine concentration also enhances the hardness significantly, which could be as a result of cementing the porous structure of the tablet, increasing the contact points between the excipients, and enhancing the inter molecular bonding forces within the tablets (chapter 3).

The viscosity (Y_3) was significantly influenced by gelatin concentration (X_1), carrageenan concentration (X_2) and their interactive term (X_1 X_2), with a p value of <0.0001 and positive large coefficients for all the terms, suggesting that increasing carrageenan and/or gelatin concentration in the formulation increases the viscosity drastically. This could be explained by the attractive electrostatic interactions between gelatin and carrageenan which depends on the concentration and ratio of both polymers (Michon et al., 1995). Accordingly, the results suggested that, at the investigated concentration ranges for both the polymers, the interaction was enhanced by increasing the total concentration of the polymers individually and more effectively by simultaneous increase of concentrations of both the polymers.

For the fourth response Y_4 (pH), significant terms were identified as X_1 (gelatin concentration) X_2 (carrageenan concentration) and X_1^2 (polynomial model of gelatin concentration). The results (**Table 5.2**) suggested that increasing gelatine concentration decreases the pH of the formulation. However, this decrease is limited as indicated by the significant influence of the positive coefficient of the polynomial model of gelatin concentration (X_1^2). Similar effect of gelatin on the pH was reported in literature (Michon et al., 2000). Carrageenan concentration (X_2) had a positive coefficient suggesting that increasing its concentration raises the pH of the formulation.

5.4.3. Revised models and surface response plots

The resulting equations for all four responses, Y_1 (disintegration time), Y_2 (Hardness), Y_3 (viscosity), and Y_4 (pH), are presented below:

$$Y_1 = + 84.4118 + 35.3049\ X_1 + 56.6747\ X_2 + 22.9657\ X_3 + 33.1818\ X_1X_2 + 12.5\ X_2X_3$$

$$Y_2 = + 13.7544 + 3.84851\ X_1 + 1.54766\ X_3$$

$$Y_3 = + 217.429 + 76.7327\ X_1 + 50.1198\ X_2 + 32.1477\ X_1X_2$$

$Y_4 = + 5.60919 - 0.0354952\ X_1 + 0.070379\ X_2 + 0.0268962 X_1^2$

Statistical analysis for testing the validity of the models is summarised in **Table 5.3**. P values for all the simulated responses were well below the significant level (<0.05) suggesting that all the revised models were significant in predicting their response values. The high value of correlation coefficients (R^2) for all four responses indicated a good fit to the raw data (observed) in the revised model. Low correlation coefficient was noticed for disintegration time possibly due to the qualitative nature of the test that depends on the visual evaluation in addition to the fact that few seconds' inaccuracy in evaluating the disintegration time can result in huge error.

Table 5.3 Summary of results for testing validity of the revised models. DF indicates: degrees of freedom; SS: sum of squares; MS: mean of square; F: Fischer's ratio; p: probability; R^2: regression coefficient.

Disintegration time						
	DF	SS	MS (variance)	F	p	R^2
Regression	5	219302	43860.3	20.4012	<0.0001	0.824
Lack of Fit	9	39007.7	4334.19	3.88643		

Viscosity						
	DF	SS	MS (variance)	F	p	R^2
Regression	3	322215	107405	77.5712	<0.0001	0.886
Lack of Fit	11	35393.4	3217.59	9.94932		

Hardness						
	DF	SS	MS (variance)	F	p	R^2
Regression	2	567.807	283.903	63.3272	<0.0001	0.803
Lack of Fit	12	129.304	10.7754	21.1665		

pH						
	DF	SS	MS (variance)	F	p	R^2
Regression	3	0.221275	0.0737584	134.959	<0.0001	0.931
Lack of Fit	11	0.0063957	0.000581427	1.10471		

Based on the revised equations, the software was used to generate response surface plots (three dimensional) that simulate the influence of the independent factors on each response individually. The graphs for disintegration time, hardness, viscosity and pH are presented in **Figures 5.2**, **5.3**, **5.4** and **5.5**, respectively. The plots can provide uninterrupted visual assessment of the change in the response surface as a function of varying the independent factors, individually and simultaneously, which is valuable to further understand the system and optimise the formulation.

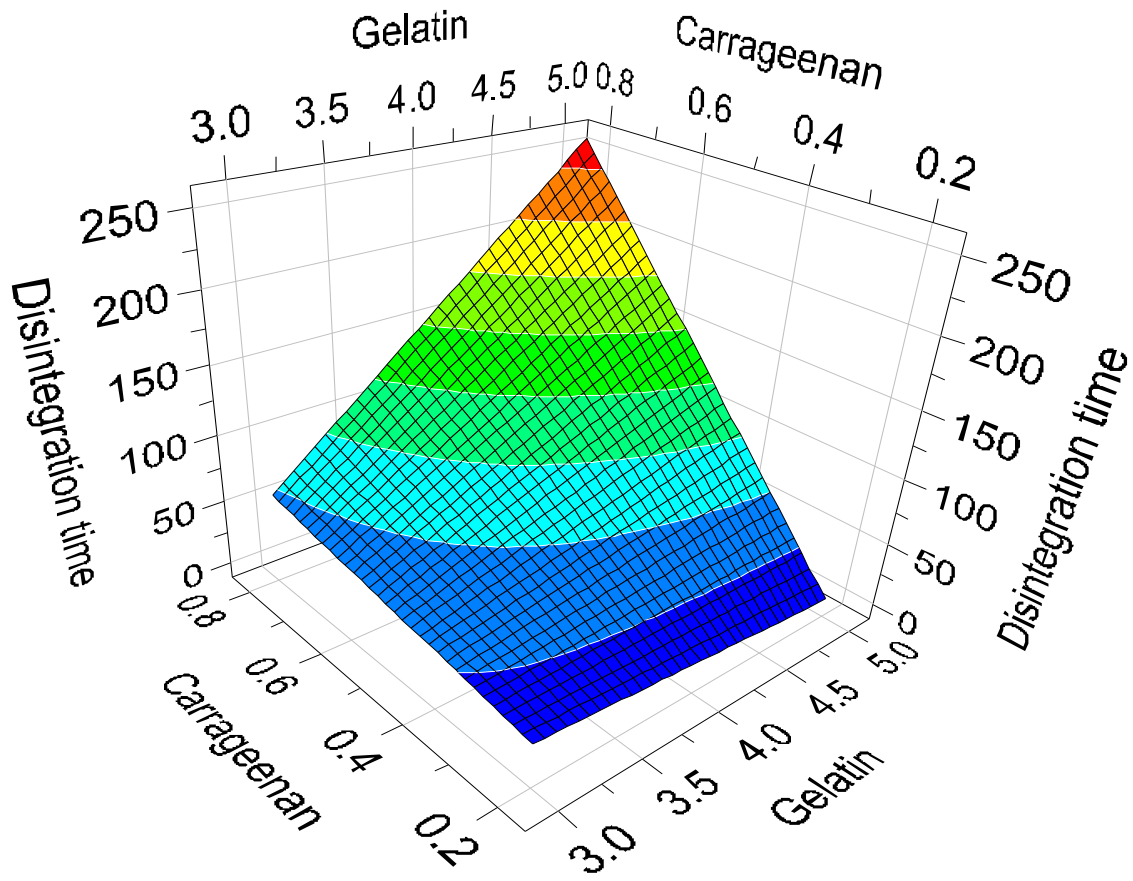


Figure 5.2 Surface response plot showing the influence of varying gelatin and carrageenan concentrations in the stock solution (%w/v) at constant concentration of alanine (3.5% w/v) on the disintegration time of the ODT.

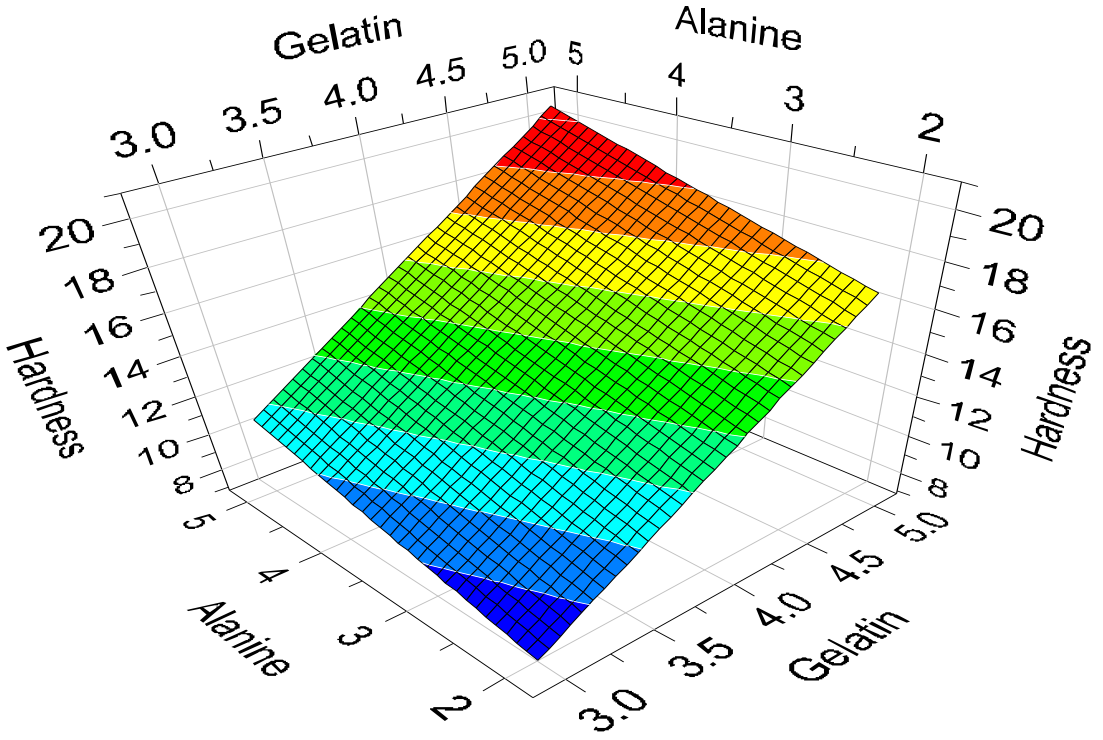


Figure 5.3 Surface response plot showing the influence of varying gelatin and alanine concentrations on the hardness of the ODT.

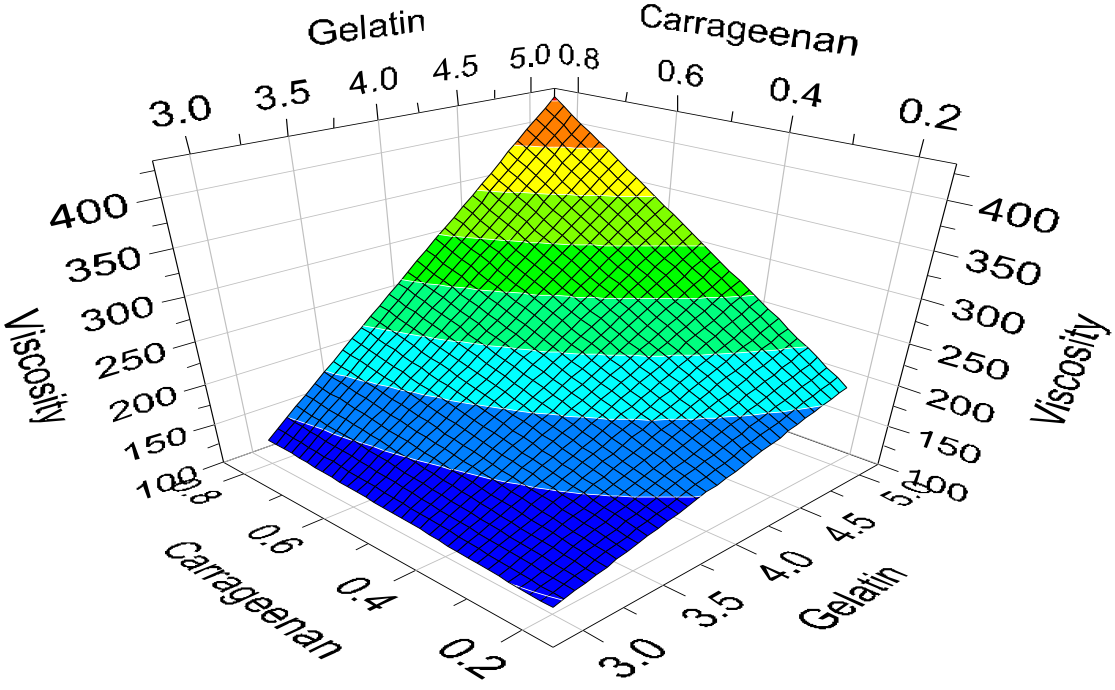


Figure 5.4 Surface response plot showing the influence of varying gelatine and carrageenan levels on the viscosity of the stock solution.

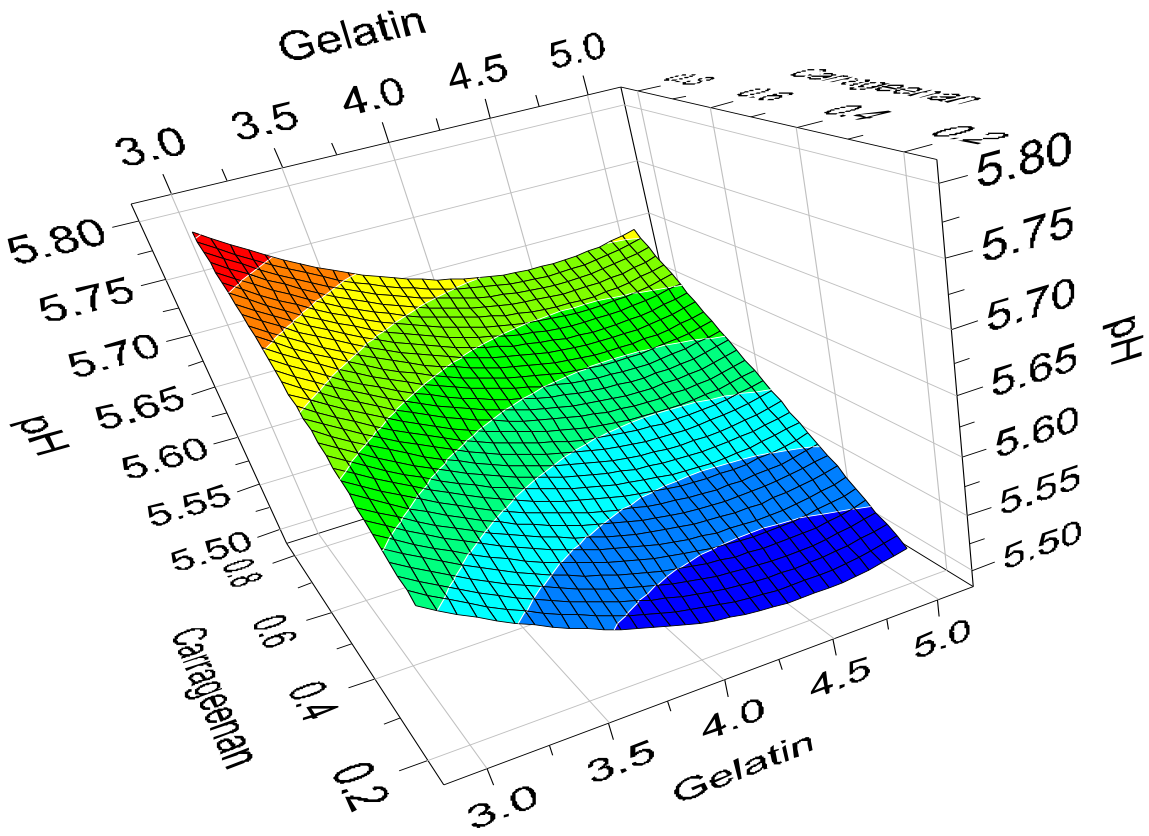


Figure 5.5 Surface response plot showing the influence of varying gelatine and carrageenan levels on the pH of the stock solution.

5.4.4. Optimum ODTs formulation

Based on the response surface plots, the software was used to perform hot spot analysis to obtain optimum formulation variables (gelatin, carrageenan and alanine concentrations) to produce ODTs with desired characteristics. The request was to minimise the disintegration time, and maximise the hardness and viscosity of the formulation, whereas the pH was excluded from the optimisation due to its limited variation in response to the studied factors. The optimal formulation was determined as 4.7% (w/v) gelatin, 0.02% (w/v) carrageenan and 3% (w/v) alanine. The observed response values of the optimised formulation compared to the predicted values are presented in **Table 5.4**. The closeness between the experimental (observed) and calculated (predicted) values of the responses can add further experimental verification to the validity of the established statistical models.

Table 5.4 Observed and predicted responses and residual values for the optimised formulation. The observed results are means, n=3.

Response	Observed	Predicted	Residual
Disintegration time (s)	14	15	-1
Hardness (N)	17.22	16.17	1.05
Viscosity (mPa.s)	172.40	181.26	-8.86
pH	5.5	5.5	0

5.4.5. Inclusion of enteric coated pellets of omeprazole

The characterisation of the enteric coated pellets of omeprazole used in the study is presented in **Table 5.5**. The results showed that the pellets were able to withstand the gastric condition (0.1 N HCl) for 2 hour with less than 10% of the total drug amount being released, which complied with the USP specification of enteric coated pellets. The dissolution profile after transferring the pellets to a pH 6.8 phosphate buffer is shown in **Figure 5.6**. Based on the optimised formulation, lyophilised ODTs containing 120.5 mg of enteric coated pellets of omeprazole (10 mg dose of omeprazole) was prepared using 18 mm diameter mould. The solution was able to suspend the pellets long enough before transferring the formulation to the freezer with no obvious settling or aggregation of the pellets. Moreover, no degradation or colour change was noticed throughout mixing, freezing and lyophilisation steps.

Table 5.5 characterisations of omeprazole enteric coated pellets. Results are mean ± SD, n=3.

Drug content % (w/w)	Drug recovery %	Density (g/cm^3)	diameter (μm)
8.27 ± 0.29	91.24 ± 1.22	1.439 ± 0.006	710 ± 40

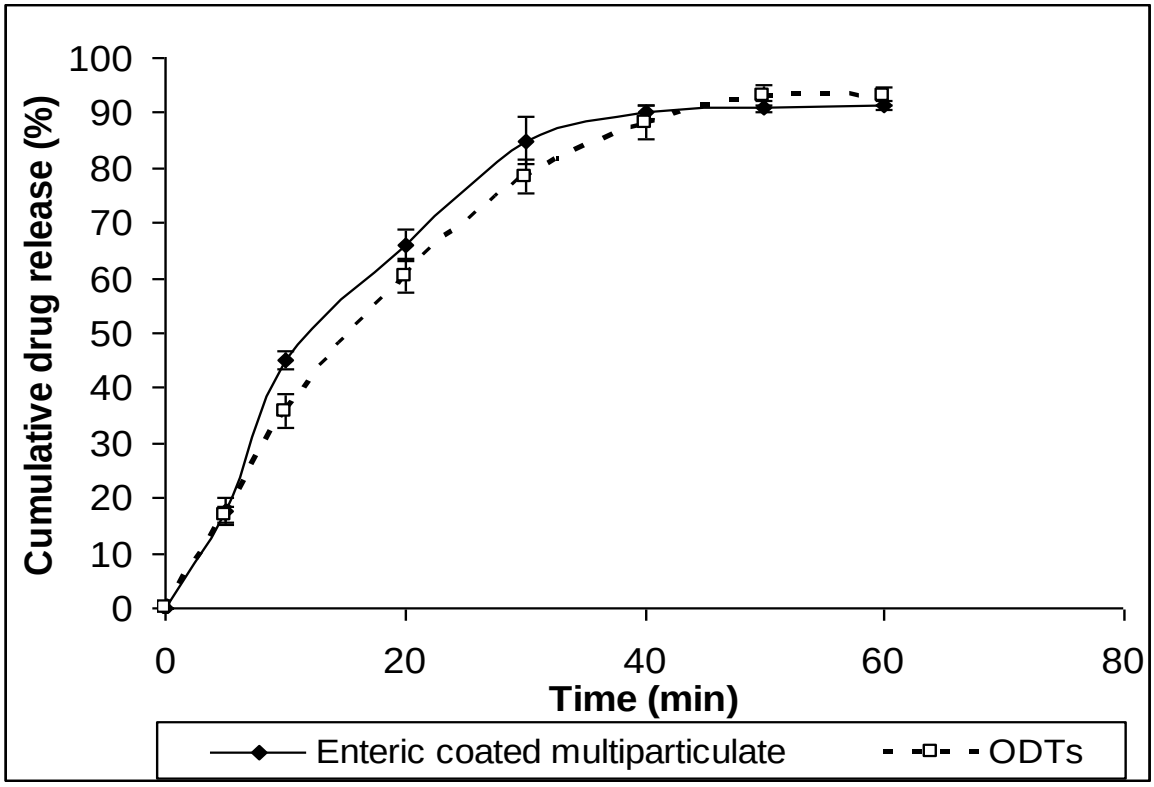


Figure 5.6 Cumulative percent of omeprazole released in phosphate buffer (pH 6.8) after 2 hours of gastric resistance study in 0.1 N HCl.

Characterisation of the tablets is summarised in **Table 5.6**. The tablets disintegrated in less than 19 seconds and had an average hardness of 17.24 ± 0.74 N (n=3). The disintegration time and hardness of the prepared tablets was not significantly different when compared to the optimised formulation without the pellets (**Table 5.5**) which suggested that the pellets did not compromise the tablets properties. The results showed no significant decrease in drug recovery after two hour in gastric condition compared to the original pellets, suggesting that the formulation and manufacturing process did not interfere with the integrity of the pellets. The dissolution profile after transferring the pellets to a pH 6.8 phosphate buffer is shown in **Figure 5.6**.

Table 5.6 Characterisations of orally disintegrating tablets containing Omeprazole pellets. Results are mean ± SD, n=3.

Disintegration time (s)	Hardness (N)	Viscosity (mPa.s)	Drug recovery %*
16 ± 3	17.2 ± 0.74	172 ± 21.3	93.14 ± 1.22

5.5. Conclusion

The central composite face centered (CCF) design applied in this study was used to provide details of the influence of independent variables on the responses. The results of analysis of variance (ANOVA) showed that all three independent variables had significant effect on the selected response. The revised model showed high degree of reliability and therefore succeeded to generate ODT formulations with optimised properties. The study showed the successful application of the combination of gelatin, carrageenan to incorporate multiparticulate drug delivery systems into lyophilised ODT formulation.

Chapter Six: Investigation of Alternative Binders for the Formulation of Lyophilised ODTs

Patents relating to this chapter

Alhusban, F., Perrie, Y., Mohammed, A. Lyophilized orally disintegrating tablets. Filed 16th November 2009.ACH/P108627GB00.

Investigation of Alternative Binders for the Formulation of Lyophilised ODTs

6.1. Introduction and Aims

Gelatin is the most common binder that has been used extensively in the formulation of lyophilised ODTs (Seager, 1998). It has been utilized in most of the commercially available lyophilised ODTs (ex. Zyprexa Zydis, Maxalt-MLT, Zelapar). Chapter 2 and 3 showed that gelatin can provide lyophilised tablets with adequate mechanical strength and short disintegration time. It is a water soluble structural protein obtained by thermal denaturation of collagen that is present inside the connective tissue (skin, cartilage and bone) of hogs, cattle and fish. In recent years, safety concerns about gelatin have been raised due to the emergence of animal diseases such as mad cow, chronic wasting and scrapie. Moreover, the use of gelatin in tablets may be unacceptable to certain patient population for example to vegetarian and/or to people with certain religious beliefs. Accordingly, the aim of the current study was to investigate the feasibility of replacing gelatin in the formulation of lyophilised ODTs with more ethically and morally acceptable components. The fundamental requirement of the new lyophilised ODT binder is the ability to produce intact tablets after freeze drying that have adequate mechanical strength and most importantly instant disintegration upon hydration. Furthermore, due to the high cost of the freeze drying process, polymers that allow short freeze drying cycle have a significant economical advantage.

Reviewing and analysing recent patents and literature in ODT formulations (chapter 1) revealed that short disintegration time could be achieved by using hydrophilic and/or hydrophobic polymers. However, in the formulation of lyophilised ODTs, high aqueous solubility is necessary to allow the easy formation of aqueous polymer solution and consequently forming continuous matrix after freeze drying. In this study, two naturally occurring hydrophilic polymers were carefully selected depending on their properties and previous applications that suggest their potential to act as a binder in the formulation of lyophilised ODTs. A progressive three-stage refinement approach was used in this study to select a new binder, optimise its concentration, determine its potential advantages as a binder

in lyophilised ODTs over gelatin, and study the influence of adding matrix supporting/disintegrating enhancing agents on the properties of the formulation. In the first stage, the candidate polymers were used individually to prepare lyophilised tablets at suitable concentration ranges and the formulation with the best characteristics was taken forward to stage 2. The second stage compared the freeze drying cycle and performance of the selected formulation with an optimised gelatin formulation. Whereas, stage 3 investigated the influence of adding matrix supporting/disintegrating enhancing agents on the properties of the formulation.

6.2. Selection of candidate polymers

The first polymer was gum arabic, which is a natural polymer harvested from the exterior of Acacia trees (Islam et al., 1997). Structurally, Gum arabic is a branched chain polysaccharide with a backbone consisting of 1,3-linked β-D galactopyranosyl units with other carbohydrates such as arabinose, glucuronic acid and rhamnose (Benke et al., 2009). Unlike most of natural gums, gum arabic is soluble in water and can yield solutions of up to 50% concentration (Cozic et al., 2009). Due to the multi functional properties, high safety profile and availability of gum arabic, it is widely used in food, cosmetic and pharmaceutical industries as emulsifying (Yadav et al., 2007), stabilising, suspending (lu et al., 2003) and encapsulating agent (Ramakrishnan et al., 2007; Kaushik and Roos, 2007). In solid oral dosage forms, gum arabic has been investigated and used as an osmotic, suspending and expanding agent in a monolithic osmotic tablet system (Lu et al., 2003) and water soluble gum in orally disintegrating films (Fuisz et al., 2008). Moreover, freeze drying of gum arabic was reported to be faster and more efficient than gelatin (Kaushik and Roos, 2007).

The second polymer was carrageenan, which is extracted from species of marine plants known as red seaweeds. Carrageenan is an anionic polysaccharides with a linear structure of repeating units of disaccharide that are connected in alternating sequences of 1,4-linked-α-D-galactose and 1,3-linked-β-D-galactose (Arda et al., 2009). Carrageenans are usually classified according to the number of sulphated groups per disaccharide: one, two or three for kappa, iota and lambda, respectively (Michon et al., 2005). It is a common ingredient in food, cosmetic and pharmaceutical products as suspending, stabilising and viscosity modifying agents. The use of carrageenans in various applications depends largely on their rheological

properties; water-soluble polymers that dissolve in either cold or hot water to form viscous solutions and consequently provide the appropriate texture for the product (Imeson, 2000). In food industry, especial in jelly candies, carrageenan is used as vegetarian alternative to gelatin (McHugh, 2003). In freeze drying of pharmaceutical products, carrageenan was investigated for its cryoprotectant activity which might be useful to protect against possible damage during the freezing and annealing steps (Choi et al., 2007), and was reported to enhance the redispersibility of freeze dried nanoparticulate systems (Kim and Lee, 2010).

6.3. Materials

Gum Arabic, gelatin from calf skin (type B, Bloom strength ~ 60), lambda carrageenan, alanine and mannitol were purchased from Sigma-Aldrich Chemicals (Pool, UK). All the materials were used as received.

6.4. Methods

6.4.1. Formulation of tablets to investigate the suitability of candidate polymers as binders in ODTs

The candidate polymers were dissolved in double distilled water at room temperature to obtain predetermined concentrations. Gum arabic was investigated at stock solution concentrations of 5%, 10%, 15%, 20% and 25% w/w. Carrageenan was investigated at concentration of 0.5%, 1. %, 1.5%, 2%, 2.5% w/w. 1.5 g of the stock solution was poured into a PEG mould (13.5mm in diameter), frozen at -80 ˚C for about 60 min, annealed for 12 hours at -15 ˚C and freeze-dried (ADVANTAGE Freeze-dryer, VIRTIS) according to an optimised regime (primary drying for 48 h at a shelf temperature of -40 ˚C, followed by secondary drying for 10 h at a shelf temperature of 20 ˚C, vacuum of 50 m Torr), which resulted in a moisture content of less than 3% w/w. All the formulations were prepared in triplicate from three independent batches.

6.4.2. Product temperature during the primary drying time

The product temperature profiles (Tb) during primary drying of the optimised formulation from the previous study (6.4.1) and the optimised gelatin formulation (5% low bloom strength)were recorded and used as an indication to compare the freeze drying conditions for the polymers. The product temperature was automatically recorded using thermo couples that were inserted in the centre bottom of the tablet. All the measurements were done in triplicate from three independent batches.

6.4.3. Formulation of tablets to the influence of the inclusion of matrix supporting/disintegrating enhancing agents

Alanine and mannitol were added individually to the optimised formulation from the previous study (6.4.1) at concentrations of 30% and 50% w/w (total solid).

6.4.4. Differential scanning calorimetry

Differential scanning calorimeter (Pyris Diamond DSC) was used to investigate the glass transition temperatures (Tg) and the crystallization events of the frozen formulations. 10–15 mg of the liquid formulation was transferred into an aluminium pan (50 µL capacity) and then sealed with an aluminium top. The sample was cooled to -65 ˚C and then heated to 20 ˚C at 5˚C/min. To determine the glass transition temperature of the maximally freeze concentrate sample (Tg'), after initial cooling to -65 ˚C, annealing for 10 min at a temperature higher than the relevant glass transition temperature (Tg) was added before carrying out the above method. Nitrogen was used as a purge gas at a flow rate of 20 mL/min. Indium and zinc were used to calibrate the heat flow and melting point onset (melting point: 156.6 °C, ΔHm: 28.42 J/g for Indium and melting point: 419.47 °C ΔHm: 108.26, J/g for Zinc). The obtained thermograms were analysed using Pyris Manager Software (version 5.00.02) where Tg and Tg' values were determined from the intersection of relative tangents to the baseline. The experiment was performed in triplicate and an empty aluminium pan was used as a reference cell for all the measurements.

6.4.5. In vitro disintegration study of the tablets

Disintegration time is the time required for ODTs to disintegrate completely without leaving any solid residue. In vitro disintegration time for lyophilised ODTs was evaluated using US pharmacopoeia monograph (<701> disintegration). Erweka (ZT3, Appartebau, GMBH) was used in this study as a disintegration apparatus and distilled water (800 mL) as disintegration medium; the disintegration medium temperature was maintained at 37 °C by thermostat. At each time, one tablet was placed in the basket rack assembly and covered by transparent plastic disk. The disintegration time was taken as the time required for ODTs to disintegrate completely without leaving any solid residue. All the measurements were carried out six times and presented as (mean ± standard deviation).

6.4.6. Mechanical properties of the tablets

The hardness of the lyophilized tablets was investigated with a texture analyzer (QTS 25: Brookfield, Essex, UK) equipped with a 25 kg load cell. The instrument was calibrated with standard weight of 500 g and 5 kg. The tablet was placed in a holder with a cylindrical hole and the hardness was taken as the peak force after 1mm penetration of 5mm diameter probe at a speed of 6 mm/min.

6.4.7. Statistical analysis

Graph Pad Instat® software was used for the statistical analysis study. Data groups were compared using one way analysis of variance (ANOVA) and pair-wise multiple comparisons method (Tukey-Kramer multiple comparison test). Standard deviation (SD) was used to report the error in the figures and texts. Probability values of 95% ($P < 0.05$) were used to determine the significant difference.

6.5. Results and discussion

6.5.1. Stage 1: the influence of polymers concentration on the formulation properties

The studied concentration ranges of arabic gum (5- 25 % w/w) and carrageenan (0.5- 2.5 %w/w) were determined based on preliminary studies that were conducted to ensure that all the formulations can be prepared easily and form intact tablets after freeze drying for full characterisation. Formulation of lower concentrations than the studied range was associated with very fragile and delicate tablets that were difficult to handle and characterise. On the other hand, higher concentrations took longer time to dissolve completely in water, gave very viscous solutions that were difficult to transfer into the mould and resulted in lyophilised tablets with very long disintegration time.

The glass transition temperature of maximally freeze concentrate samples (Tg') of formulations based on varied concentrations of gum arabic and carrageenan are summarised in **Tables 6.1** and **6.2**, respectively. The results showed that formulations based on gum arabic had Tg' values of about -14.00 ˚C irrespective of the concentration of the polymer (one way ANOVA/Tukey- Kramer: $p>0.05$). Similarly, all formulation based on varied concentration of carrageenan displayed T'g values (around -34.50 ˚C) that were not significantly different (one way ANOVA/Tukey- Kramer: $p>0.05$). The results suggested that each polymer has a distinctive Tg' with no significant influence of the polymer concentration on the Tg' (**Figure 6.1**).

The glass transition temperature of maximally freeze concentrate formulation (Tg') is crucial parameter to determine the freeze drying conditions that ensure the formation of intact tablets. Usually, lyophilisation of formulations at temperature 1 to 3 ˚C higher than their Tg' (collapse temperature) results in the collapse of their structure (Pikal and Shah, 1990), and in turn formulations with low Tg' are required to be freeze dried at low shelf temperature and consequently take longer time for the freeze drying cycle to finish (Rambhatla et al., 2006). Accordingly, formulations based on gum arabic are expected to have more efficient freeze drying cycle as they can tolerate higher shelf temperature and consequently shorter primary drying time than carrageenan formulation.

Table 6.1 The glass transition temperature of maximally freeze concentrate samples (Tg') of formulations based on varied concentrations of gum arabic.

Concentration of gum arabic (% w/w)	Tg'
5	-13.88 ± 0.33
10	-13.70 ± 0.34
15	-13.79 ± 0.41
20	-13.75 ± 0.20
25	-13.69 ± 0.27

Table 6.2 The glass transition temperature of maximally freeze concentrate samples (Tg') of formulations based on varied concentrations of carrageenan.

Concentration of carrageenan (% w/w)	Tg'
0.5	-34.42 ± 0.54
1	-34.23 ± 0.46
1.5	-34.74 ± 0.34
2	-34.66 ± 0.29
2.5	-34.69 ± 0.24

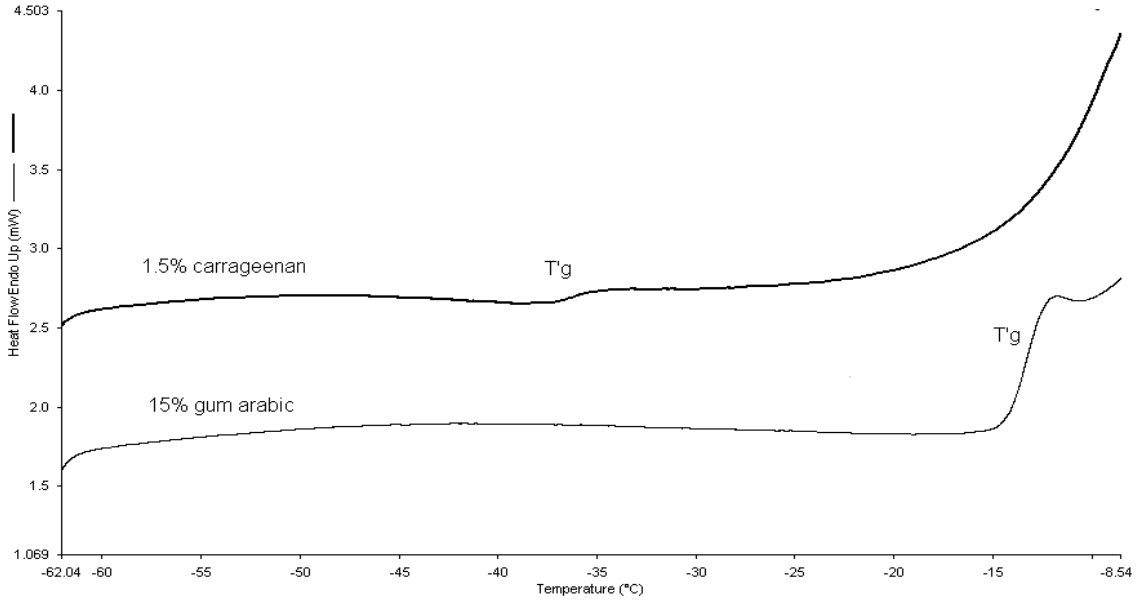


Figure 6.1 DSC heating scans of aqueous solutions of gum arabic and carrageenan show the glass transition temperature of maximally freeze concentrate samples.

The hardness of ODTs based on gum arabic and carrageenan are presented in **Figures 6.2** and **6.3**, respectively. Similar trend was followed by both polymers; increasing the concentration of the polymer in the formulation increased the hardness significantly (one way ANOVA/Tukey-Kramer: $p>0.05$). However, wide variations in the improvement were obtained for each polymer, which can be attributed mainly to the physicochemical properties of the polymer and its concentration range in the study. In case of tablets made from gum arabic, the hardness was found to progressively increase from 0.56 ± 0.15 N in the 5% formulation to reach 9.22 ± 0.22 N in the 25% formulation (**Figure 6.2**) compared to a lesser improvement in case of carrageenan, from 0.37 ± 0.05 N in the 0.5% formulation to a maximum hardness of 2.39 ± 0.26 N in the 2.5% formulation (**Figure 6.3**)**.** Carrageenan was found to be suitable to formulate lyophilised ODTs only at low concentration due to the formulation limitations that were mentioned earlier, therefore fluffy and highly porous tablets were produced after freeze drying as very low mass of carrageenan (1-2 mg) was distributed in a relatively large volume of the tablet (1.5 ml). In case of gum arabic much higher mass of the polymer could be incorporated in the tablets, consequently resulting in closer polymer networks and high hardness values.

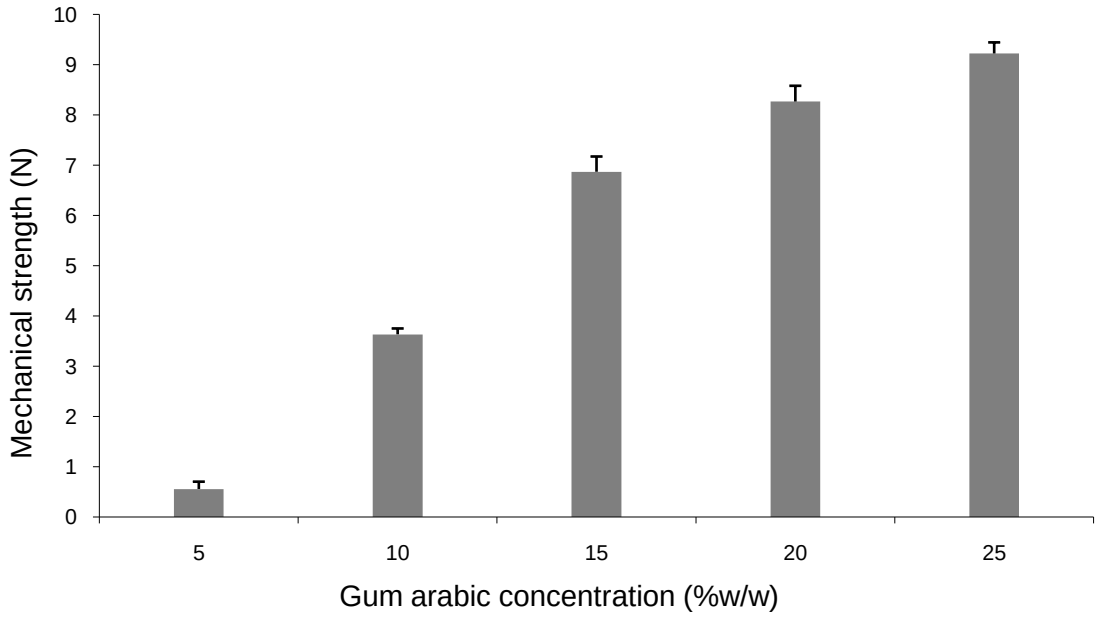


Figure 6.2 Mechanical properties of lyophilised tablets based on varied concentration of gum arabic. Results are mean ± SD, n=3.

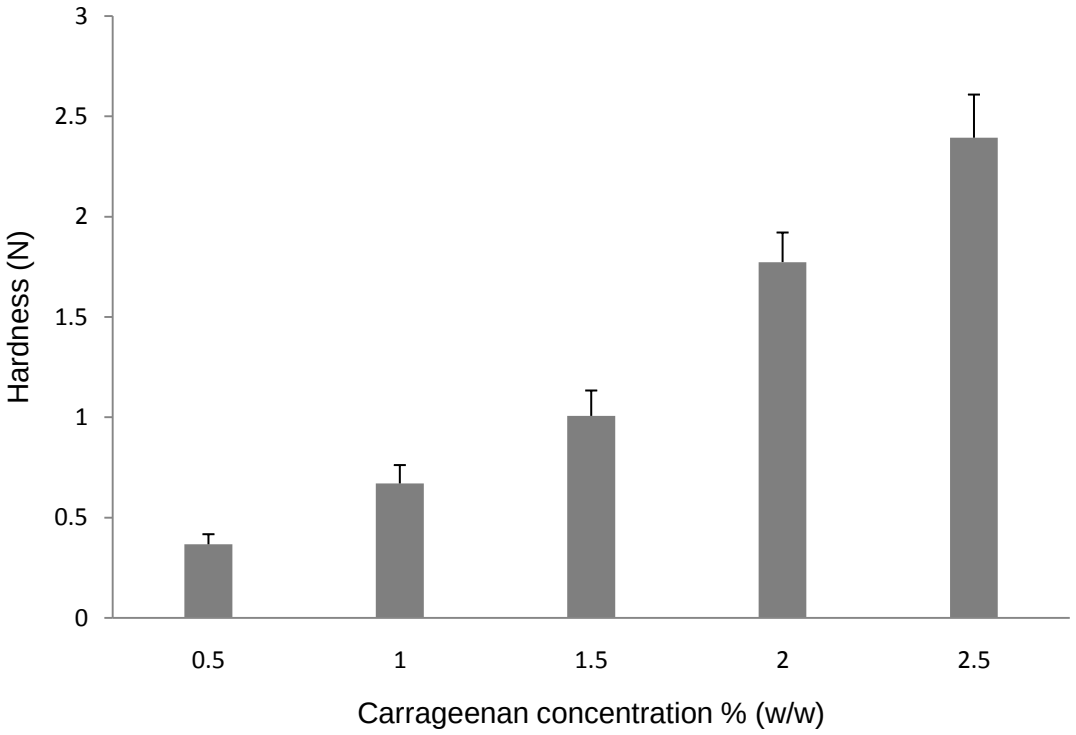


Figure 6.3 Mechanical properties of lyophilised tablets based on varied concentration of carrageenan. Results are mean ± SD, n=3.

With regard to the disintegration time of the tablets, the results showed (**Figures 6.4** and **6.5**) that increasing the concentration of the polymers in the formulation resulted in significant increase in the disintegration time ($p>0.05$). The average disintegration time of tablets based on gum arabic gradually rose from 2 s in the 5% formulation to reach a maximum time of 51 s in the 25% formulation. In case of tablets based on varied concentration of carrageenan, the disintegration time increased more substantially with each increment in the polymer concentration to reach a maximum time of 190s for the highest concentration (2.5%). The results could be explained as increasing the polymer concentration decreases the porosity of the tablets and therefore more time is needed for the disintegrating media to penetrate through the tablets. The difference in the disintegration time between tablets made from gum arabic and carrageenan could be attributed to their differences in the wettability (Fukami et al., 2006), molecular weight (Chen et al., 2006) and inner structural characteristics of the tablet after freeze drying (chapters 2 and 4).

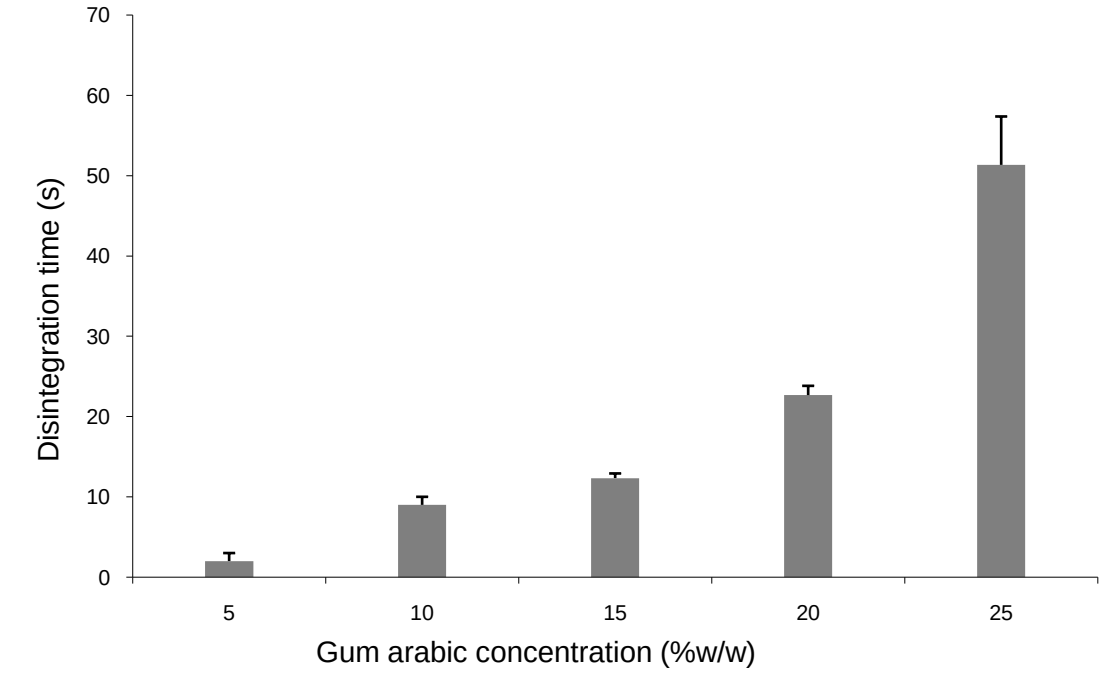


Figure 6.4 The effect of gum arabic concentration on the disintegration time of lyophilised ODTs. Results are mean ± SD, n=3.

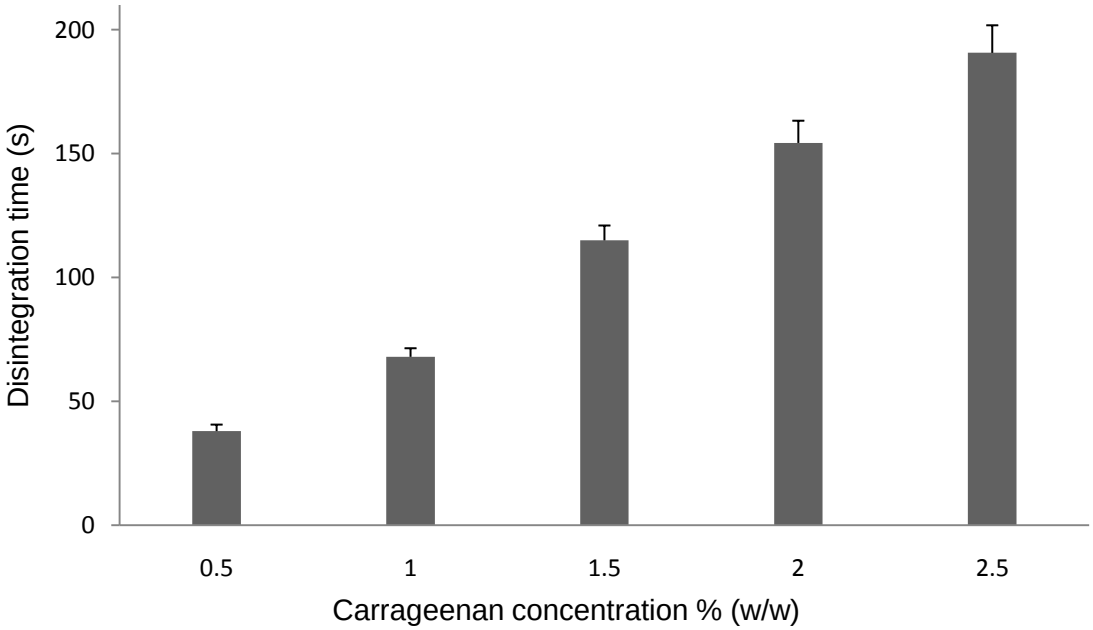


Figure 6.5 The effect of carrageenan concentration on the disintegration time of lyophilised ODTs. Results are mean ± SD, n=3.

Selection of the polymer and its ideal concentration to be taken forward to Stage II of the study depended on finding the best balance between hardness and disintegration time of the tablets. A formula termed the Lyophilised Tablet Index (LTI = hardness/disintegration time) took both the above mentioned factors into consideration and was used in decision making (chapter 2). The LTI values of tablets based on gum arabic and carrageenan are summarised in **Tables 6.3** and **6.4**, respectively. The results suggested that tablets made from 15% gum arabic achieved the highest LTI value and consequently was selected to be taken forward to stage II. Moreover this formulation is expected to offer efficient freeze drying cycle due to its high Tg' as explained earlier.

Table 6.3 Lyophilised tablet index (LTI) of tablets based on varied concentration of gum arabic.

Concentration of gum arabic % (w/w)	LTI
5	0.278
10	0.404
15	0.557
20	0.365
25	0.180

Table 6.4 Lyophilised tablet index (LTI) of tablets based on varied concentration of carrageenan.

Concentration of carrageenan % (w/w)	LTI
0.5	0.010
1	0.010
1.5	0.009
2	0.011
2.5	0.013

6.5.2. Stage 2: comparing the freeze drying cycle and performance to lyophilised tablets based on gelatin

The second stage was aimed to explore the benefits of using gum arabic over gelatin, which is used extensively as a binder in ODT formulation (chapters 2, 3 and 4). The first advantages was observed in the first step of the preparation process, where gum arabic showed complete and fast dispersion in water at room temperature, in contrast to gelatin formulations, where heating (above 40 ˚C) is necessary. Another drawback of using gelatin as matrix forming agent in the formulation of lyophilised ODTs is the long freeze drying cycle (chapter 2). To compare the freeze drying cycle of the optimised formulations based on gum arabic (15% w/w) and gelatin (5 % of low bloom strength), thermal probes were used to monitor the product temperature during the freeze drying process (**Figure 6.6**). Monitoring the product temperature during the freeze drying process is a valuable technique to understand and correlate the heat and mass transfer processes (Tang et al., 2005). Moreover, it gives an estimation of the end of the primary drying (sublimation), which appears as a sudden increase in the product temperature due to the absence of ice crystals that can be sublimed (Schneid et al., 2009). The results (**Figure 6.6**) showed that the product temperatures of the gelatin based formulation were always around 10 to 15 ˚C higher than the shelf temperature (-40 ˚C) suggesting high resistance to the sublimation process (Tang and Pikal, 2004) and consequently slower sublimation rate. Therefore, the end point of the primary drying took long time (an average of 2840 ± 104 min) to appear (**Figure 6.6**). On the other hand, tablets based on gum arabic had temperatures close to the shelf temperature during the primary drying, suggesting more efficient sublimation process (less resistance to sublimation) than gelatin. As a result, the end point of tablets based on gum arabic appeared in drastically shorter time (an average of 680 ± 74 min).

These advantages of using gum arabic did not compromise the actual performance of the tablets in terms of disintegration time and mechanical properties. The optimised concentration of gum arabic (15%) achieved higher LTI value of 0.54 compared to 0.47 for tablets made from low bloom strength gelatin at a concentration 5%, which was the highest for tablet made from gelatine (chapter 2).

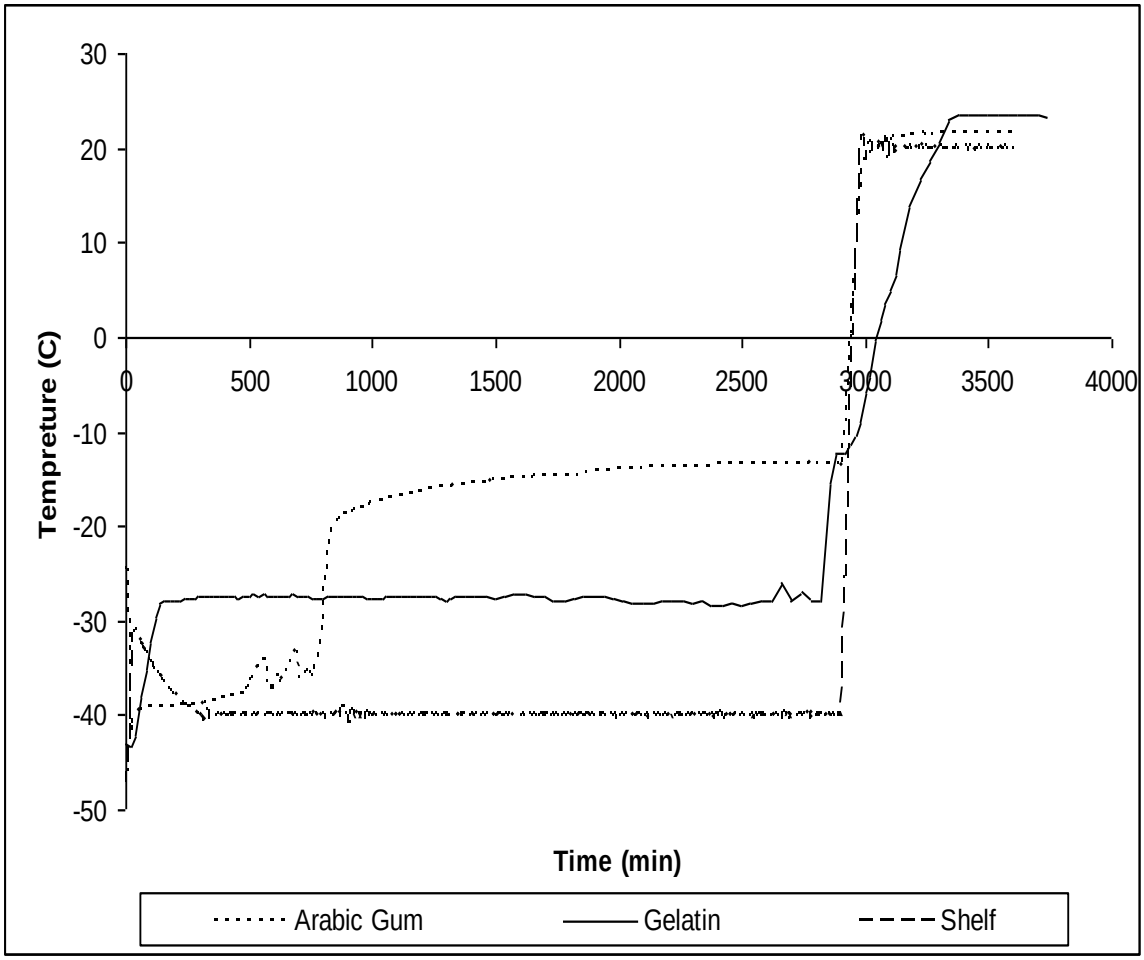


Figure 6.6 The temperature profiles of tablets based on gum arabic and gelatin during their freeze drying cycles.

Moreover, gum arabic as a natural polymer that extracted from acacia trees can overcome some safety and ethical concerns imposed by the origin of gelatin, which comes from the hydrolysed product of animal collagen tissues, such as skin, tendon, ligament and bones. Compared to tablet based on gelatin, gum arabic has no risk of causing animal origin diseases (CJD) and at the same time is more suitable to vegetarian and people with certain religious beliefs.

6.5.3. Stage 3: The influence of the inclusion of matrix supporting/disintegrating enhancing agents

The third stage of the study involved the addition of matrix supporting/disintegrating enhancing agents to the 15% gum arabic formulation brought forward from Stage I (control). Alanine and mannitol were chosen on the basis of our previous work and they were included in the formulation at two concentrations, 30 and 50% w/w, as they showed the best performance in enhancing the hardness and reducing the disintegration time simultaneously (chapter 2 and 3).

The thermal properties of the frozen formulations are summarized in **Table 6.5**. At concentration of 30%, both mannitol and alanine showed lower Tg values than the 15% gum arabic with no crystallisation events (Cr) in the heating scan. However, after annealing both formulation displayed Tg' at temperatures higher than their Tg, which is attributed to their crystallisation in the annealing step. At concentration of 50% (w/w), both formulation showed crystallization (Cr) in the heating steps and consequently showed Tg' at temperatures comparable to the 15% gum arabic alone.

The disintegration time results (**Figure 6.7**) showed that addition of mannitol or alanine at concentration of 30% (w/w) achieved instant disintegrations of about 4-5 s, which are significantly shorter than the formulation of 15% gum arabic alone ($p>0.01$), which could be attributed to the high wetting properties of these two materials (chapter 2 and 3). However, inclusion 50% of mannitol and alanine showed slightly longer disintegration time compared to the 30%, which can be explained as a result of decreasing the total porosity of the tablets at this concentration (chapter 2 and 3).

Table 6.5 Thermal properties of frozen solutions of gum arabic (15% w/w) after the addition of matrix supporting/disintegrating enhancing agents. Results are mean ± SD, n=3.

Excipient	Concentration (% w/w)	Tg (˚C)	Cr (˚C)	Tg' (˚C)
Alanine	30	-32.8 ± 0.3	-	-15.3 ± 0.3
Alanine	50	-44.9 ± 0.2	-36.2 ± 0.3	-14.7 ± 0.1
Mannitol	30	-26.3 ± 0.2	-	-15.9 ± 0.1
Mannitol	50	-41.1 ± 0.2	-28.4 ± 0.1	-14.8 ± 0.1

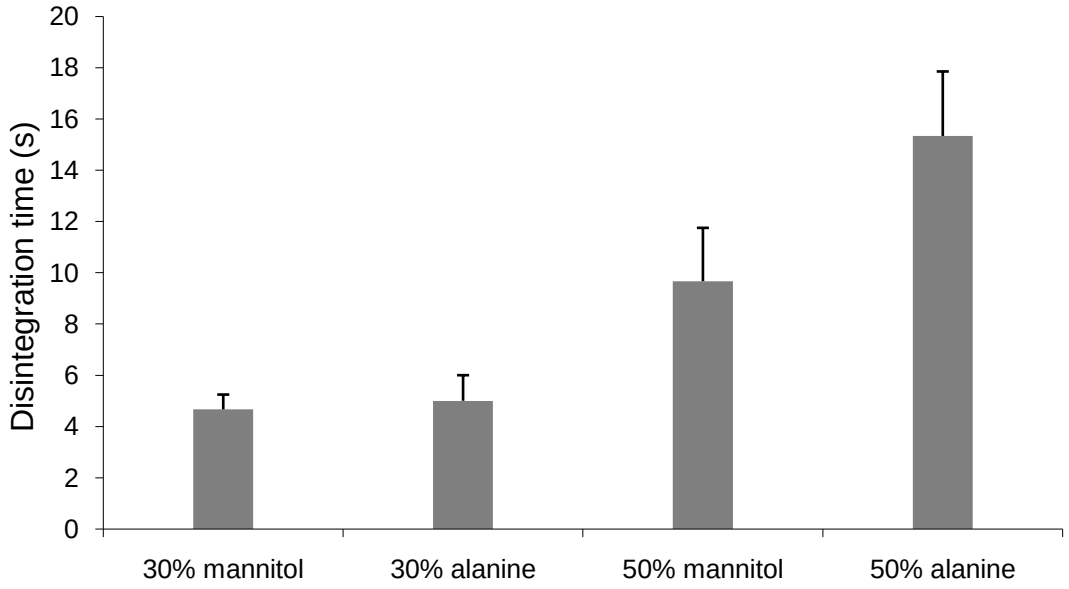


Figure 6.7 The disintegration time of ODTs based on gum arabic after the addition of matrix supporting/disintegrating enhancing agents. Results are mean ± SD, n=3.

With regards to the mechanical properties (**Figure 6.8**), addition of 30% alanine and mannitol appeared to have no significant improvement on the hardness ($p>0.05$). However, increasing the concentration to 50% enhanced the hardness significantly compared to the control formulation ($p<0.05$).

In order to evaluate the effect of inclusion of amino acids on the hardness and disintegration simultaneously and compare it to the control formulation (15% gum arabic only), Relative lyophilised tablets index (RLTI) were calculated according to the following equation:

$$RLTI = (H/DT) \div (H^{\circ}/DT^{\circ})$$

Where H: hardness of the tested tablet, DT: disintegration time of the tested tablet, H°: hardness of the control tablets, DT°: disintegration time of the control tablet.

The RLTI value provided a ratio indicative of whether the new formulation was better than the control. Values over than 1 indicate improvements over the control whereas lower than 1 suggest retardation in the overall tablet properties. Also, RLTI values can evaluate the degree of improvement for all the formulations, in basis of higher value the better formulation.

The RLTI values are presented in **Table 6.6**. The results suggested that inclusion of alanine and mannitol in concentrations range from 30% to 50% (w/w) improved the overall tablets properties, which confirmed their role in the formulation as matrix supporting/disintegrating enhancing agents.

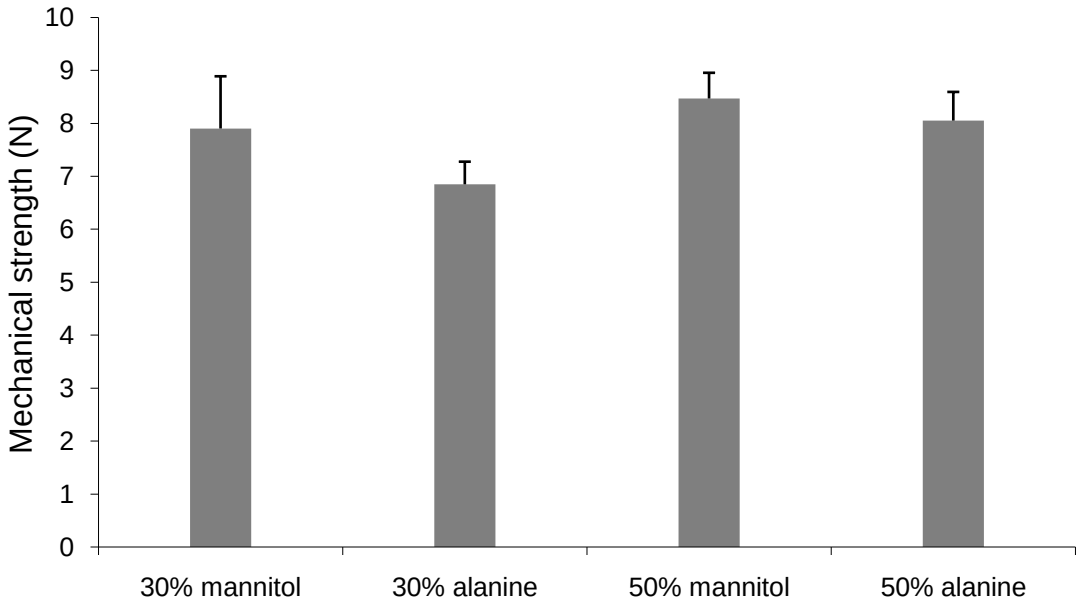


Figure 6.8 The hardness of ODTs based on gum arabic after the addition of matrix supporting/disintegrating enhancing agents. Results are mean ± SD, n=3.

Table 6.6 The relative lyophilised tablet index of tablets based on 15% gum arabic and mannitol or alanine.

Excipient	Concentration % (w/w)	RLTI
Alanine	30	2.46
Alanine	50	0.94
Mannitol	30	3.04
Mannitol	50	1.57

6.6. Conclusion

The current study suggests that gum arabic has an outstanding potential to be used as a binder in the formulation of lyophilised ODTs. Arabic gum showed immediate dispersion in either cold or hot water to form low viscosity solutions, which allowed the incorporation of high concentration of polymer and simplified the formulation process at the same time. The use of gum arabic as a binder was found to provide elegant freeze dried tablets with rapid disintegration time and sufficient mechanical strength to withstand manual handling. Tablets based on 15% w/w gum arabic achieved the best balance between hardness the disintegration time. Compared to gelatin formulation, the tablets based on gum arabic showed superior performance in term of disintegration time and hardness. Moreover, tablets comprising of gum arabic were prepared using a shorter freeze drying cycles than those with Gelatin. Inclusion of matrix supporting/disintegrating enhancing agents further enhanced the tablet characteristics.

Chapter Seven: Formulation Design and Optimization of Lyophilised ODTs Incorporating Hydrophilic and Hydrophobic Drugs Using Gum Arabic as a Binder

Patents relating to this chapter

Alhusban, F., Perrie, Y., Mohammed, A. Lyophilized orally disintegrating tablets. Filed 16th November 2009.ACH/P108627GB00.

Formulation Design and Optimization of Lyophilised ODTs Incorporating Hydrophilic and Hydrophobic Drugs Using Gum Arabic as a Binder

7.1. Introduction and Aims

Despite recent advances in the formulation of lyophilised ODTs, the number of products on the market is limited by type and dose of active drugs. The limitation is primarily due to the multiple factors associated with incorporating new active ingredients that influence the manufacturing process as well as the quality and performance of the lyophilised ODTs. Our previous results showed that incorporation of amino acids, even at low concentration, drastically affected the porosity, wettability and intermolecular bonding of the tablets and consequently the freeze drying cycle, disintegration time and mechanical properties (chapter 3). Similarly, incorporating active drugs is expected to influence all aspects of the formulation. Moreover, inclusion of high doses of hydrophobic drugs is an additional challenge, due to difficulties in keeping the drug particles suspended before freezing the formulation, as the presence of high concentration of hydrophobic drug in aqueous environment increases the chance of drug particle aggregation and sedimentation (Frenkel et al., 2005), which affects the homogeneity of the formulation in liquid state and consequently the consistency of drug content within the batch. Adding suspending agents and surfactant to address this challenge may complicate the formulation process and optimisation. For hydrophilic drugs, a limitation in the maximum dose is imposed by the plasticising effect of the drug molecules on the matrix system that lowers the glass transition temperature or eutectic melting temperature and consequently lowers the collapse temperature which necessitates longer freeze drying regimes at lower temperatures to produce intact products (Seager, 1998). However, the use of gum arabic and alanine as the main excipients in the formulation of lyophilized ODTs can offer numerous advantages that can overcome the limitations and facilitate the formulation. The self emulsifying (Li et al., 2010) and suspending (Lu et al., 2003) properties of gum arabic can be useful in increasing the dose of hydrophobic drugs that can be incorporated without compromising the consistency of drug content, disintegration time and dissolution profile of the tablets. On the other hand, alanine, due to its tendency to crystallise in the frozen state

(chapter 3), can facilitate the freeze drying process through concealing the plasticising effect of active drugs, and consequently increases the dose of hydrophilic drugs that can be incorporated in lyophilised dosage forms (Seager, 1998).

Successful development of new pharmaceutical formulations requires extensive and comprehensive research to determine significant factors in formulation, understand their effects (individually and collectively), and optimise them to obtain high quality products. For lyophilised ODTs, traditional experimentation approach can be time and material consuming and consequently is associated with high cost, due to the existence of multiple factors that influence the formulation performance and manufacturing process. Recently, factorial design of experiment (DoE) supported by statistical software has been reported as an efficient and powerful tool in the development and optimization of pharmaceutical dosage forms (Nagarwal et al., 2009). Factorial design evaluates the influence of various formulation parameters and their interaction with the lowest number of experiments, hence reducing the cost and time of the work (Bhavsar et al., 2006). Moreover, factorial design of experiment is considered an essential part of quality by design paradigm (QbD) which is recommended by the FDA as a new regulatory requirement for approval of generic drugs (Yu, 2008).

The main objective of the current study was to investigate the feasibility of incorporating therapeutics doses of active drugs in lyophilised ODTs based on gum arabic and alanine. To achieve this aim, full factorial design (3^2) was adapted to evaluate the influence of concentration of two independent variables, alanine and the active drug, on five crucial responses, disintegration time, Tg', hardness, friability and drug content. Quantitative estimation of the significant model terms (linear, polynomial and interactive) was used to build statistical model for each response that can describe the relationship between the dependant and independent variables. These models were used to optimise the concentration of alanine and the drug to maximize the quality of the formulation. Further, ODTs containing therapeutic doses of the drugs were prepared based on the optimised formulations, their short term stability was assessed and their dissolution profiles were compared to commercially available products.

Four drugs with varied physicochemical and therapeutic properties were selected for the study, namely 5,5-diphenylhydantoin, ranitidine HCl, ibuprofen and loperamide HCl. The formulation of these drugs as ODTs, in addition to improving patient compliance,

demonstrates potential pharmaceutical benefits, such as enhancing the dissolution profile of the drugs and providing rapid onset of action.

5,5-diphenylhydantoin (phenytoin) is a white crystalline powder which is practically insoluble in water with a molecular weight of 206.3 g/mol (BP, 2005). 5,5-diphenylhydantoin is an antiepileptic drug which is used in the treatment of epilepsy. It is available in parental, suspension, capsule, and chewable tablet formulations which are indicated for the control of generalized tonic-clonic and complex partial seizures (Katzung., 2007).

Ranitidine HCl is supplied as white to pale yellow granular substance that is freely soluble in water with a molecular weight of 350.87 g/mol (BP, 2005). It is a histamine H_2 receptor antagonist that is used to relieve and prevent heart burn associated with acid indigestion and sour stomach, and as short term treatment of active duodenal ulcer. Ranitidine HCl works by inhibiting H2 receptor at the parietal cell that is lining the stomach lumen, hence fast dissolution of the formulation in the stomach is necessary to exhibit its therapeutics response. The drug is available as immediate release formulations including conventional and effervescent tablets, capsules, and solution formulations (Katzung., 2007).

Ibuprofen (MW of 350.87 g/mol) is a white crystalline powder that is practically insoluble in water (BP, 2005). Ibuprofen is a nonsteroidal anti-inflammatory drug that possesses analgesic and antipyretic properties. It is available as conventional and chewable tablets, capsule, ODT and suspension formulations (Katzung., 2007).

Loperamide HCl (MW of 350.87 g/mol) is a white powder that is slightly soluble in water (BP, 2005). It is indicated for the control and symptomatic relief of acute nonspecific diarrhoea (Katzung., 2007).

7.2. Materials

Gum Arabic, alanine, ranitidine HCl, 5,5-diphenylhydantoin, ibuprofen, loperamide HCl, sodium-octansulphonate, triethylamine, ammonium hydroxide, sodium-octansulphonate, triethylamine, and sodium lauryl sulphate were purchased from Sigma-Aldrich Chemicals (Pool, UK). Tris(hydroxymethyl)amino-methane was supplied from ICN Biomedicals (Ohio, USA). Nurofen Meltlets (200 mg ibuprofen), Zantac™ Relief (75mg ranitidine HCl), Epanutin® Infatabs

(50mg phenytoin), and Imodium® Instant (2mg loperamide HCl) were obtained from a local pharmacy. All the chemicals were of analytical grade.

7.3. Methods

7.3.1. Full factorial design

The statistical experimental design in this study was performed using MODDE software version 8 (Umetrics Inc., NJ, USA). For each drug (ranitidine HCl, 5,5-diphenylhydantoin, ibuprofen, loperamide HCl), a 3^2 randomised full factorial design of experiment was used to study the influence of 2 factors, each at 3 levels, and experimental trials were performed in triplicate at all 9 possible combinations (27 experimental runs in total). The concentration of alanine (X_1) and the drug (X_2) were selected as independent variables. The three factorial levels for each independent factors, low, medium and high, were coded as -1, 0 and 1, respectively. The disintegration time (Y_1), Tg' (Y_2), hardness (Y_3), friability (Y_4) and drug content (Y_5) were investigated as dependant variables (responses).

7.3.2. Preparation of ODTs for factorial design experiments

To prepare the stock solution, the binder (gum arabic) was dissolved in double distilled water at room temperature to obtain a concentration of 15 % w/w. Alanine was added to the solution at the designated concentration as a percentage of the dissolved gum Arabic. With constant stirring on a magnetic stirrer, the active drug was added slowly at the designated concentration, as a percentage of the dissolved gum arabic. The resulted formulation was subjected to shear homogenisation at 5000 rpm for 10 min to obtain uniform solution (in case of ranitidine HCl) or suspension (in case of 5,5-diphenylhydantoin, ibuprofen and loperamide HCl). A constant mass of 1.10 g of the homogenised formulation was poured in a tablet mould with internal diameter of 13.50 mm, frozen at -80 °C for about 60 minutes, annealed in -20 ^{o}C a pre-cooled freezer for 12 hours and then transferred back to the -80 ^{o}C freezer. The frozen formulation was freeze-dried (ADVANTAGE Freeze-dryer, VIRTIS) according to an optimized regime (primary drying for 16 hours at shelf temperature of -35 °C and secondary drying for 1 hour at shelf temperature of 10 °C and vacuum of 50 mTorr).

The optimised formulation for each drug was formulated by the same method and the observed (experimental) and the predicted (from the model) values for the responses were compared to evaluate the validity of the model.

7.3.3. Preparation of ODTs for the dissolution and stability studies

Based on the optimised formulation for each drug, lyophilised ODTs containing therapeutic dose of the drugs were formulated to carry out dissolution and stability studies. 50 mg 5,5-diphenylhydantoin ODTs was prepared in 18 mm diameter tablet mould, 75 mg ranitidine HCl in 20 mm mould, 200 mg ibuprofen in 20 mm mould, and 2 mg loperamide HCl in 13.5 mm mould. Accurate mass of the homogenised formulation required to obtain the therapeutic dose was poured in the designated tablet mould. The samples were subjected to the same protocol of freezing and freeze drying as above.

7.3.4. Differential scanning calorimetry studies

Differential scanning calorimetry (Pyris Diamond DSC and Intracooler 2P: Perkin Elmer, Wellessey, USA) was used to determine the glass transition temperature (Tg) and crystallisation event of the formulation in its frozen state (before freeze drying). 10-15mg of the liquid formulation were loaded into aluminium pans, cooled to -65 °C and then heated to 20 °C at 5 °C/min with a nitrogen purge of 20ml/min. To determine the glass transition temperature of the maximally freeze concentrate sample (Tg'), after initial cooling to -65 °C, annealing step for 15 min at -15 °C was performed before carrying out the above method. An empty aluminium pan was used as reference for all measurements.

The resulting plots were analysed by Pyris manager software. Tg and Tg' values were determined from the intersection of relative tangents to the baseline. All the measurements were done in triplicate from independently prepared samples.

The DSC was calibrated for temperature and heat flow using standard samples of indium (melting point: 156.6 ˚C, ΔHm: 28.42 J/g) and Zinc (melting point: 419.5 ˚C, ΔHm: 108.26 J/g).

7.3.5. Disintegration time

The disintegration time of the tablets was measured using a USP disintegration tester (Erweka, ZT3). Distilled water (800 ml) kept at 37 °C was used as a medium and the basket was raised and lowered at a fixed frequency of 30 cycles/min. One tablet was tested at a time.

7.3.6. Mechanical properties of the tablets

The hardness of the lyophilized tablets was investigated with a texture analyzer (QTS 25: Brookfield, Essex, UK) equipped with a 25 kg load cell. The instrument was calibrated with standard weight of 500 g and 5 kg. The tablet was placed in a holder with a cylindrical hole and the hardness was taken as the peak force after 1mm penetration of 5mm diameter probe at a speed of 6 mm/min.

Friability of the tablets was evaluated by tumbling a sample of 5 tablets in a USP friabilator (Sotax, model F2, Basel, Switzerland) for 4 minutes at 25 rpm. The tablets were brushed gently, reweighed and the friability was calculated as a percentage of weight loss to the initial weight.

Friability= $(W_o - W)/ W_o \times 100\%$

7.3.7. Moisture content

The moisture content of the freeze-dried tablets was determined by thermogravimetric analysis (TGA) (Pyris 1 TGA: Perkin-Elmer, Waltham, USA). The TGA was calibrated for temperature, furnace and weight using standard calibrants of Alume and Nickel. The tablets were cut into small pieces, loaded onto TGA platinum pan and placed into the pre-equilibrated furnace at 30 °C. After equilibration at this temperature, the samples were heated at a rate of 10 ˚C/min to 150 °C and held isothermally at this temperature for 1 minute.

7.3.8. Drug content

For 5,5-diphenylhydantoin ODTs, after complete disintegration in 10 mL double distilled water in a 500mL beaker, 400 mL of an extraction solvent, a mixture of acetonitrile/water (80:20, v/v), was added gradually with constant stirring on a magnetic stirrer. The solution was

transferred to 500mL volumetric flask and the extraction solvent was added to make up the volume. After good shaking, the solution was filtered through a 0.45 µm nylon filter (CHROMACOL LTD, Herts, UK) in autosampler vials for HPLC assay.

Ranitidine HCl tablets was dissolved in 1 L double distilled water, shaken for 30 min, and filtered through a 0.45 µm nylon filter (CHROMACOL LTD, Herts, UK) in autosampler vials for HPLC assay.

For ibuprofen, the tablet disintegrated by adding 10 mL double distilled water in a 100mL beaker, 80 mL of an extraction solvent, a mixture of methanol/water (80:20, v/v), was added gradually with constant stirring on a magnetic stirrer. The solution was transferred to 100mL volumetric flask and the extraction solvent was added to make up the volume. After good shaking, the solution was filtered through a 0.45 µm nylon filter (CHROMACOL LTD, Herts, UK) in autosampler vials for HPLC assay.

For loperamide HCl, the tablet disintegrated by adding 5 mL double distilled water in a 100mL beaker, 80 mL of the mobile phase (see HPLC method) was added gradually with constant stirring on a magnetic stirrer. The solution was transferred to 100mL volumetric flask and the mobile phase was added to make up the volume. After good shaking, the solution was filtered through a 0.45 µm nylon filter (CHROMACOL LTD, Herts, UK) in autosampler vials for HPLC assay.

7.3.9. HPLC analysis

HPLC analysis of the selected drugs was carried out using Reverse phase HPLC (Dionex AS 50 autosampler with GP50 gradient pump HPLC System: Dionex, UK) at room temperature using a Gemini 5 µm, 4.6 x 150 mm, column (Phenomenex La Luna: Phenomenex, Torrance, USA).

5,5-diphenylhydantoin was analysed using acetonitrile: water (90:10. v/v) as a mobile phase at a flow rate of 1.0 mL/min, with a sample injection volume of 5 µL and UV detection at 213 nm (Gupta and Myrdal, 2005). Under these HPLC conditions the retention time for 5,5-diphenylhydantoin was 1.94 minutes. The concentration of 5,5-diphenylhydantoin in the tablets was determined by reference to a calibration curve constructed by diluting a stock solution (1mg/mL) of 5,5-diphenylhydantoin using the mobile phases to obtain serial

concentrations in a range from 1.0 to 100.0 µg/ml. The calibration curve was performed in triplicate and resulted in a linear correlation in the studied concentration range (r^2=0.99).

Ranitidine HCl was analysed using a mobile phase consisting of acetonitrile: phosphate buffer (20:80, v/v). The buffer prepared as 10 mM phosphate and adjusted to pH 7.1 with 0.1 N sodium hydroxide (Shah et al., 2006). The mobile phase flow rate was 1 ml/min, the injection volume was 20 µl and the UV absorbance was at 230 nm. The retention time was 4.78 min. The concentration of ranitidine HCl in the tablets was determined by reference to a calibration curve constructed from dilutions of a stock solution (1mg/mL), using the mobile phases, in a concentration range between 10 to 100 µg/mL. The calibration curve was performed in triplicate and resulted in a linear correlation in the studied concentration range (r^2=0.99).

Ibuprofen was analysed using a mixture of methanol: water (80:20. v/v) as a mobile phase at a flow rate of 1.0 mL/min, with a sample injection volume of 5 µL and UV detection at 230 nm. Under these HPLC conditions the retention time for Ibuprofen was 2.47 minutes. The concentration of the drug in the tablets was determined by reference to a calibration curve constructed from dilutions of stock solution (1mg/mL) in a range between 100 and 500 µg/ml. The calibration curve was performed in triplicate and resulted in a linear correlation in the studied concentration range (r^2=0.99).

Loperamide HCl was analysed using a mobile phase consisting of an aqueous solution of 0.1% sodium-octansulphonate, 0.05% triethylamine and 0.1% ammonium hydroxide: acetonitrile (45:55, v/v) at a flow rate of 1.5 mL/min, with a sample injection volume of 20 µL and UV detection at 226 nm (Savic et al., 2009). Under these HPLC conditions the retention time was 2.07 minutes. The concentration of the drug in the tablets was determined by reference to a calibration curve constructed from dilutions of stock solution (1mg/mL) in a range between 10 and 100 µg/ml. The calibration curve was performed in triplicate and resulted in a linear correlation in the studied concentration range (r^2=0.99).

7.3.10. Dissolution studies

The dissolution profiles of the prepared ODTs that contained therapeutic doses of the drugs and the commercial products were evaluated in a USP dissolution apparatus II (Erweka DT 600,

Heusenstamm, Germany). The dissolution conditions and dissolving medium for each drug were as prescribed in the USP.

For 5,5-diphenylhydantoin (phenytoin) tablets (50 mg), the dissolution medium was 0.05 M tris buffer (900ml, 37 ˚C), which was prepared by dissolving 60.5 g of tris(hydroxymethyl)amino-methane in 10 liters of double distilled water, adjusting the pH to 9.0 by phosphoric acid, and dissolving 100 g of sodium lauryl sulphate. The dissolution experiment was for 2 hours at rotational speed of 100rpm (USP, 2003). At fixed time intervals, 5 ml samples were withdrawn and replaced with fresh medium (37 ˚C). The concentration of the drug in the filtered samples was analysed using HPLC assay (see HPLC method) and the cumulative drug release was calculated.

For ranitidine HCl tablets (75 mg), the dissolution was performed in 900 mL deionised water (37 ˚C) for 45 minutes and at rotational speed of 50rpm (USP, 2003). At fixed time intervals, 5 ml samples were withdrawn and replaced with fresh medium (37 ˚C). The samples were filtered through a 0.45 µm nylon filter (CHROMACOL LTD, Herts, UK) and diluted with water, when necessary. The amount of drug dissolved was analysed by Jenway 6405 UV/Vis spectrophotometer (Bibby Scientific Limited, Staffordshire, UK) at the wavelength of maximum absorbance (314 nm) and the cumulative drug release was calculated.

For ibuprofen tablets (200 mg) the dissolution was performed in 900 mL phosphate buffer (pH 7.2, 37 ˚C) for 60 minutes and at rotational speed of 50rpm (USP, 2003). At fixed time intervals, 5 ml samples were withdrawn and replaced with fresh medium (37 ˚C). The samples were filtered through a 0.45 µm nylon filter (CHROMACOL LTD, Herts, UK) and diluted with the medium (phosphate buffer), when necessary. The amount of drug dissolved was analysed by Jenway 6405 UV/Vis spectrophotometer (Bibby Scientific Limited, Staffordshire, UK) at the wavelength of maximum absorbance (221 nm) and the cumulative drug release was calculated.

For loperamide HCl, the dissolution was performed in 900 mL of 0.01 N hydrochloric acid (37 ˚C) for 30 minutes and at rotational speed of 50 rpm (USP, 2003). At fixed time intervals, 5 ml samples were withdrawn and replaced with fresh medium (37 ˚C). The concentration of the drug in the filtered samples was analysed using HPLC assay (see HPLC method) and the cumulative drug release was calculated.

7.3.11. Stability studies

Short term stability studies were performed on the optimised ODTs that contain therapeutic dose of the drugs. The tablets were stored in air-tight amber glass bottles with a tight lid and were kept in a climatic cabinet (Firlabo, model SF BVEHF, Meyzieu, France) with a storage condition of 40 ˚C and 75% RH. After 3 months, the samples were evaluated for moisture content, disintegration time, mechanical properties and drug content.

7.4. Results and discussion

7.4.1. Factorial design

The formulation of lyophilised orally disintegrating tablets (ODTs) consists of a water soluble binder, which gives shape and provides mechanical strength to the tablets. Matrix supporting/ disintegration enhancing agents to fortify the porous framework provided by the water soluble polymer and accelerate the disintegration of the ODT. Preliminary screening studies (chapter 6) were conducted to select and optimise the choice and concentration of the binder and the matrix supporting/disintegration enhancing agent. The results suggested that gum arabic has a superior profile as a binder, whereas alanine and mannitol showed favourable performance as matrix supporting/disintegration enhancing agents over a concentration range of 20- 50 % (w/w). The concentration of gum arabic was fixed at 15% (w/w) as this concentration provides elegant tablets in a very short freeze drying cycle and achieves the best balance between mechanical property and disintegration time (the highest lyophilised tablet index). Alanine and active drug concentrations were further investigated in this study as independent factors for their influence on ODT characteristics using a 3^2 randomised full factorial design of experiment. For each active drug, three levels of alanine (X_1) and the active drug (X_2) concentrations were defined depending on the physicochemical characteristics of the drug and the required dose (see below). Five crucial responses in the development of ODTs were evaluated for each formulation including disintegration time (Y_1), Tg' (Y_2), hardness (Y_3), friability (Y_4) and drug content (Y_5). A quadratic statistical model incorporating all main, interactive and polynomial terms was used to evaluate the influence of the studied factors (independent factors) on the responses (dependent variables).

$$Y_i = b_0 + b_1 X_1 + b_2 X_2 + b_{12} X_1X_2 + b_{11} X_1^2 + b_{22} X_2^2, \quad (1)$$

Where Y_i is the response (dependent variable), b_0 is the arithmetic mean response of the 27 trials, and b_1 and b_2 are the estimated coefficient for the factors X_1 and X_2, respectively. The main effects (X_1 and X_2**)** represent the average result of changing one factor at a time from its low to high value while keeping the other factor at its centre point. The interaction terms (X_1X_2) show the change in the response when both factors are varied simultaneously. The polynomial terms (X_1^2 and X_2^2) express non linear correlations with the response.

7.4.2. ODTs of 5,5-diphenylhydantoin

7.4.2.1. Experimental design

To optimise the properties of lyophilised ODTs that contain 50 mg of 5,5-diphenylhydantoin, three concentration levels of drug, low (10%w/w) medium (20%w/w) and high (30%w/w), and three concentration levels of alanine, low (20%w/w) medium (30%w/w) and high (40%w/w), were used in the factorial design experiment. These concentrations of 5,5-diphenylhydantoin were designed to allow the production of lyophilised ODTs with size range comparable to the standard tablets sizes. The designed dimensions (diameter × thickness) of lyophilised tablets containing 50 mg 5,5-diphenylhydantoin are 18.00mm × 21.13 mm, 18.00 mm × 10.57 mm and 18.00 mm × 7.05 mm for 10, 20 and 30% (w/w) formulations, respectively.

Based on a 3^2 randomised full factorial design, 9 formulations were prepared in triplicates (27 experiments (9*3) in total). The design and the results from the 27 experiments are presented in **Table 7.1**. The results showed that the disintegration time of the tablets varied from 2 to 9 s, the Tg' of the formulation was between -24.82 to -14 ˚C, the hardness varied from 2.8 to 6.98 N, friability from 6.01 to 19.78 % and drug content from 95.12 to 106.95 %. The results showed that disintegration time, Tg', hardness and friability showed wide variations in responses (**Figure 7.1**) suggesting that these responses are strongly dependent on the selected independent factors. In case of drug content, the results seem to be unsystematic and random and might be explained as experimental errors.

Table 7.1 Full factorial design worksheet for 5,5-diphenylhydantoin study. The concentrations (%w/w) of 5,5diphenylhydantoin and alanine are percentages of the mass of gum arabic dissolved in the stock solution.

Exp Name	Run order	5,5-diphenylhydantoin (%)	Alanine (%)	Disintegration time (s)	Tg' (˚C)	Hardness (N)	Friability (%)	Drug recovery (%)
N1	16	10	20	7	-24.82	2.80	9.59	99.84
N2	23	30	20	5	-23.82	3.37	19.78	98.10
N3	1	10	40	3	-15.04	5.29	7.62	102.69
N4	13	30	40	6	-14.81	6.00	10.44	104.85
N5	18	10	30	2	-15.42	3.49	7.12	103.30
N6	21	30	30	3	-15.08	4.95	10.35	104.78
N7	11	20	20	4	-24.50	4.42	16.78	103.81
N8	19	20	40	4	-14.09	5.80	12.57	101.45
N9	5	20	30	7	-15.55	4.23	15.58	95.29
N10	2	10	20	8	-24.17	3.32	8.46	104.93
N11	27	30	20	3	-23.90	3.54	16.45	95.12
N12	24	10	40	2	-15.31	4.34	6.33	98.43
N13	8	30	40	6	-14.56	6.44	10.36	100.39
N14	22	10	30	2	-15.32	4.58	6.20	103.44
N15	10	30	30	2	-15.27	5.27	11.57	101.24
N16	4	20	20	4	-24.37	3.38	15.42	101.40
N17	14	20	40	6	-14.21	5.57	10.98	104.77
N18	6	20	30	9	-15.47	4.57	13.51	99.94
N19	3	10	20	5	-24.46	3.24	9.02	105.97
N20	9	30	20	4	-24.12	4.02	17.38	96.55
N21	26	10	40	3	-15.27	4.83	6.01	99.71
N22	20	30	40	7	-14.82	6.98	10.97	99.11
N23	15	10	30	3	-15.08	3.84	7.46	101.55
N24	7	30	30	2	-14.85	5.57	12.52	103.88
N25	12	20	20	5	-24.29	3.81	15.63	106.95
N26	25	20	40	4	-14.00	5.02	11.12	103.43
N27	17	20	30	5	-15.68	3.97	13.44	97.13

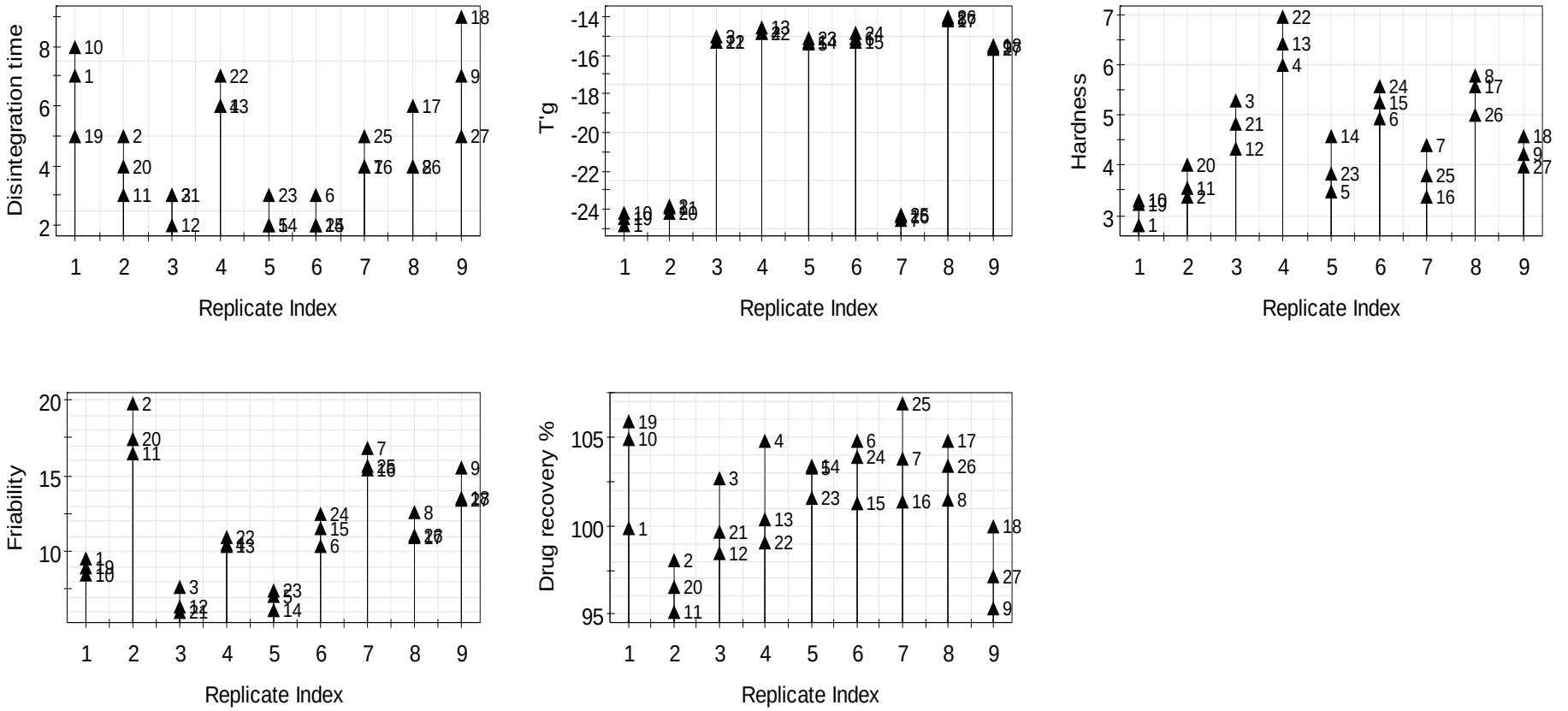


Figure 7.1 Replicate plots of the responses. The values of the response are plotted vs. experimental runs displaying the variation in the response for replicated experiments.

7.4.2.2. Analysis of variance (ANOVA)

Analysis of variance (ANOVA) was performed to evaluate the significance of the quadratic models (linear, interactive and polynomial) on the responses and to establish their quantitative effects. **Table 7.2** summarises the effects of the model terms and associated p values for all five responses. At a 95% confident level, a model was considered significant if the p value <0.05. The results indicate that the disintegration time of the tablets was significantly affected only by the interactive model between 5,5-diphenylhydantoin and alanine (X_1X_2), whereas the rest of the model terms had no significant contribution in determining the disintegration time (p>0.05). This can be explained as alanine and 5,5-diphenylhydantoin concentrations simultaneously affect the factors that control the disintegration of the ODTs in an interactive way. The total concentration of both materials influences the porosity of the tablets which controls the diffusion of the disintegrating media into the tablets (Bi et al., 1999). At the same time, alanine by itself, because of its high wettability property (chapter 3), enhances the disintegration by wicking mechanism of disintegration (Fukami et al., 2006) whereas, 5,5-diphenylhydantoin provides the hydrophobic moiety inside the tablet and therefore promotes the disintegration by the repulsive mechanism (Guyot-Hermann and Ringard, 1981). The results therefore indicate that the interactive terms were the most significant factors in the disintegration time and accordingly the shortest disintegration time can be achieved by balancing both concentrations simultaneously.

Table 7.2 The quantitative factor effects and associated p value for the responses.

Term	Y_1		Y_2		Y_3		Y_4		Y_5	
	Effect	P value	Effect	P value	Effect	P value	Effect	P value	Effect	P value
X_2	0.1500	0.6465	0.1709	0.0191	0.4799	<0.0001	2.3928	<0.0001	-0.7601	0.2351
X_1	-0.1933	0.5552	3.9898	<0.0001	0.8501	<0.0001	-1.9381	<0.0001	0.1205	0.8482
X_2^2	-0.8630	0.0773	-0.0660	0.5035	0.0102	0.9353	-2.4227	<0.0001	-0.2248	0.8044
X_1^2	0.5921	0.2164	-2.8927	<0.0001	0.0500	0.6899	0.7697	0.0266	0.3033	0.7384
X_1X_2	1.1076	0.0029	-0.0087	0.9004	0.1942	0.0375	-0.8613	0.0011	1.3846	0.0404

In case of the Tg', X_1, X_2 and X_1^2 are significant model terms. X_1 (alanine concentration) has a large positive coefficient (3.9898) suggesting that increasing alanine concentration significantly increases the Tg' value, which can be explained by the tendency of high concentrations of alanine to crystallise in the frozen formulation (**Figure 7.2**). X_2 (5,5-diphenylhydantoin concentration) has a smaller positive coefficient (0.1709) suggesting that increasing 5,5-diphenylhydantoin concentration significantly increases the Tg' but to a lower extent than alanine. This antiplastcising effect might be a result of the low solubility (hydrophobic) nature of 5,5-diphenylhydantoin in the hydrophilic environment of the tablet mixture (Mao et al., 2008). On the other hand, the polynomial terms X_1^2 (alanine concentration) has a large negative coefficient which reflects the inability of alanine to crystallise at low concentration and consequently shows plasticising behaviour in the system (lower Tg') (**Figure 7.2**). The interactive term (X_1X_2) appears to have no significant effect on the Tg', possibly due to the limited solubility of 5,5-diphenylhydantoin.

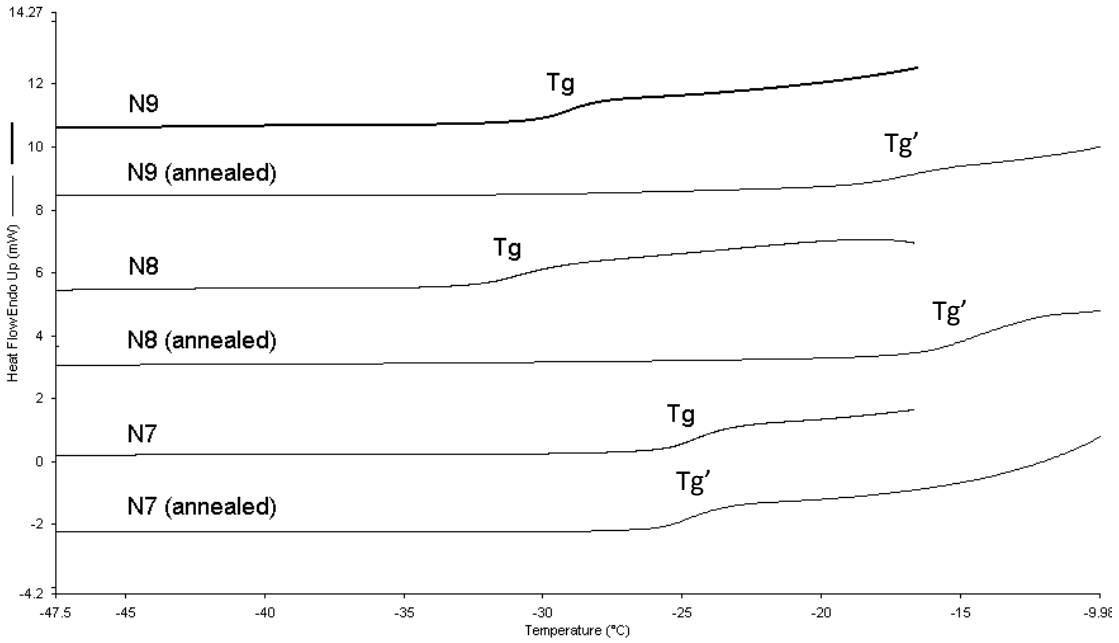


Figure 7.2 Overlaid DSC heating scans of frozen formulations based on high (N8), medium (N9) and low (N7) alanine concentration with medium concentration of 5,5-diphenylhydantoin.

For the third response Y_3 (hardness) linear terms X_1 (alanine concentration) and X_2 (5,5-diphenylhydantoin concentration) were identified as the most significant factors with p value < 0.00001, whereas the interactive term X_1X_2 was less significant with p value (0.0375) just below the significant level ($p<0.05$). The quantitative estimation (**Table 7.2**) of the significant terms indicated that increasing alanine concentration was the most effective way to enhance the hardness of the ODTs with a positive coefficient of 0.8501, which confirms the role of alanine as a matrix supporting agent (chapter 3). Increasing 5,5-diphenylhydantoin concentration (X_2), also, enhances the hardness but to a lesser extent than alanine, as indicated with the smaller positive coefficient (0.4799). Minimal degree of improvement was seen as a result of interaction between alanine and 5,5-diphenylhydantoin. These results can be explained in terms of intermolecular bonding force and contact points between the excipients within the tablets (Adolfsson and Nyström, 1996). The high degree of improvement in the hardness associated with increasing alanine concentration in the tablet might be a result of enhancement of both factors; the intermolecular bonding force, possibly through initiating hydrogen bonds with the binder (gum arabic) as both contain hydrogen bond donors and acceptors, and contact points between the excipients, as a result of decreasing the porosity. Increasing 5,5-diphenylhydantoin may increase the contact point within between the excipients but it is not expected to make strong bonds due to its high hydrophobic nature. Therefore, the improvement in the hardness associated with increasing 5,5-diphenylhydantoin concentration was smaller than alanine.

Significant influence for response Y_4 (friability) was exhibited by X_1 ($p<0.0001$), X_2 ($p<0.0001$), X_1X_2 ($p<0.01$), X_1^2 ($p<0.05$) and X_2^2 ($p<0.0001$), suggesting that all these model terms affect the friability but by varied significant levels depending on p value. The factor which had the most detrimental effect on the friability (increasing the friability) was X_2 (5,5-diphenylhydantoin concentration) as suggested by its large positive coefficient, suggesting that incorporation of high concentration of 5,5-diphenylhydantoin concentration increased the friability of the ODTs. However, this effect seemed to be dependent on the concentration of 5,5-diphenylhydantoin as indicated by the negative coefficient of the polynomial term of 5,5-diphenylhydantoin concentration (X_2^2). The study also indicated that increasing alanine concentration (X_1) was an efficient way to reduce the friability of the tablet, as X_1 had high negative coefficient (-1.9381) which further confirms its role as matrix supporting agent. However, the significant influence of X_1^2 (polynomial term of alanine concentration) with positive coefficient limits the reduction of the friability by increasing alanine concentration to a

certain level. The interactive terms X_1X_2 had a negative coefficient of -0.8613, suggesting a reduction in the friability which might be due to increase in the contact points between the excipients inside the tablets (alanine and 5,5-diphenylhydantoin).

ANOVA results (**Table 7.2**) indicated that all the model terms including linear, interactive and polynomial, had no significant influence on the drug content ($p>0.05$) suggesting that the results were randomly distributed and the variation was only due to experimental errors in formulation and/or detection.

7.4.2.3. Revised model and surface response plots

After analysing the influence of all the quadratic model terms on the dependent variables (responses), the insignificant terms were omitted to generate a revised model that included only model terms that have a significant influence. The resulting equations for all five responses, Y_1 (disintegration time), Y_2 (Tg'), Y_3 (hardness), Y_4 (friability) and Y_5 (drug content), are presented below:

$Y_1 = + 4.742 + 1.108\ X_1X_2$

$Y_2 = -15.235 + 3.990\ X_1 + 0.171\ X_2 - 2.893\ X_1^2$

$Y_3 = +4.48426 + 0.850X_1 + 0.480X_2 + 0.194X_1X_2$

$Y_4 = +13.172 + -1.938\ X_1 + 2.393\ X_2 - 0.861\ X_1X_2 + 0.770\ X_1^2 - 2.423\ X_2^2$

$Y_5 = 101.334$

The results for testing the validity of the model are summarised in **Table 7.3**. P values for all the simulated responses were below the significant level (<0.05) suggesting that all the revised models were significant in predicting their response values. The high value of correlation coefficient (R^2) for Tg', hardness and friability indicate a good fit to the revised model. Low correlation coefficient were noticed for the disintegration time possibly due to the qualitative nature of the test that depends on the visual evaluation in addition to the fact that few seconds' inaccuracy in evaluating the disintegration time can cause huge error, as the disintegration time is very short.

Table7.3 Summary of results for testing validity of the revised models. DF indicates: degrees of freedom; SS: sum of squares; MS: mean of square; F: Fischer's ratio; p: probability; R^2: regression coefficient.

Disintegration time						
	DF	SS	MS (variance)	F	p	R^2
Regression	5	45.988	9.198	3.403	0.021	0.448
Lack of Fit	3	36.086	12.029	10.477		

Tg'						
	DF	SS	MS (variance)	F	p	R^2
Regression	5	519.451	103.890	881.505	< 0.0001	0.995
Lack of Fit	3	1.9121	0.637	20.382		

Hardness						
	DF	SS	MS (variance)	F	p	R^2
Regression	5	25.756	5.151	26.901	< 0.0001	0.865
Lack of Fit	3	0.844	0.281	1.593		

Friability						
	DF	SS	MS (variance)	F	p	R^2
Regression	5	346.007	69.201	53.041	<0.0001	0.927
Lack of Fit	3	10.388	3.463	3.664		

Based on the revised equations, the software was used to generate response surface plots (three dimensional) that simulate the influence of the independent factors on each response individually. The graphs for disintegration time, Tg', hardness and friability are presented in **Figures 7.3**, **7.4**, **7.5** and **7.6**, respectively. These plots can provide uninterrupted visual assessment of the change in the response surface as a function of varying the independent factors, individually and simultaneously, which is valuable to further understand the system and optimise the formulation.

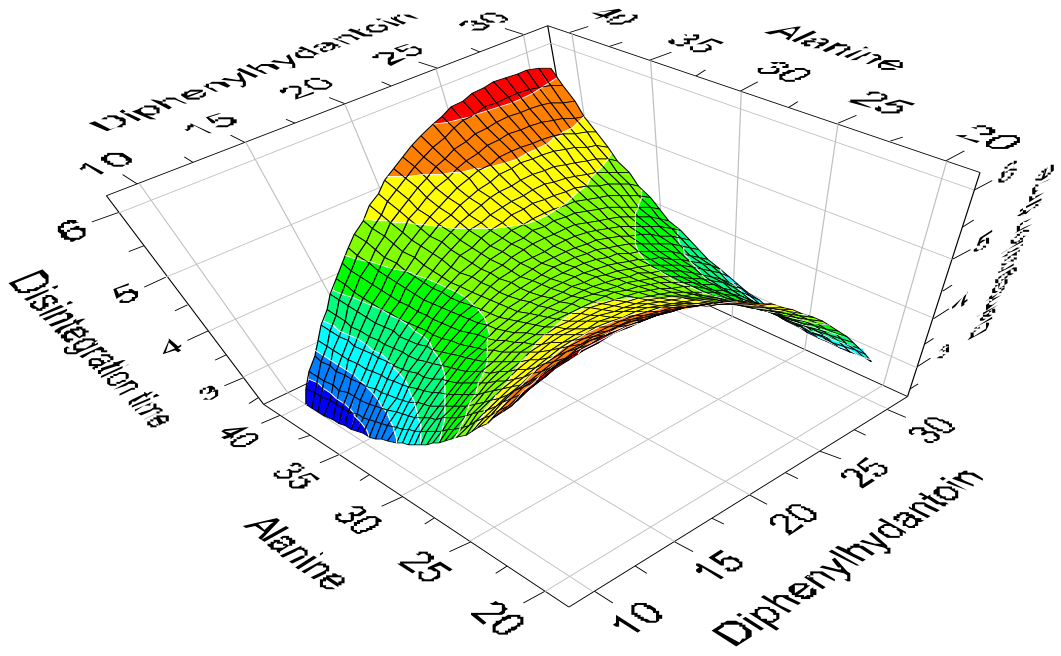


Figure 7.3 Surface response plot showing the influence of varying alanine and 5,5-diphenylhydantoin concentration on the disintegration time of the ODT.

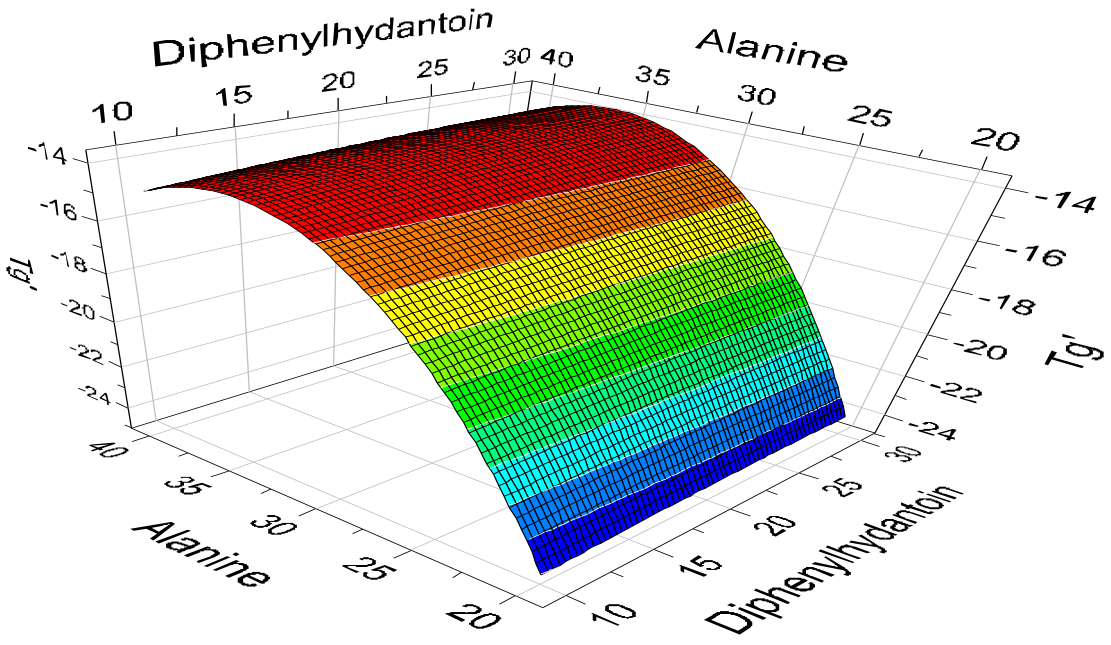


Figure 7.4 Surface response plot showing the influence of varying alanine and 5,5-diphenylhydantoin concentration on the Tg'.

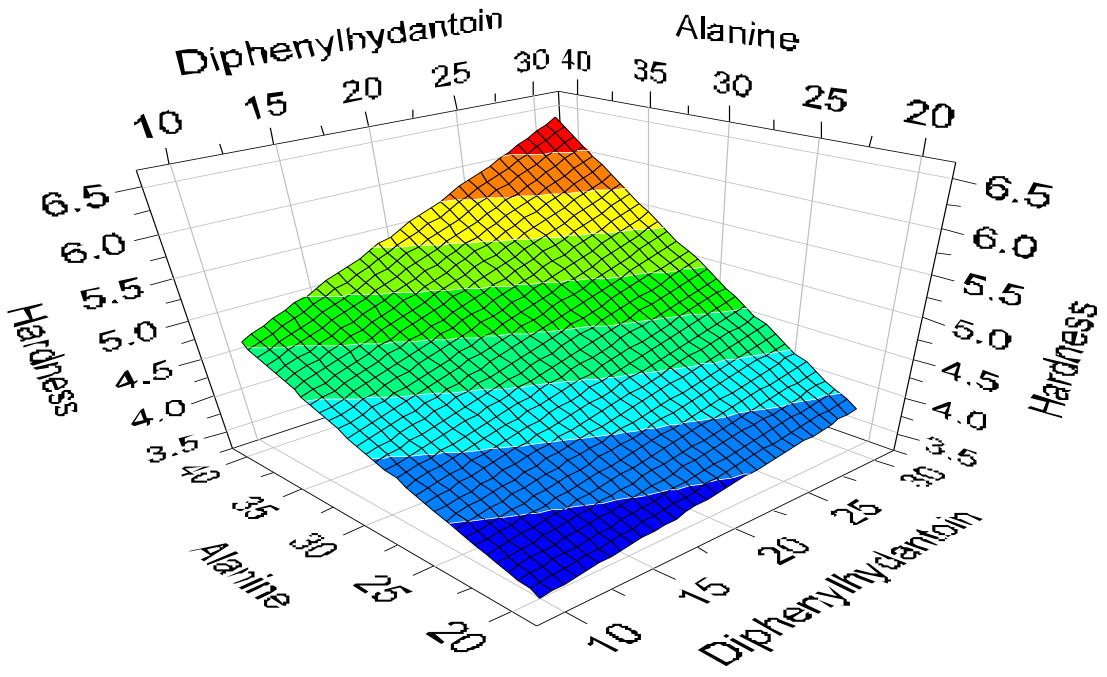


Figure 7.5 Surface response plot showing the influence of varying alanine and 5,5-diphenylhydantoin concentration on the hardness of the ODT.

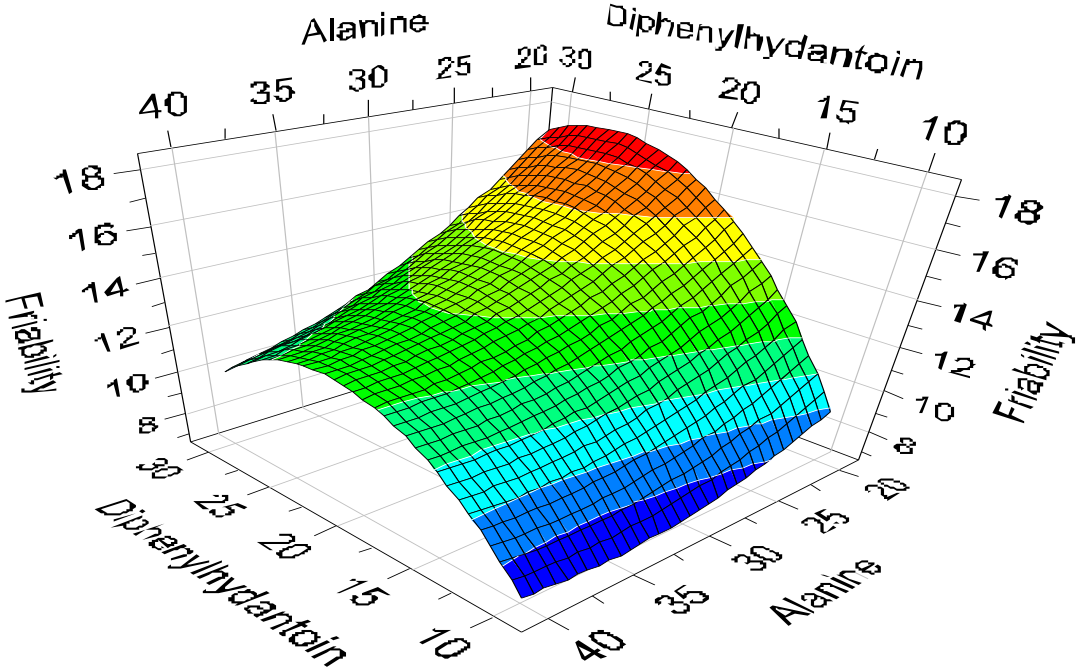


Figure 7.6 Surface response plot showing the influence of varying alanine and 5,5-diphenylhydantoin concentration on the friability of the ODT.

7.4.2.4. Optimum ODTs formulation

Based on the response surface plots, the software performed hot spot analysis to determine the optimum formulation variables (alanine and 5,5-diphenylhydantoin concentration) to produce ODTs with short disintegration time, high Tg', high hardness and low friability. The optimal formulation was determined as 40% (w/w) alanine and 30 % (w/w) 5,5-diphenylhydantoin concentration. The observed response values of the optimised formulation compared to the predicted values are presented in **Table 7.4**. The characterisation results were verified experimentally and only small differences were found between the experimental (observed) and calculated (predicted) values.

7.4.2.5. Formulation of 50 mg 5,5-diphenylhydantoin lyophilised ODTs

Based on the optimised formulation, lyophilised ODTs containing 50 mg dose of 5,5-diphenylhydantoin were prepared using 18 mm diameter mould. The characterisation summary of the tablets is presented in **Table 7.5**. As expected, the tablets showed instant disintegration using the USP apparatus (less than 6 seconds) without leaving any lumps or gritty particles in the disintegration vessel. The mechanical properties of the tablets suggest the need for specialised packaging to provide the tablets with extra protection against possible mechanical stresses during storage and handling by patients. The average thickness of the dried tablets was 7.45 mm (SD=0.31, n=6).

Table 7.4 Observed and predicted (from the revised model) responses and residual values for the optimised formulation. The observed results are means, n=3.

Response	Observed	Predicted	Residual
Disintegration time (s)	5	5.90	-0.90
Tg' (˚C)	-14.6	-14.52	0.08
Hardness (N)	6.52	6.45	0.07
Friability (%)	9.82	10.09	-0.27
Drug content (%)	101.52	102.68	-0.94

Table 7.5 Characterisation of the prepared lyophilised ODTs after 0 and 3 months at 40 ˚C and 75% RH. Results are means ± SD, n=3.

Parameters	Time interval (months)	
	0	3
Moisture content (%)	2.04 ± 0.47	2.25 ± 0.32
Disintegration time (s)	5 ± 1	6 ± 1
Hardness (N)	6.41 ± 0.56	6.28 ± 0.42
Friability (%)	10.45 ± 2.75	9.2 ± 1.83
Drug content (%)	101.24 ± 3.89	99.12 ± 4.02

7.4.2.6. Dissolution studies

The dissolution profiles of the prepared lyophilised tablets and commercial chewable tablets (Epanutin® Infatabs) that contain 50 mg 5,5-diphenylhydantoin (phynetoin) are presented in **Figure 7.7**. According to the US Pharmacopeia, not less than 70% of the dose should be dissolved within 120 min under the prescribed dissolution conditions. The results showed that both products satisfied the USP criteria by far and showed fast dissolution rate with around 60% of drug release in less than 5 min, which might be explained as a consequence of crushing the chewable tablets (Epanutin Infatabs) to fine powder before performing the dissolution test and the instant disintegration of the lyophilised tablets. However, the lyophilised tablets showed higher dissolution efficiency with complete drug release in about 10 min **(Figure 7.7).** This can possibly be attributed to the intrinsic emulsifying properties of gum arabic (Yadav et al., 2007) that enhanced the extent and rate of dissolution.

7.4.2.7. Stability studies

The lyophilised ODTs of 5,5-diphenylhydantoin were subject to short term stability studies. There was no change noticed in appearance or smell. The results (**Table 7.5**) indicated that no significant change in moisture content, disintegration time, drug content and mechanical properties were observed suggesting that the formulation were chemically and physically stable.

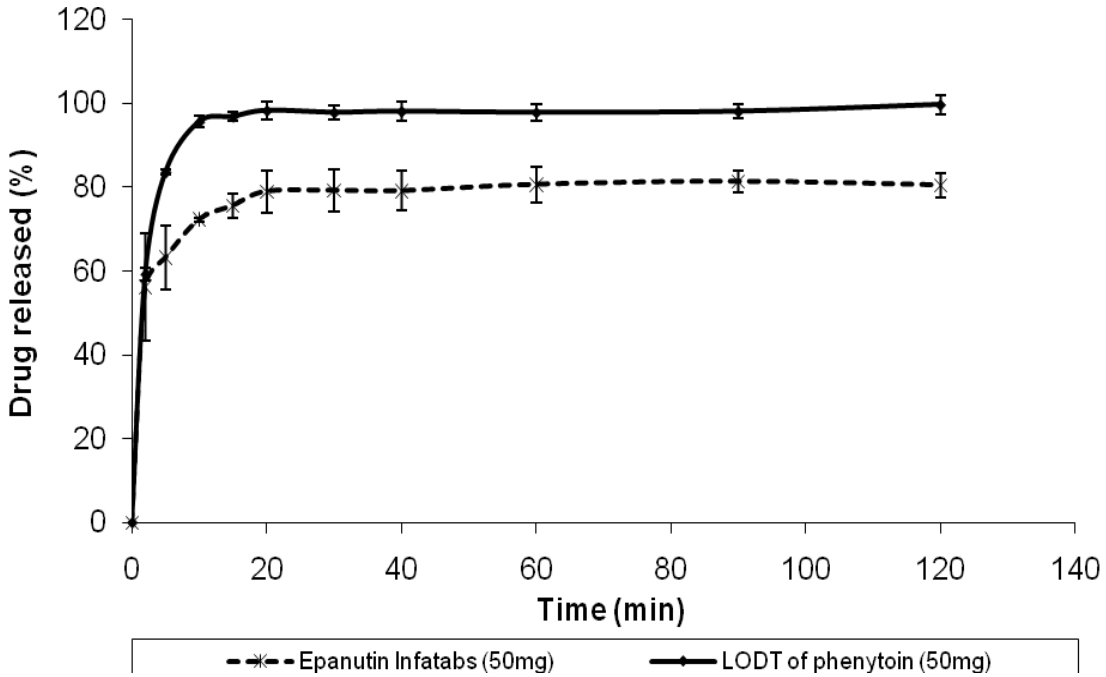


Figure 7.7 Dissolution profiles of commercially available chewable tablets (Epanutin Infatabs) and formulated lyophilised orally disintegrating tablets (LODT) of 5,5-diphenylhydantoin. Results are means ± SD, n=3.

7.4.3. ODTs of ranitidine HCl

7.4.3.1. Experimental design

To optimise the properties of lyophilised ODTs that contain 75 mg of ranitidine HCl, three concentration levels of the drug, low (10% w/w) medium (25% w/w) and high (40% w/w), and three concentration levels of alanine, low (20% w/w) medium (40% w/w) and high (60% w/w), were used in the factorial design experiment. These concentrations of ranitidine HCl were designed to allow the production of lyophilised ODTs in size comparable to standard tablet. The dimensions (diameter × thickness) of lyophilised tablets containing 75 mg ranitidine HCl were 20.00mm × 13.54 mm, 20.00 mm × 5.41 mm and 20.00 mm × 3.38 mm for 10, 25 and 45 % (w/w) formulations, respectively.

The studied alanine concentration range (up to 60% w/w) was higher than the concentration range required for finding the best balance between disintegration time and mechanical

properties (as in the case of incorporating hydrophobic drugs). This is mainly due to the high water solubility of ranitidine HCl that is expected to exhibit a plasticising effect on the system and consequently lower the collapse temperature of the formulation in a concentration dependant matter (higher concentration lower collapse temperature). Therefore, alanine crystallization in the frozen state is necessary to give stability to the formulation, protect against possible collapse and produce elegant lyophilised products (chapter 3). Our previous research showed that the crystallisation of alanine might be retarded by the presence of highly soluble moiety in the system (chapter 4) and higher concentration of alanine was required to crystallise. Accordingly the influence of alanine on the formulation characteristics was investigated at high concentration.

Based on a 3^2 randomised full factorial design, 9 formulations were prepared in triplicates (27 experiments (9*3) in total). The design and the results from the 27 experiments are presented in **Table 7.6**. The results showed that the disintegration time of the tablets varied from 3 to 8 s, the Tg' of the various formulations was between -33.65 to -14.05 ˚C, the hardness varied from 1.53 to 9.00 N, friability from 1.62 to 22.87 % and drug content from 88.38 to 102.57 %. The results showed that disintegration time, Tg', hardness and friability showed wide variations (**Figure 7.8**) suggesting that these responses were strongly dependent on the selected independent factors. In case of drug content, the results seem to be unsystematic and random suggesting independency from the studied factors and therefore the variation might be explained as experimental errors.

7.4.3.2. Analysis of variance (ANOVA)

Analysis of variance (ANOVA) was performed to evaluate the significance of the quadratic models (linear, interactive and polynomial) on the responses and to establish quantitative values of their effects. **Table 7.7** summarises the effects of the model terms and associated p values for all five responses. At a 95% confident level, a model was considered significant if the p value <0.05. The results indicate that the disintegration time of the tablets was significantly affected only by alanine concentration in a linear way (X_1) and ranitidine HCl concentration in a polynomial way (X_2^2), whereas the rest of the model terms had no significant contribution in determining the disintegration time (p>0.05). Alanine concentration has a positive coefficient (+0.4304) suggesting that increasing the concentration of alanine increases the disintegration

Table 7.6 Full factorial design worksheet for ranitidine HCl study. The concentrations (%w/w) of ranitidine HCl and alanine are percentages of the mass of gum arabic dissolved in the stock solution.

Exp Name	Run Order	Ranitidine HCl (%)	Alanine (%)	Disintegration time (s)	Tg' (˚C)	Hardness (N)	Friability (%)	Drug recovery (%)
N1	1	10	20	5	-26.29	3.29	10.77	101.06
N2	22	40	20	4	-27.58	3.95	21.75	100.16
N3	26	10	60	4	-14.12	3.65	14.57	88.38
N4	7	40	60	5	-18.5	2.89	17.54	102.57
N5	11	10	40	5	-14.25	8.60	2.58	99.75
N6	20	40	40	5	-33.37	1.53	15.47	90.76
N7	13	25	20	6	-26.25	4.28	18.02	101.58
N8	9	25	60	8	-17.33	6.50	12.84	99.60
N9	4	25	40	7	-17.48	6.86	14.54	94.46
N10	12	10	20	5	-26.24	3.82	11.25	91.96
N11	17	40	20	3	-28.27	3.63	19.24	95.54
N12	18	10	60	5	-14.05	2.79	11.45	99.86
N13	19	40	60	5	-18.29	2.50	19.21	91.81
N14	2	10	40	5	-14.54	6.67	2.02	95.85
N15	6	40	40	4	-33.65	1.72	13.45	102.12
N16	14	25	20	4	-26.72	3.90	16.23	92.83
N17	24	25	60	6	-17.41	6.76	10.54	90.34
N18	10	25	40	6	-17.74	8.43	16.89	90.76
N19	15	10	20	5	-25.93	3.75	9.84	98.07
N20	8	40	20	4	-28.81	3.36	22.87	97.59
N21	3	10	60	4	-14.51	3.21	14.29	95.43
N22	23	40	60	5	-19.02	3.14	21.54	94.85
N23	21	10	40	5	-14.67	7.48	1.62	102.52
N24	25	40	40	5	-32.83	1.87	19.54	97.02
N25	27	25	20	5	-26.41	4.30	17.73	97.57
N26	16	25	60	8	-17.52	6.91	13.98	92.86
N27	5	25	40	5	-17.37	9.00	21.54	94.09

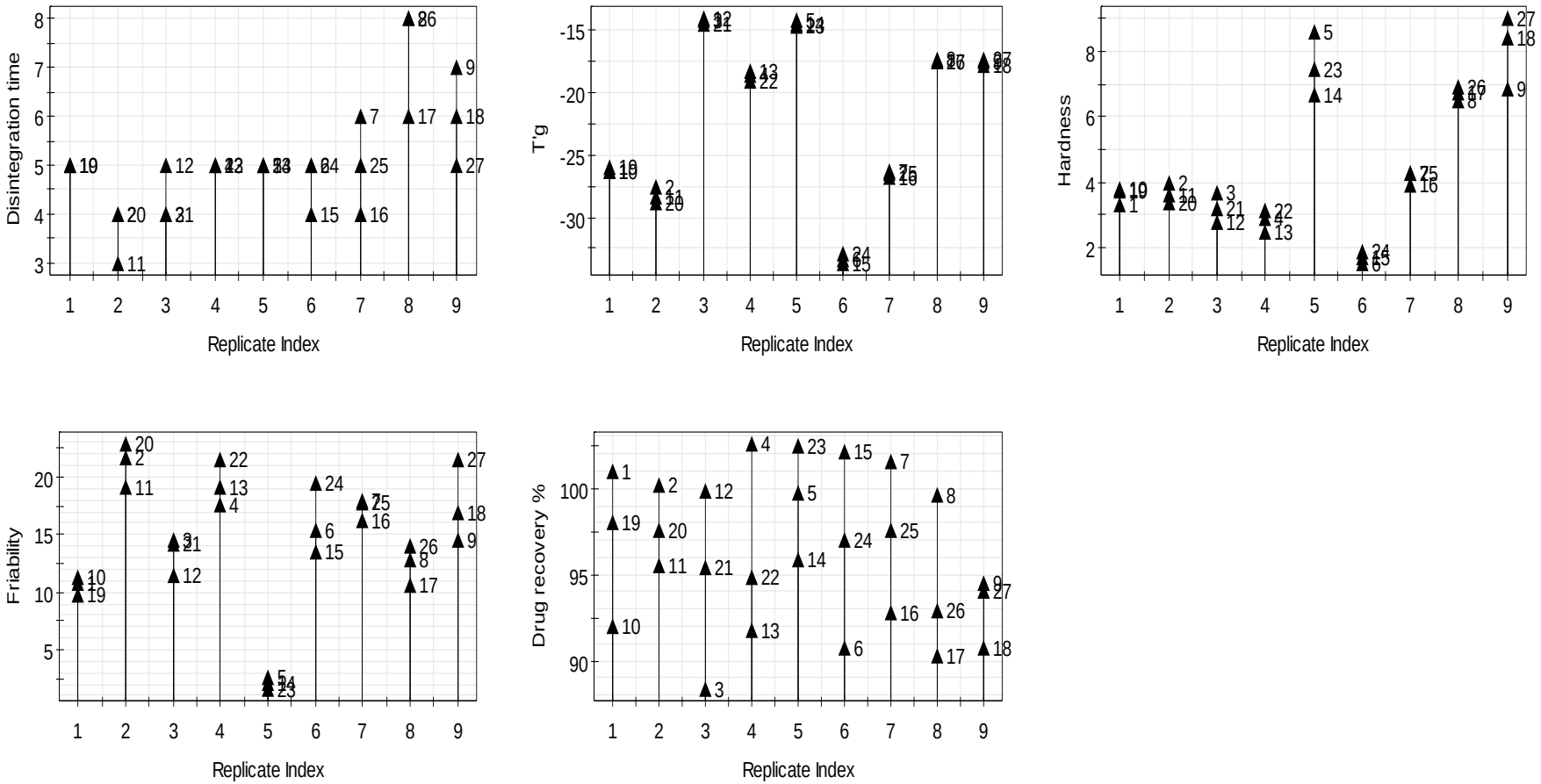


Figure 7.8 Replicate plots of the responses. The values of the response are plotted vs. experimental runs displaying the variation in the response for replicated experiments.

time. This is in line with our previous research studying the influence of alanine concentration on placebo lyophilised tablets (chapter 3), where a parabolic relation between disintegration time and alanine concentration between 20 and 40 % (w/w) was followed by steady increase at higher concentrations. However, the parabolic relation was not observed in this study as no alanine concentration was investigated between 20 and 40% (w/w) and therefore the polynomial terms of alanine concentration (X_1^2) showed no significant effect. On the other hand, negative coefficient of polynomial term for ranitidine HCl suggested that a very short disintegration time could be achieved at low (10%w/w) and high (40%w/w) concentrations of ranitidine HCl whereas the intermediate concentration (25%w/w) was associated with significant increase in the disintegration time. At low concentration of ranitidine HCl, the lyophilised tablets have higher porosity and consequently fast diffusion of the disintegrating medium and short disintegration time, whereas at high concentration, the presence of high concentration of highly soluble moiety (ranitidine HCl) might be the trigger for fast disintegration.

In case of Tg' of the formulation, the results showed that both alanine and ranitidine HCl had significant linear influences (X_1 and X_2, consequently) (**Table7. 7**). X_1 (alanine concentration) has a positive coefficient (+2.3154) suggesting that increasing alanine concentration significantly increases the Tg' value, which could be explained by the tendency of high concentrations of alanine to crystallise in the frozen formulation (**Figure 7.9**). X_2 (ranitidine HCl concentration) has a negative coefficient (-3.6864) suggesting that increasing ranitidine HCl

Table 7.7 The quantitative factor effects and associated p value for the responses.

Term	Y_1		Y_2		Y_3		Y_4		Y_5	
	Effect	P value	Effect	P value	Effect	P value	Effect	P value	Effect	P value
X_2	-0.1377	0.4053	-3.6864	0.0008	-0.7962	0.0371	4.2209	<0.0001	0.0453	0.9589
X_1	0.4304	0.0148	2.3154	0.0231	0.7809	0.0406	-1.0925	0.1496	-0.5833	0.5092
X_2^2	-1.0722	0.0002	-2.4891	0.0817	-1.3371	0.0170	-1.5877	0.1463	1.6103	0.2123
X_1^2	-0.1474	0.5351	-2.1090	0.1363	-0.5115	0.3325	2.1040	0.0417	0.1365	0.9142
X_1X_2	0.1161	0.4900	0.4633	0.6353	0.1807	0.6252	-0.0826	0.9127	-0.1651	0.8538

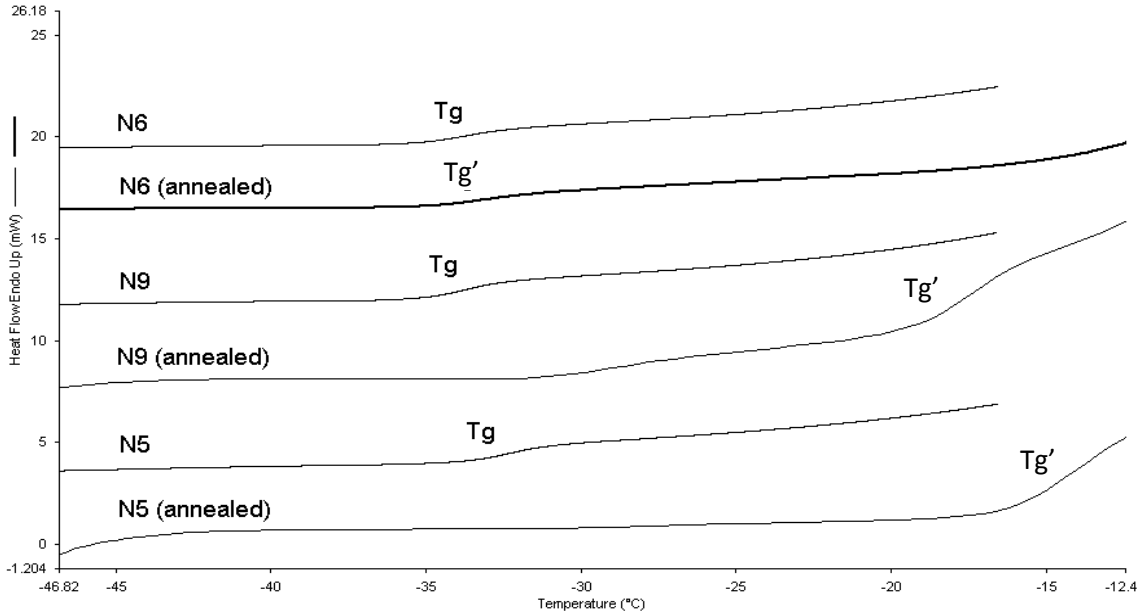


Figure 7.9 Overlaid DSC heating scans of frozen formulations based on high (N6), medium (N9) and low (N5) ranitidine HCl concentration with medium concentration of alanine.

concentration in the formulation significantly lowered the Tg' value. The large degree of negative influence of ranitidine HCl to reduce the Tg' of the formulation drastically could be attributed to its high aqueous solubility that retards or prevents crystallisation of alanine in the frozen formulation (**Figure 7.9**) and to a lesser extent, exhibits its own plasticising effect in the system. Moreover, the DSC results also confirmed the need for high concentrations of alanine (>40% w/w) to stabilise formulations (increase their collapse temperature) that contain high concentrations of ranitidine HCl (40% w/w).

For the third response Y_3 (hardness), ANOVA results suggested that increasing alanine concentration (X_1) in the formulation enhanced the hardness of the tablets significantly, as the linear terms X_1 showed a positive coefficient with p value >0.05 (**Table 7.7**). However, this enhancement was highly dependent on the incorporated concentration of ranitidine HCl, which was found to influence the hardness in linear (X_2) and polynomial (X_2^2) patterns. The linear term (X_2) had a negative coefficient suggesting that incorporating higher concentration of ranitidine HCl in the formulation decreased the hardness of the tablets. However, this behaviour was suspended at optimum concentration (around 25% w/w) as indicated by the large negative coefficient of the polynomial term (X_2^2) suggesting a higher value for hardness at this concentration (**Table 7.7**).

In term of friability, ranitidine concentration X_2 was identified as the most significant factor with p value < 0.0001. The large positive coefficient value (+4.2209) of this linear term (**Table 7.7**) indicated that a substantial deterioration in the friability (increasing the friability) was associated with increasing the concentration of ranitidine HCl in the formulation. Alanine on the other hand was able to protect the tablet against friability but only in a polynomial pattern (X_1^2), which suggested that an optimum concentration of alanine was required to achieve the lowest possible friability (around 40% w/w).

The results of the mechanical properties (hardness and friability) can be explained by the various factors that influence hardness of the tablet including intermolecular bonding forces and contact points between the excipients within the tablets (Adolfsson and Nyström, 1996). The improvement in the mechanical properties that were associated with increasing alanine concentration in the tablet might be a result of synergism in both factors; the intermolecular bonding force, possibly through initiating hydrogen bonds with the binder (gum Arabic) as both contain hydrogen bond donors and acceptors, and contact points between the excipients, as a result of decreasing the porosity.

ANOVA results (**Table 7.7**) indicated that all the model terms including linear, interactive and polynomial, had no significant influences on the drug content ($p>0.05$) suggesting that the results were randomly distributed and the variation was due to experimental errors in formulation and/or analysis.

7.4.3.3. Revised model and surface response plots

After analysing the influence of all the quadratic model terms on the dependent variables (responses), the insignificant terms were omitted to generate a revised model that included model terms which have significant influence. The resulting equations for all five responses, Y_1 (disintegration time), Y_2 (Tg'), Y_3 (hardness), Y_4 (friability) and Y_5 (drug content), are presented below:

$$Y_1 = +6.2855 + 0.4304 X_1 - 1.0722 X_2^2$$

$$Y_2 = -17.3926 + 2.3154 X_1 - 3.6864 X_2$$

$$Y_3 = +6.4020 + 0.7809 X_1 - 0.7962 X_2 - 1.3371 X_2^2$$

$Y_4 = +13.9958 + 4.2209\ X_2 + 2.1040\ X_1^2$

$Y_5 = 94.5923$

The results of testing the validity of the model are summarised in Table **7.8**. P values for all the simulated responses were below the significant level (<0.05) suggesting that all the revised models were significant in predicting their response values. The high value of correlation coefficient (R^2) for Tg', hardness and friability indicated a good fit to the revised model. Lower correlation coefficient was obtained for the disintegration time possibly due to the qualitative nature of the test that depends on the visual evaluation in addition to the fact that few seconds' inaccuracy in evaluating the disintegration time can cause huge error.

Based on the revised equations, the software generated response surface graphs that simulate the influence of the independent factors on each response individually for each factor including disintegration time Tg', hardness and friability are presented in **Figures 7.10**, **7.11**, **7.12** and **7.13**, respectively.

Table 7.8 Summary of results for testing validity of the revised models. DF indicates: degrees of freedom; SS: sum of squares; MS: mean of square; F: Fischer's ratio; p: probability; R^2: regression coefficient.

Disintegration time						
	DF	SS	MS (variance)	F	p	R^2
Regression	5	21.6666	4.3333	6.9999	0.001	0.625
Lack of Fit	3	4.3333	1.4444	3		

Tg'						
	DF	SS	MS (variance)	F	p	R^2
Regression	5	814.856	162.971	11.2691	<0.0001	0.782
Lack of Fit	3	301.812	100.604	960.103		

Hardness						
	DF	SS	MS (variance)	F	p	R^2
Regression	5	78.2386	15.6477	6.5128	0.001	0.688
Lack of Fit	3	44.9559	14.9853	49.0529		

Friability						
	DF	SS	MS (variance)	F	p	R^2
Regression	5	606.545	121.309	9.5754	<0.0001	0.695
Lack of Fit	3	190.996	63.6655	15.2698		

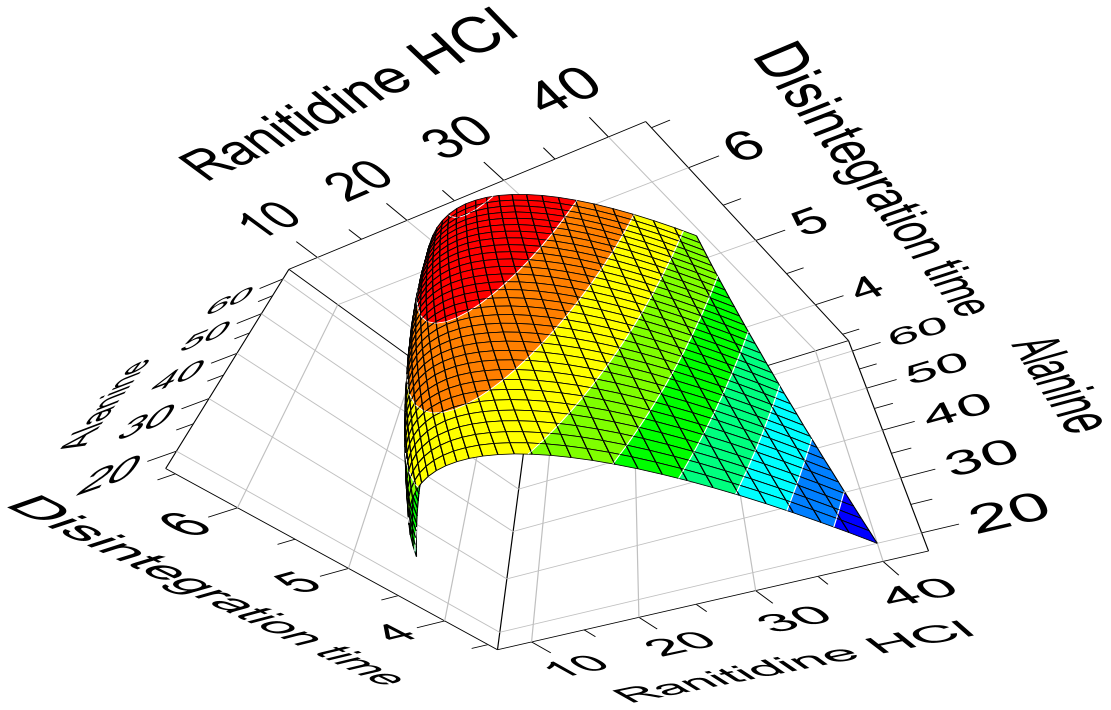


Figure 7.10 Surface response plot showing the influence of varying alanine and ranitidine HCl concentration on the disintegration time of the ODT.

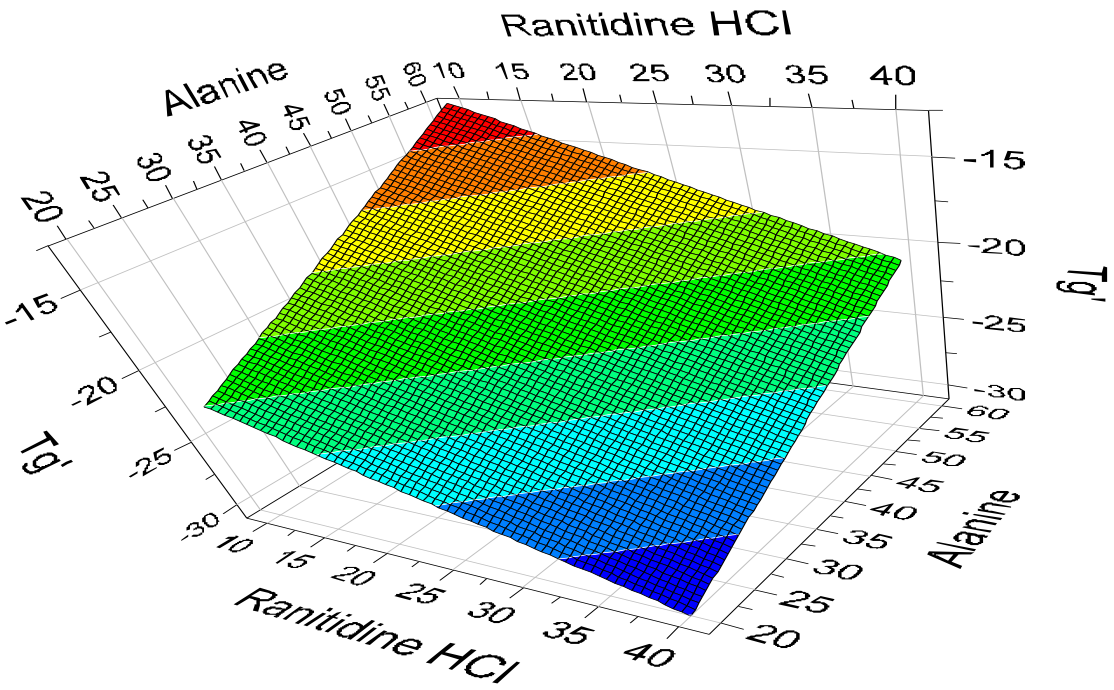


Figure 7.11 Surface response plot showing the influence of varying alanine and ranitidine HCl concentration on the Tg'.

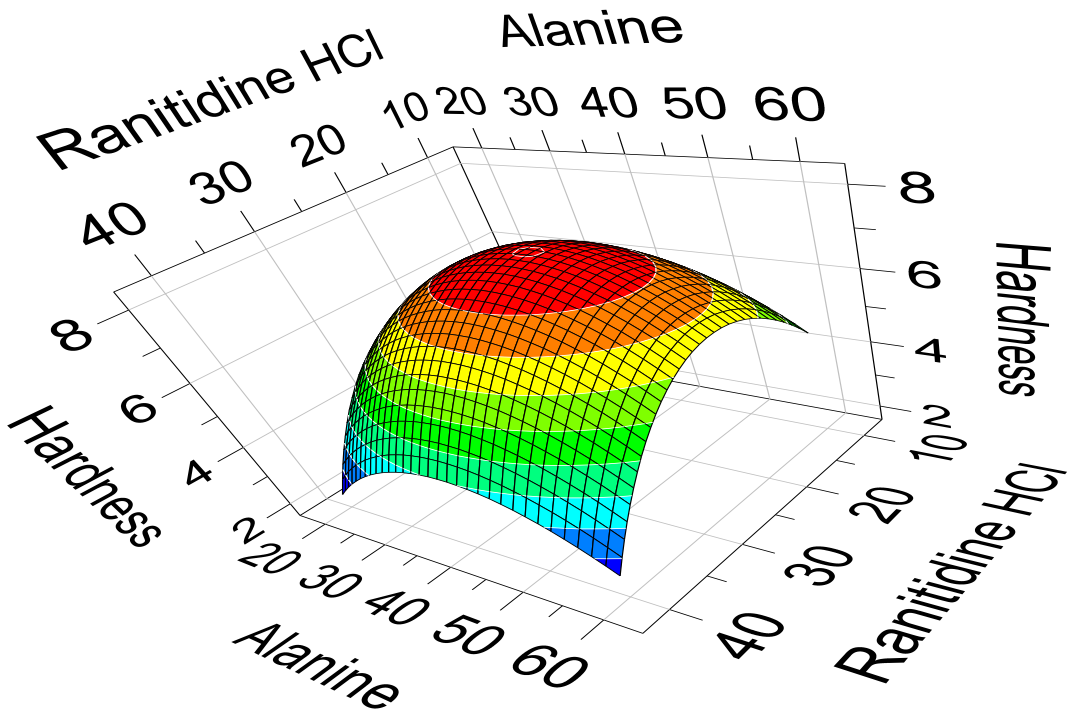


Figure 7.12 Surface response plot showing the influence of varying alanine and ranitidine HCl concentration on the hardness of the ODT.

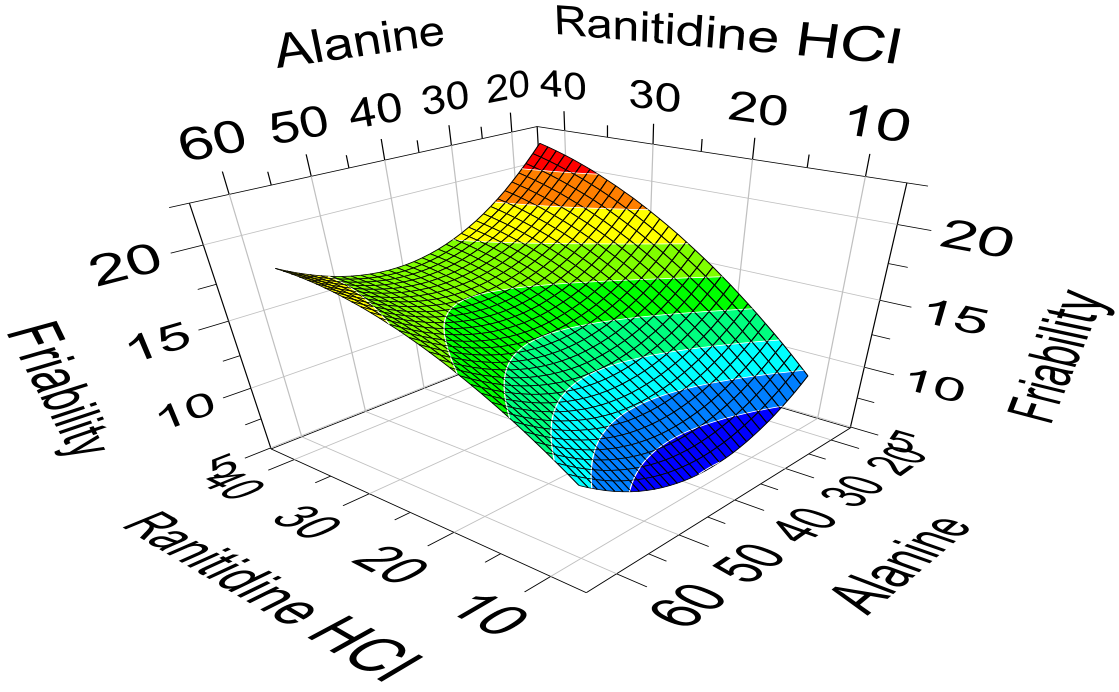


Figure 7.13 Surface response plot showing the influence of varying alanine and ranitidine HCl concentration on the friability of the ODT.

7.4.3.4. Optimum ODTs formulation

Based on the response surface plots, the software was used to perform hot spot analysis to obtain optimum formulation variables (alanine and ranitidine HCl concentrations) to produce ODTs with short disintegration time, high Tg', high hardness and low friability. The optimal formulation was determined as 40% (w/w) alanine and 15 % (w/w) ranitidine HCl. The observed response values of the optimised formulation compared to the predicted values are presented in **Table 7.9**. The closeness of the actual (observed) and calculated (predicted) values verified the established statistical models experimentally.

7.4.3.5. Formulation of 75 mg ranitidine HCl lyophilised ODTs

Based on the optimised formulation, lyophilised ODTs containing 75 mg dose of ranitidine HCl were prepared using 20 mm diameter mould. Characterisation summary of the tablets is presented in **Table 7.10**. As expected, the tablets showed instant disintegration using the USP apparatus (less than 4 seconds) without leaving any lumps or gritty particles in the disintegration vessel. The mechanical properties of the tablets suggest the need for specialised packaging to withstand possible external mechanical stresses during storage and handling by patients.

Table 7.9 Observed and predicted (from the revised model) responses and residual values for the optimised formulation. The observed results are means, n=3.

Response	Observed	Predicted	Residual
Disintegration time (s)	4.00	5.50	-1.50
Tg' (˚C)	-14.87	-15.72	0.85
Hardness (N)	7.09	6.13	0.96
Friability (%)	3.85	8.55	-4.70
Drug content (%)	96.57	95.51	1.06

Table 7.10 Characterisation of the prepared lyophilised ODTs after 0 and 3 months at 40 ˚C and 75% RH. Results are means ± SD, n=3.

Parameters	Time interval (months)	
	0	3
Moisture content (%)	1.85 ± 0.51	2.21 ± 0.42
Disintegration time (s)	5 ± 1	6 ± 1
Hardness (N)	7.41 ± 0.70	6.88 ± 0.52
Friability (%)	4.45 ± 2.75	2.98 ± 1.83
Drug content (%)	97.24 ± 2.93	96.18 ± 4.24

7.4.3.6. Dissolution studies

The dissolution profiles of the prepared lyophilised tablets and commercial compressed tablets (Zantac™ Relief) that contain 75 mg ranitidine HCl are presented in **Figure 7.14**. According to the USP, not less than 80% of the drug should dissolve within 45 min under the prescribed dissolution conditions. The results showed that both products satisfied the USP criteria with complete dissolution in less than 45 min. However, the lyophilised tablets showed faster dissolution rate with 100% drug release in about 5 min compared to 20 min for the compressed tablets (**Figure 7.14**) Due to the high solubility of ranitidine HCl in the aqueous media, the slow dissolution rate of the compressed tablets could be attributed to their slow disintegration (about 13 min) compared to instant disintegration of the lyophilised tablet.

7.4.3.7. Stability studies

Short term stability studies of the lyophilised ODTs of ranitidine HCl are summarised in **Table 7.10**. The results indicated no significant change in appearance, moisture content, disintegration time, drug content and mechanical properties, suggesting that the formulation was chemically and physically stable.

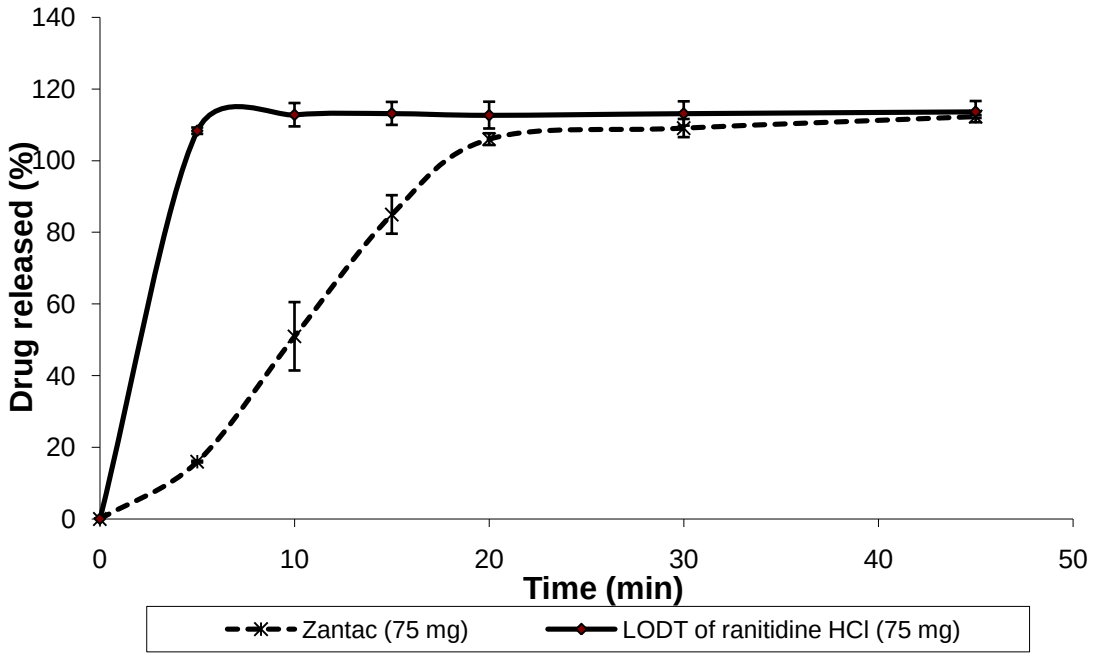


Figure 7.14 Dissolution profiles of commercially available compressed tablets (Zantac 75mg) and formulated lyophilised orally disintegrating tablets (LODT) of ranitidine HCl. Results are means ± SD, n=3.

7.4.4. ODTs of ibuprofen

7.4.4.1. Experimental design

To optimise the properties of lyophilised ODTs containing 200 mg of ibuprofen, three concentration levels of drug, low (10% w/w) medium (25% w/w) and high (40% w/w), and three concentration levels of alanine, low (20% w/w) medium (30% w/w) and high (40% w/w), were used in the factorial design experiment. These concentrations of ibuprofen were designed to allow the production of lyophilised ODTs in tablet size ranges comparable to the standard tablet sizes. The designed dimensions (diameter × thickness) of lyophilised tablets containing 200 mg ibuprofen are 20.00mm × 36.00 mm, 20.00 mm × 14.44 mm and 20.00 mm ×9.00 mm for 10, 25 and 40% (w/w) formulations, respectively.

Based on a 3^2 randomised full factorial design, 9 formulations were prepared in triplicates (27 experiments (9*3) in total). The design and results from the 27 experiments are presented in **Table 7.11**.

Table 7.11 Full factorial design worksheet for ibuprofen study. The concentrations (%w/w) of ibuprofen and alanine are percentages of the mass of gum arabic dissolved in the stock solution.

Exp Name	Run Order	Ibuprofen (%)	Alanine (%)	Disintegration time	Tg'	Hardness	Friability	Drug recovery %
N1	22	10	20	3	-25.27	1.87	13.84	108.00
N2	9	40	20	5	-25.19	3.60	13.12	98.037
N3	13	10	40	9	-15.91	3.62	18.55	101.15
N4	12	40	40	16	-15.90	3.93	14.41	99.38
N5	24	10	30	7	-16.13	3.51	25.19	103.55
N6	14	40	30	4	-16.28	3.33	32.15	103.62
N7	3	25	20	11	-25.22	3.11	27.42	102.35
N8	6	25	40	8	-15.54	3.56	20.68	106.12
N9	21	25	30	10	-17.06	2.60	30.47	103.17
N10	20	10	20	3	-25.18	2.32	11.92	107.15
N11	11	40	20	6	-25.28	4.37	10.38	95.91
N12	23	10	40	12	-15.73	3.01	19.54	100.01
N13	10	40	40	18	-15.50	4.92	13.27	99.38
N14	5	10	30	6	-16.08	3.53	23.66	104.69
N15	19	40	30	4	-16.17	3.11	33.47	103.32
N16	27	25	20	12	-24.99	2.52	25.45	103.64
N17	16	25	40	7	-15.28	3.70	18.94	102.47
N18	4	25	30	9	-16.94	2.32	31.61	105.25
N19	18	10	20	4	-24.52	1.98	14.39	103.61
N20	8	40	20	6	-25.37	3.23	8.75	100.08
N21	25	10	40	10	-15.51	2.86	16.31	103.55
N22	1	40	40	12	-15.69	3.94	17.00	101.06
N23	7	10	30	6	-15.99	3.01	25.19	101.42
N24	17	40	30	5	-16.61	3.87	32.15	102.76
N25	2	25	20	12	-25.43	3.16	27.42	102.18
N26	26	25	40	9	-15.42	4.27	20.68	105.07
N27	15	25	30	7	-17.22	2.11	29.39	104.96

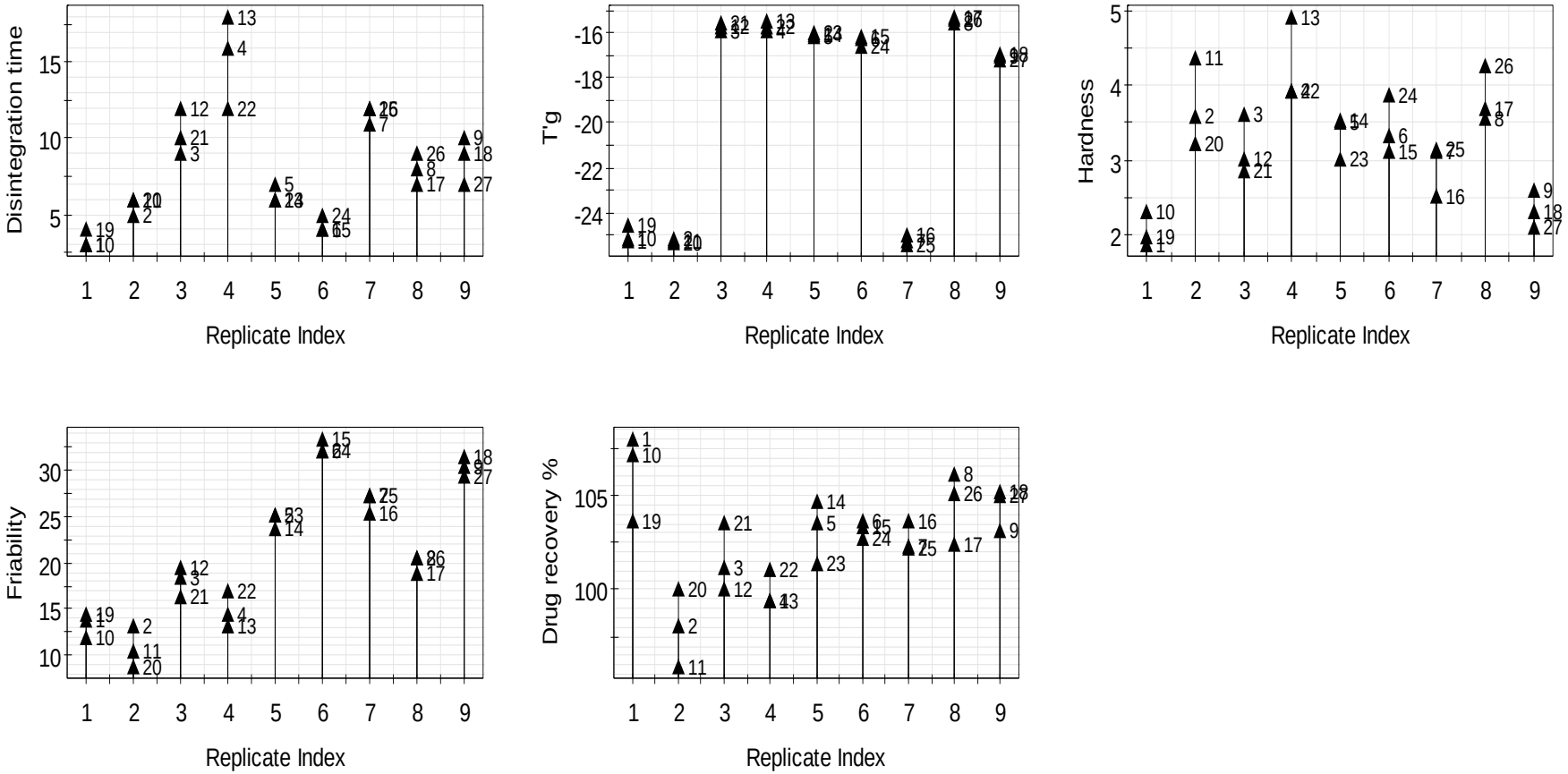


Figure 7.15 Replicate plots of the responses. The values of the response are plotted vs. experimental runs displaying the variation in the response for replicated experiments.

The results (**Table 7.11**) showed that the disintegration time of the tablets varied from 3 to 18 s, the Tg' of the formulation was between -25.43 to -15.28 ˚C, the hardness varied from 1.87 to 4.92 N, friability from 8.75 to 33.47 % and drug content from 95.91 to 108.00 %. The variation and repeatability (**Figure 7.15**) of the disintegration time, Tg', hardness and friability of the formulations suggest that these responses were considerably dependent on the selected independent factors. In case of drug content, the results seem to be unsystematic and random possibly due to experimental errors.

7.4.4.2. Analysis of variance (ANOVA)

Analysis of variance (ANOVA) was performed to evaluate the significance of the quadratic models (linear, interactive and polynomial) on the responses and to estimate their quantitative effects. **Table 7.12** summarises the effects of the model terms and associated p values for all five responses. At a 95% confidence level, a model was considered significant if the p value <0.05. The results indicate that the disintegration time of the tablets was significantly affected by the linear, polynomial models of alanine concentration(X_1 and X_1^2, respectively) and the polynomial model of ibuprofen concentration (X_2^2). The positive coefficients of both models for alanine concentration suggested a parabolic relationship with the disintegration time, which is typical for such disintegrating enhancing agent as a balance between porosity and wettability properties of the lyophilised tablets achieves the shortest disintegration time (chapter 3). Increasing alanine concentration in the formulation decreases the porosity of the tablets but, at the same time, enhances the wettability suggesting the need for concentration optimisation to achieve fast disintegration of the tablets (chapter 3).

Ibuprofen concentration showed significant influence on the disintegration time only through its polynomial model (X_2^2). The negative coefficient of X_2^2 might suggest that incorporation of ibuprofen in the formulation retards the disintegration only at intermediate concentration at around 30% w/w (**Table 7.11**), whereas incorporation of low and high concentration promotes disintegration. This could be explained as incorporation of low concentration of ibuprofen (20% w/w) had minimum influence on tablets' properties that control the disintegration (wettability and porosity) and therefore the disintegration seemed to be controlled only by alanine concentration. Incorporation of higher concentration (30% w/w) decreased the porosity and increased the hydrophobicity inside the tablets which together resulted in an increase in disintegration time. However, increasing the concentration of ibuprofen to 40%

Table 7.12 The quantitative factor effects and associated p value for the responses.

Term	Y_1		Y_2		Y_3		Y_4		Y_5	
	Effect	P value	Effect	P value	Effect	P value	Effect	P value	Effect	P value
X_2	0.7977	0.1169	-0.0193	0.7836	0.3628	0.0031	0.2799	0.7292	-1.4650	0.0611
X_1	2.3439	<0.0001	3.9605	<0.0001	0.3616	0.0032	0.3098	0.7017	-0.1048	0.7894
X_2^2	-2.1855	0.0053	0.1947	0.0656	0.1775	0.2700	-4.6493	0.0006	-1.3719	0.1228
X_1^2	2.2438	0.0044	-2.7165	<0.0001	0.2146	0.1854	-8.2485	<0.0001	-0.9698	0.0971
X_1X_2	0.5714	0.2632	0.1631	0.0617	-0.1646	0.1522	-0.1098	0.8938	0.9610	0.0840

(w/w) showed faster disintegration, possibly due to the emergence of additional repulsive forces between the hydrophobic (ibuprofen) and hydrophilic (alanine) molecules inside the tablets that promote disintegration (Guyot-Hermann and Ringard, 1981).

The Tg' of the formulation was significantly influenced by the linear (X_1) and polynomial (X_1^2) models of alanine concentration. X_1 (alanine concentration) has a large positive coefficient (3.9618) suggesting that increasing alanine concentration drastically increases the Tg' value of the formulation, which could be explained by the tendency of high concentrations of alanine to crystallise in the frozen formulation (**Figure 7.16**). The polynomial terms X_1^2 (alanine concentration) has a negative large coefficient (-2.7165) which can be attributed to the inability of alanine to crystallise at low concentration and consequently resulting in plasticising the system (lower Tg'). On the other hand, both models of ibuprofen (linear and polynomial) and its interactive model with alanine (X_1X_2) showed no significant effect on the Tg', possibly due to the very low aqueous solubility of ibuprofen.

For the third response Y_3 (hardness), the significant independent factors were identified as the linear terms of alanine concentration(X_1) and ibuprofen concentration(X_2) with a respective p value of 0.0031and 0.0025 (**Table 7.12**). Both models had positive coefficient suggesting that increasing the concentration of alanine and/or ibuprofen in the formulation enhanced the hardness of the tablets. The results can be explained by the various factors that influence the hardness of the tablet including intermolecular bonding force and contact points between the excipients within the tablets. The high degree of improvement in the hardness associated with increasing alanine concentration in the tablet might be a result of additive effect of both

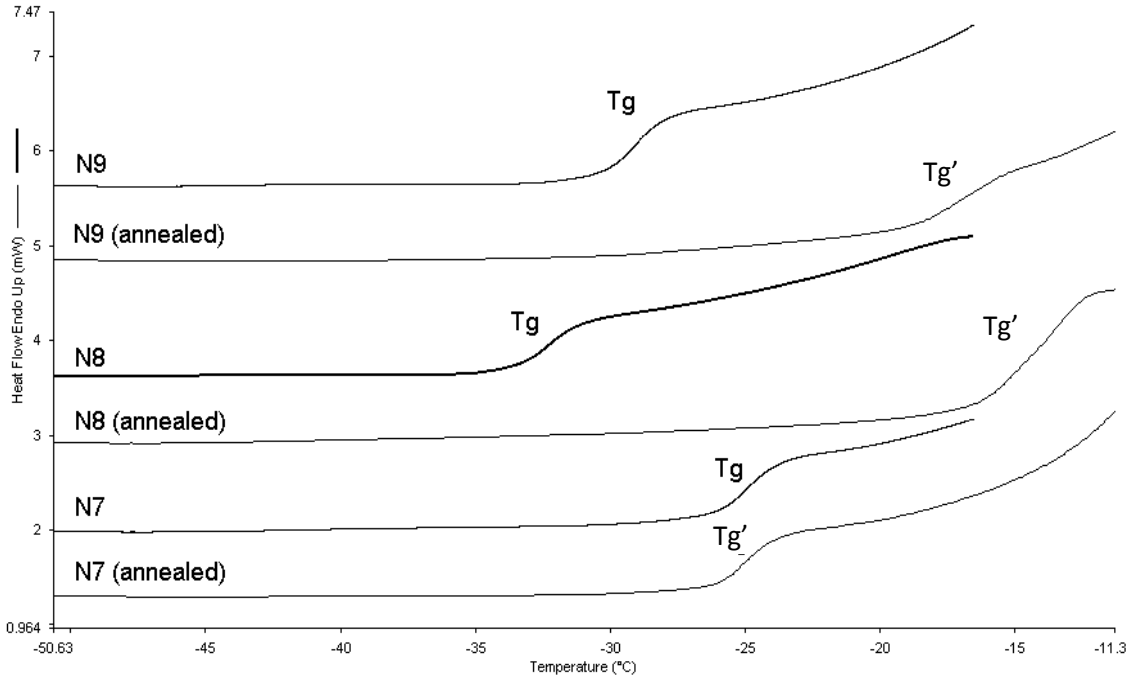


Figure 7.16 Overlaid DSC heating scans of frozen formulations based on high (N8), medium (N9) and low (N7) alanine concentrations with medium concentration of ibuprofen.

factors; intermolecular bonding force, possibly through hydrogen bonds with the binder (gum arabic) as both contain hydrogen bond donors and acceptors, and contact points between the excipients, as a result of decrease in the porosity (chapter 3). However increasing ibuprofen concentration improved the hardness possibly as a result of increase in the contact points between the excipients.

In terms of friability of the tablets, only the polynomial model of alanine (X_1^2) and ibuprofen (X_2^2) concentrations showed significant influence with p value <0.0001and 0.0006, respectively (**Table 7.12**). The high level of significant influence and the large negative coefficient (-8.2485) of X_1^2 (alanine concentration) suggested that optimising alanine concentration was the most effective way to reduce the friability of the tablets. However, this protection against friability was not linear (polynomial) as a consequence of high friability values that were recorded for tablets with intermediate concentration of alanine (30% w/w). Similarly, ibuprofen concentration influenced the friability in a negative polynomial way, as tablets with low (20%) and high (40%) concentration had lower friability than tablets containing intermediate concentration (30%).

ANOVA results (**Table 7.12**) indicated that all alanine and ibuprofen concentration models including linear, interactive and polynomial, had no significant influence on the drug content ($p>0.05$) suggesting that the results were randomly distributed and the variation was possibly due to experimental errors in formulation and/or detection.

7.4.4.3. Revised model and surface response plots

After analysing the influence of all the quadratic model terms on the dependent variables (responses), the insignificant terms were omitted to generate a revised model that included model terms with significant influence. The resulting equations for all five responses, Y_1 (disintegration time), Y_2 (Tg'), Y_3 (hardness), Y_4 (friability) and Y_5 (drug content), are presented below:

$$Y_1 = + 8.5735 + 2.3440\ X_1 + 2.2438\ X_1^2 - 2.1855\ X_2^2$$

$$Y_2 = - 16.6609 + 3.9605\ X_1 - 2.7165\ X_1^2$$

$$Y_3 = + 2.8580 + 0.3616\ X_1 + 0.3628\ X_2$$

$$Y_4 = + 33.7294 - 8.2485\ X_1^2 + -4.6493\ X_2^2$$

$$Y_5 = + 104.9170$$

The results for testing the validity of the model are summarised in table 13. P values for all the simulated responses were below the significant level (<0.05) suggesting that all the revised models were significant in predicting their response values. The high value of correlation coefficient (R^2) for the disintegration time, Tg' and friability indicate a good fit to the revised model.

Table 7.13 Summary of results for testing validity of the revised models. DF indicates: degrees of freedom; SS: sum of squares; MS: mean of square; F: Fischer's ratio; p: probability; $\mathbf{R^2}$: regression coefficient.

Disintegration time						
	DF	SS	MS (variance)	F	p	R^2
Regression	5	290.386	58.077	9.3882	<0.001	0.691
Lack of Fit	3	96.577	32.192	17.3838		

Tg'						
	DF	SS	MS (variance)	F	p	R^2
Regression	5	501.347	100.269	797.576	<0.001	0.995
Lack of Fit	3	1.84301	0.614335	13.8734		

Hardness						
	DF	SS	MS (variance)	F	p	R^2
Regression	5	8.51981	1.70396	5.58357	0.002	0.571
Lack of Fit	3	3.51459	1.17153	7.28648		

Friability						
	DF	SS	MS (variance)	F	p	R^2
Regression	5	1127.16	225.432	13.6202	<0.001	0.764
Lack of Fit	3	311.884	103.961	52.427		

Based on the revised equations, the software was used to generate response surface graphs that simulated the influence of the independent factors on each response individually. The graphs for disintegration time Tg', hardness and friability are presented in **Figures 7.17**, **7.18**, **7.19** and **7.20**, respectively.

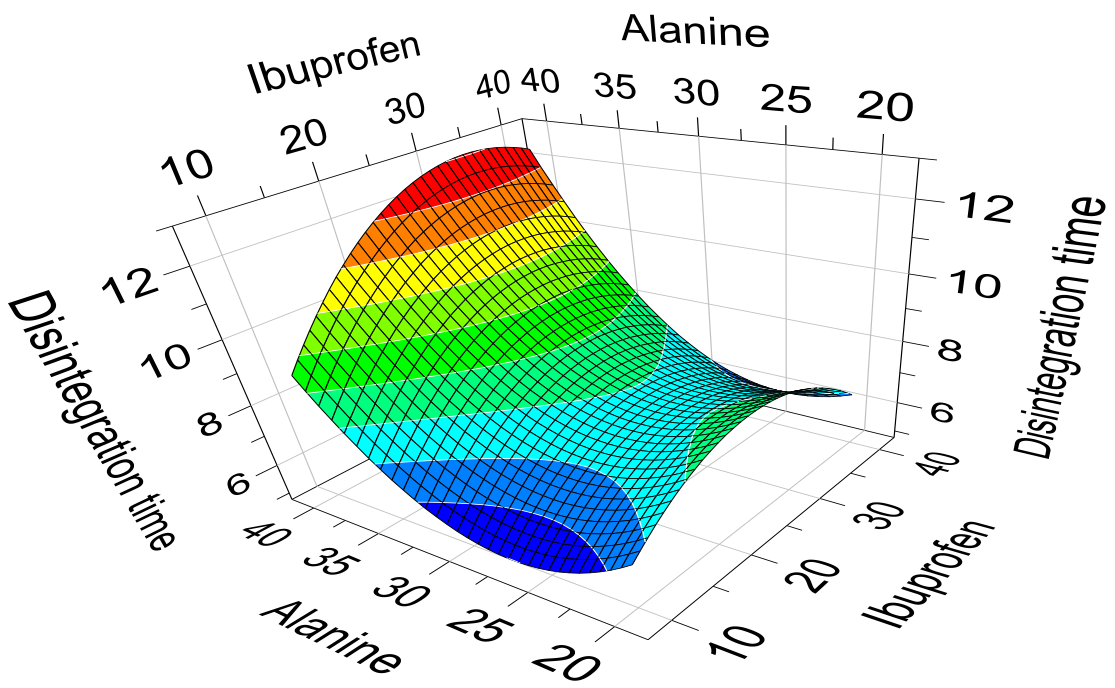


Figure 7.17 Surface response plot showing the influence of varying alanine and ibuprofen concentration on the disintegration time of the ODT.

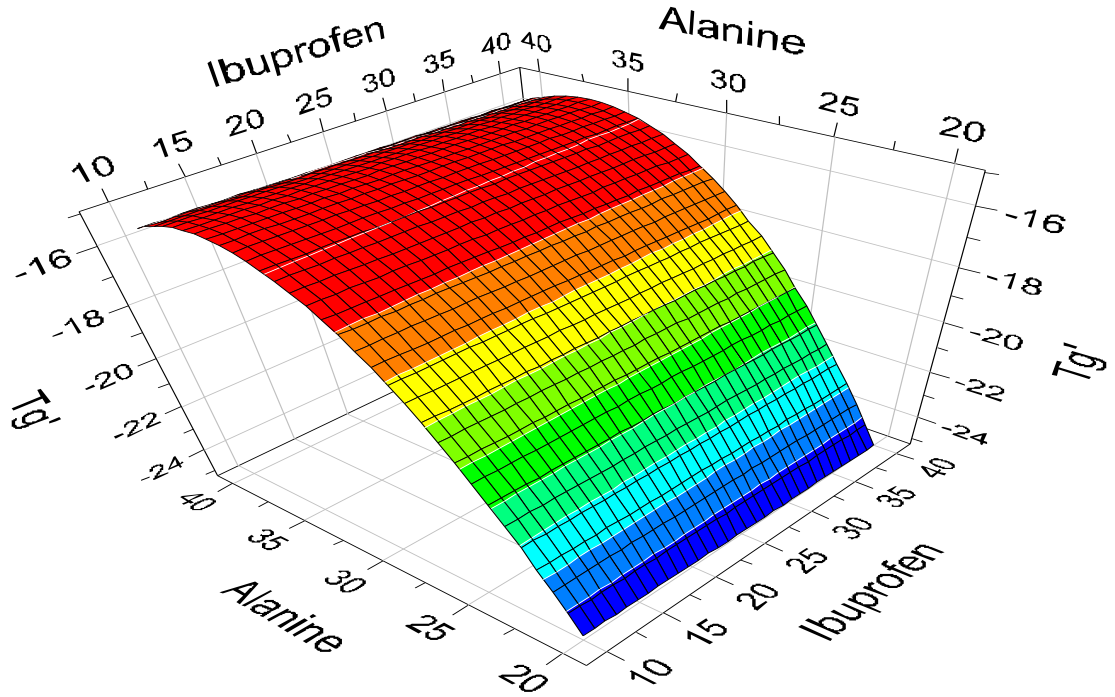


Figure 7.18 Surface response plot showing the influence of varying alanine and ibuprofen concentration on the Tg'.

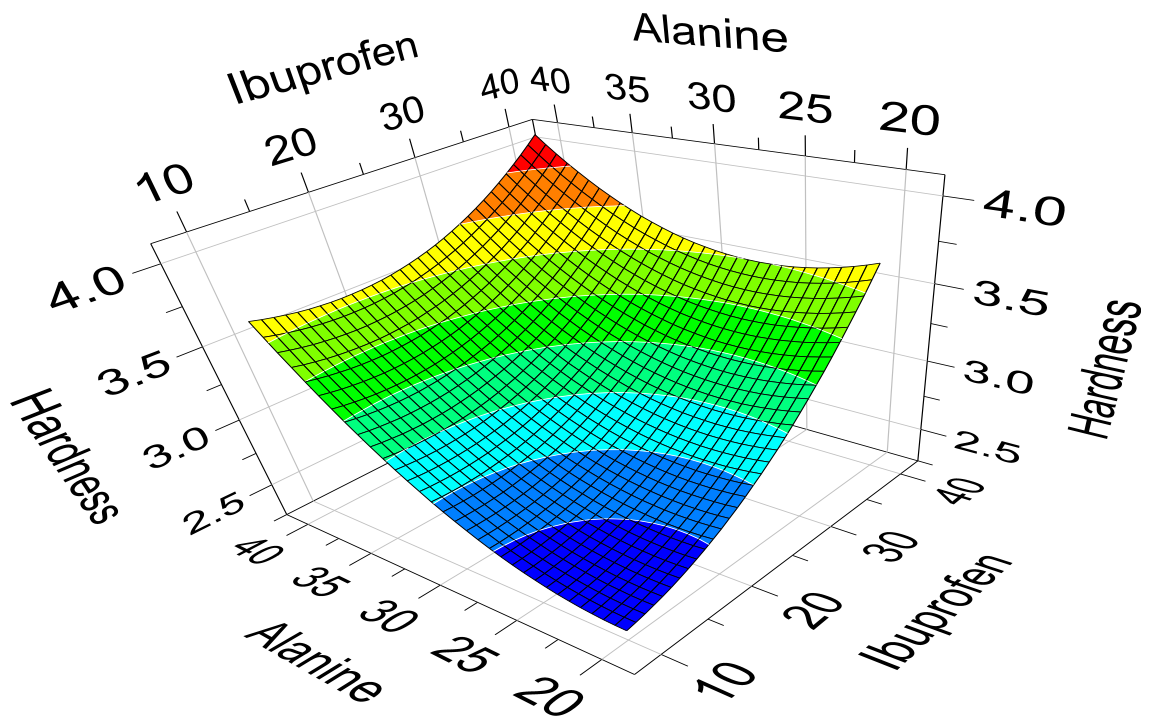


Figure 7.19 Surface response plot showing the influence of varying alanine and ibuprofen concentration on the hardness of the ODT.

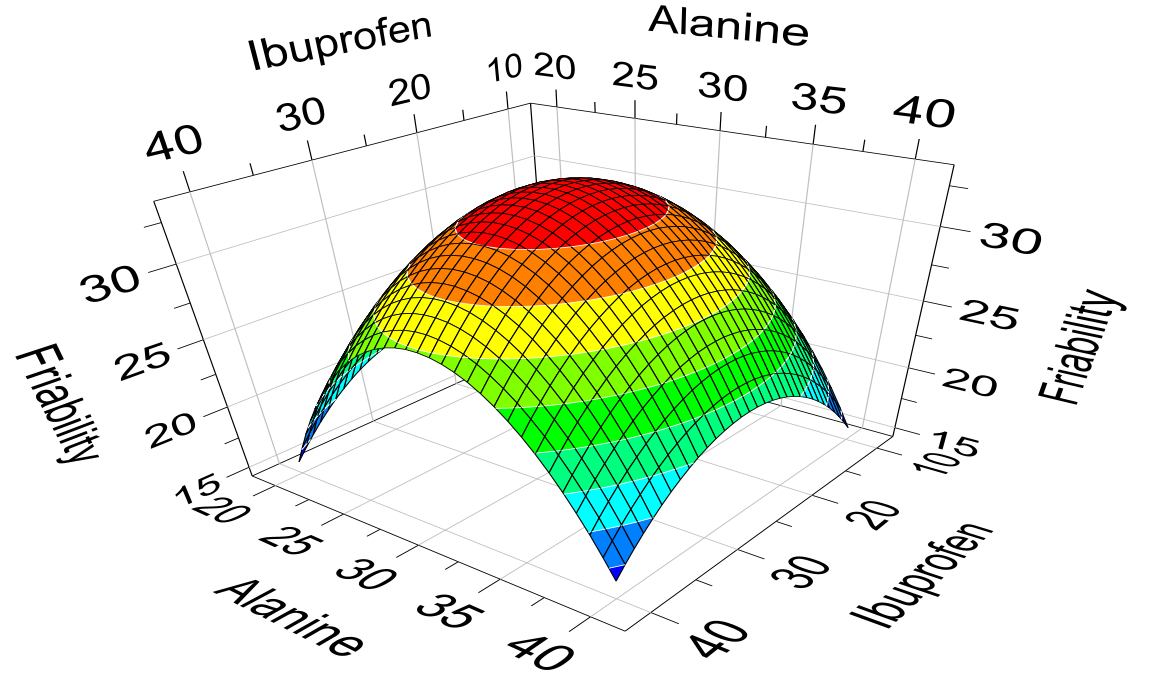


Figure 7.20 Surface response plot showing the influence of varying alanine and ibuprofen concentration on the friability of the ODT.

7.4.4.4. Optimum ODTs formulation

Based on the response surface plots, the software was used to perform hot spot analysis to find the optimum formulation variables (alanine and ibuprofen concentrations) to produce ODTs with optimum characteristics such as short disintegration time, high Tg', high hardness and low friability. The optimal formulation was determined as 40% (w/w) alanine and 40 % (w/w) ibuprofen concentration. The observed values of the responses of the optimised formulation compared to the predicted values are presented in **Table 7.14**. The characterisation results verified, experimentally, the established statistical models, as only small differences were observed between the actual (observed) and calculated (predicted) values.

7.4.4.5. Formulation of 200 mg ibuprofen lyophilised ODTs

Based on the optimised formulation, lyophilised ODTs containing 200 mg dose of ibuprofen were prepared using 18 mm diameter mould. The characterisation summary of the tablets is presented in **Table 7.15**. As expected, the tablets showed instant disintegration using the USP apparatus (less than 16 seconds) without leaving any lumps or gritty particles in the disintegration vessel. The average thickness of the dried tablets was 9.12 mm (SD=0.27, n=6).

Table 7.14 Observed and predicted (from the revised model) responses and residual values for the optimised formulation. The observed results are means, n=3.

Response	Observed	Predicted	Residual
Disintegration time (s)	15	13	2
Tg' (˚C)	-15.64	-15.33	-0.31
Hardness (N)	5.01	4.05	0.96
Friability (%)	12.94	15.79	-2.85
Drug content (%)	102.37	104.92	-2.55

Table 7.15 Characterisation of the prepared lyophilised ODTs after 0 and 3 months at 40 ˚C and 75% RH. Results are means ± SD, n=3.

Parameters	Time interval (months)	
	0	3
Moisture content (%)	1.87 ± 0.42	2.27 ± 0.39
Disintegration time (s)	16 ± 3	17 ± 3
Hardness (N)	5.57 ± 0.61	5.08 ± 0.52
Friability (%)	13.94 ± 3.34	11.82 ± 2.57
Drug content (%)	97.54 ± 2.32	101.12 ± 3.05

7.4.4.6. Dissolution studies

The dissolution profiles of the prepared lyophilised tablets and commercial compressed ODTS (Nurofen Meltlets) that contain 200 mg ibuprofen are presented in **Figure 7.21**. According to the US Pharmacopeia, not less than 70% of the dose should dissolve within 120 min under the prescribed dissolution conditions. The results showed that both products satisfied the USP criteria as the required 70% release was reached in less than 10 min, which could be attributed to their fast disintegration in the dissolution medium. However, the lyophilised tablets showed faster dissolution rate with around 90% drug release after 5 min compared to around 50% in case of compressed ODTs (**Figure 7.21**). The quicker disintegration of the lyophilised tablets could not fully explain their faster dissolution rate, as the difference was only about 30 seconds. Therefore, the results might be attributed to the presence of gum Arabic in the lyophilised tablets which has intrinsic emulsifying properties that can enhance the extent and rate of dissolution of hydrophobic drugs (Yadav et al., 2007).

7.4.4.7. Stability studies

The results from short term stability studies of the lyophilised ODTs are summarised in **Table 7.15**. The results indicated that no significant change in appearance, moisture content, disintegration time, drug content and mechanical properties was observed, which may suggests that the formulation were chemically and physically stable.

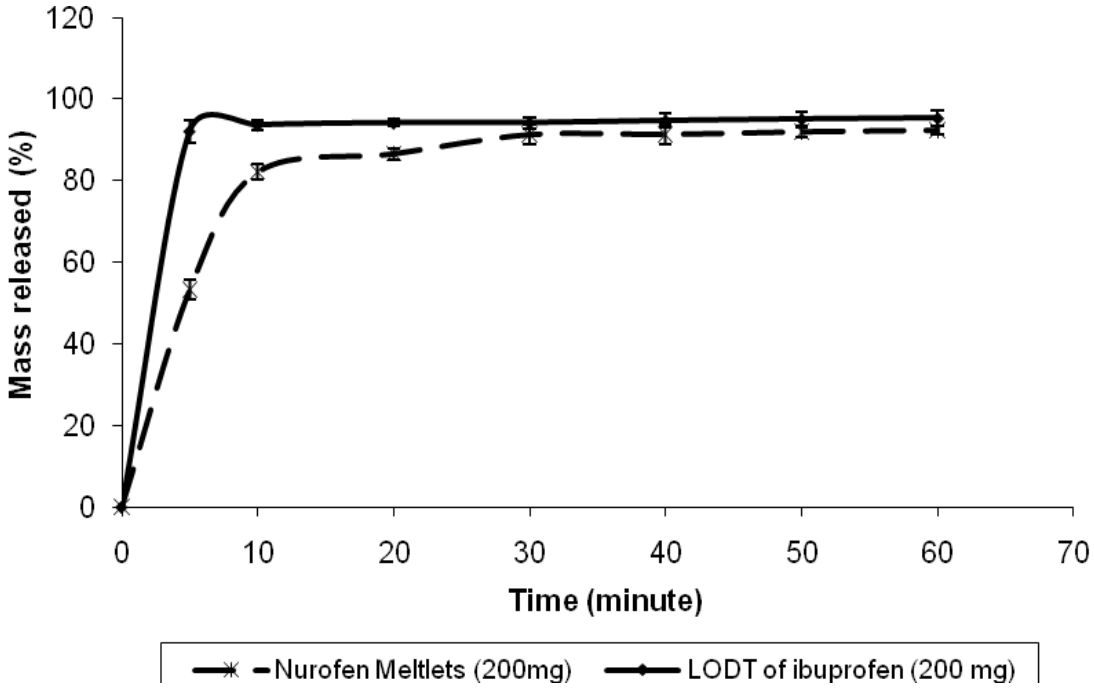


Figure 7.21 Dissolution profiles of commercially available compressed ODTs (Nurofen Meltlets) and formulated lyophilised orally disintegrating tablets (LODT) of ibuprofen. Results are means ± SD, n=3.

7.4.5. ODTs of loperamide HCl

7.4.5.1. Experimental design

To optimise the properties of lyophilised ODTs containing 2 mg of loperamide HCl , three concentration levels of the drug, low (1 %w/w) medium (2 %w/w) and high (3 %w/w), and three concentration levels of alanine, low (20 %w/w) medium (30 %w/w) and high (40 %w/w), were used in the factorial design experiment. These concentrations of loperamide HCl were designed to allow the production of lyophilised ODTs in tablet with sizes comparable to the standard tablets. The designed dimensions (diameter × thickness) of lyophilised tablets containing 2 mg loperamide HCl are 13.50mm × 7.91 mm, 13.50 mm × 3.96 mm and 13.50 mm × 2.64 mm for 1, 2 and 3% (w/w) formulations, respectively.

Based on a 3^2 randomised full factorial design, 9 formulations were prepared in triplicates (27 experiments (9*3) in total). The design and the results from the 27 experiments are presented in **Table 7.16**. The results showed that the disintegration time of the tablets was between 3 to 6 s, the Tg' of the formulation was between -25.04 to -13.06 ˚C, the hardness varied from 1.71 to 5.52 N, friability from 7.32 to 26.80 % and drug content from 94.10 to 109.76 %. The variation and repeatability (**Figure 7.22**) of the Tg', hardness and friability of the formulations suggest that these responses were considerably dependent on the selected independent factors. In case of disintegration time and drug content, the results seem to be unsystematic and random possibly due to experimental errors.

7.4.5.2. Analysis of variance (ANOVA)

Analysis of variance (ANOVA) was performed to evaluate the significance of the quadratic models (linear, interactive and polynomial) on the responses and to establish their quantitative effects. **Table 7.17** summarises the effects of the model terms and associated p values for all five responses. At a 95% confident level, a model was considered significant if the p value <0.05.

The results indicate that the disintegration time of the tablets was independent from the studied concentration ranges of alanine and loperamide HCl, as all the model terms showed no significant influence on the disintegration time ($p>0.05$). This could be attributed to the instant disintegration of all the prepared tablets (3 to 6 seconds) as a result of using a pre-optimised concentrations of the binder (gum arabic) and disintegrating enhancing agent (alanine). Additionally low concentrations of loperamide HCl were incorporated in the formulation which did not affect the disintegration time. Moreover, the qualitative nature of the disintegration test which depends on the visual evaluation in addition to the fact that few seconds' inaccuracy in evaluating the disintegration time may cause huge error.

Table 7.16 Full factorial design worksheet for loperamide HCl. The concentrations (%w/w) loperamide HCl and alanine are percentages of the mass of gum arabic dissolved in the stock solution.

Exp Name	Run Order	Loperamide HCl (%)	Alanine (%)	Disintegration time	Tg'	Hardness	Friability	Drug recovery %
N1	6	1	20	4	-24.11	1.71	19.42	103.39
N2	22	3	20	5	-24.96	2.30	15.84	95.67
N3	10	1	40	5	-13.06	5.16	14.12	97.91
N4	16	3	40	5	-14.71	3.18	9.29	94.95
N5	19	1	30	5	-15.04	3.79	14.89	101.13
N6	11	3	30	4	-15.24	2.84	25.84	104.00
N7	21	2	20	3	-24.75	3.06	20.82	100.25
N8	18	2	40	4	-14.06	4.17	9.70	100.71
N9	1	2	30	6	-15.15	2.95	22.80	103.72
N10	5	1	20	3	-24.22	1.81	18.87	99.13
N11	24	3	20	6	-25.04	2.05	14.95	97.49
N12	27	1	40	4	-13.27	5.52	13.20	109.76
N13	12	3	40	3	-14.74	3.66	8.97	99.01
N14	20	1	30	4	-15.11	4.71	17.43	99.94
N15	2	3	30	3	-15.31	2.54	26.80	100.10
N16	3	2	20	5	-24.68	3.56	19.59	103.09
N17	9	2	40	3	-14.12	3.90	10.11	95.43
N18	15	2	30	4	-15.17	3.29	21.76	97.68
N19	14	1	20	4	-24.18	2.36	20.8	102.16
N20	25	3	20	4	-24.99	2.71	17.52	97.07
N21	23	1	40	4	-13.12	5.03	11.19	96.58
N22	13	3	40	5	-14.65	3.82	7.32	94.10
N23	26	1	30	4	-15.02	3.92	15.44	98.36
N24	4	3	30	4	-15.30	2.89	25.41	97.16
N25	7	2	20	4	-24.69	2.76	18.84	102.27
N26	8	2	40	3	-14.15	3.54	10.97	102.74
N27	17	2	30	4	-15.19	2.52	23.19	96.08

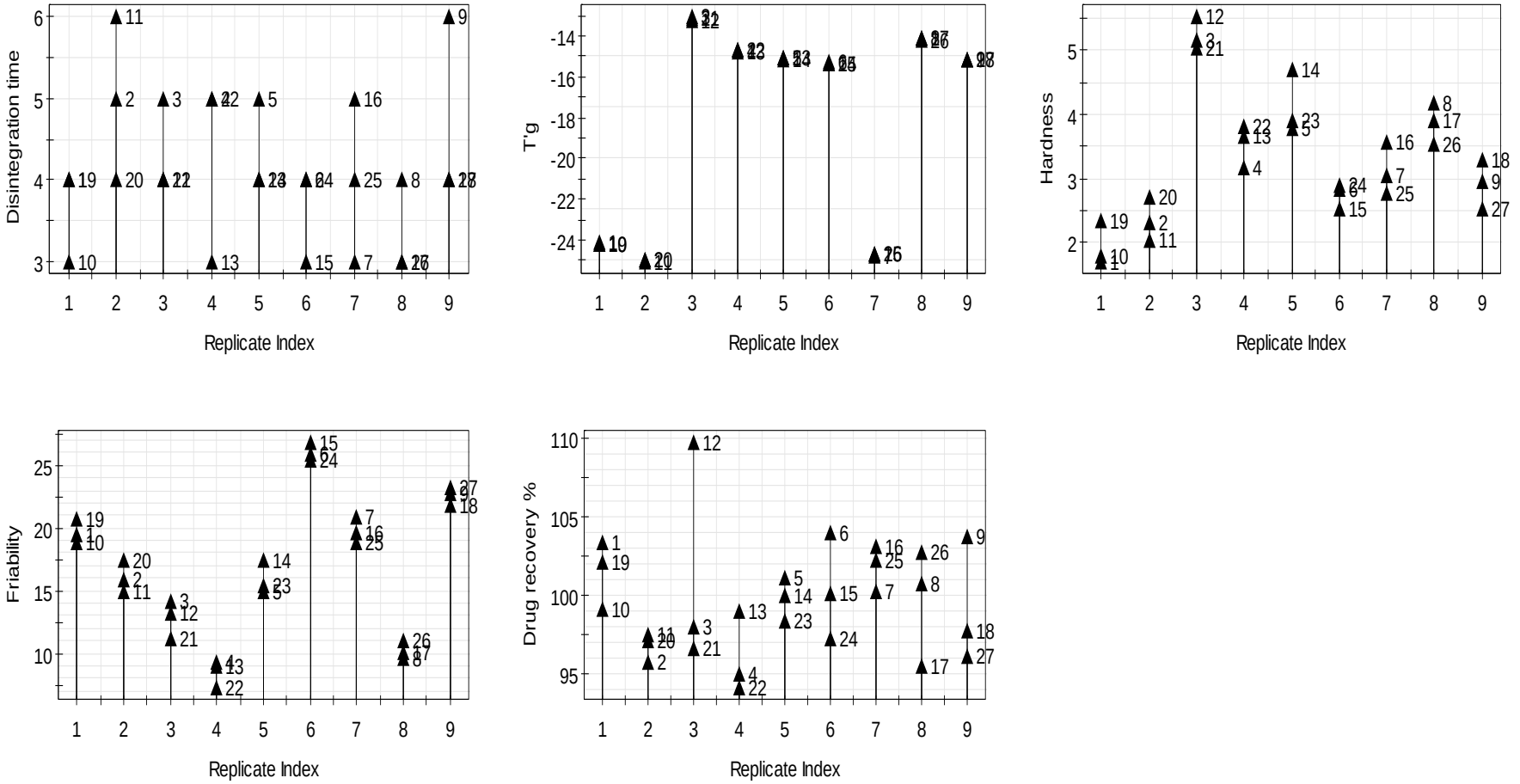


Figure 7.22 Replicate plots of the responses. The values of the response are plotted vs. experimental runs displaying the variation in the response for replicated experiments.

In case of Tg' of the formulation, the results showed that both independent factors (alanine and loperamide HCl concentrations) had significant influence (**Table 7.17**). The linear model of alanine concentration (X_1) had a large positive coefficient (4.4282) suggesting that increasing alanine concentration in the formulation drastically increases the Tg' value, which could be explained by the tendency of high concentrations of alanine to crystallise in the frozen formulation (**Figure 7.23**). However, upon increasing the concentration of alanine above a threshold level, the minimum concentration required to initiate crystallisation, the increase in Tg' is limited as indicated from the negative effect of the polynomial term of alanine concentration (-2.8572). Moreover, the negative coefficient of polynomial terms of alanine concentration (X_1^2) could be attributed to the inability of alanine to crystallise at low concentration and consequently showed plasticising behaviour in system (lower Tg'). Loperamide HCl concentration appeared to influence the Tg' significantly only by its linear model (X_2) with a negative coefficient of -0.347082, suggesting that increasing loperamide HCl concentration in the formulation significantly lowers the Tg'. Compared to ranitidine HCl (see above), loperamide HCl showed less plasticising effects on the system possibly due to its lower aqueous solubility or to the fact that a smaller concentration was used in the experiments.

Table 7.17 The quantitative factor effects and associated p value for the responses.

Term	Y_1		Y_2		Y_3		Y_4		Y_5	
	Effect	P value	Effect	P value	Effect	P value	Effect	P value	Effect	P value
X_2	0.0969	0.6549	-0.3471	<0.0001	-0.3752	0.0062	0.2874	0.7129	-1.3139	0.1344
X_1	-0.0916	0.6725	4.4282	<0.0001	0.7230	<0.0001	-3.3212	0.0003	-0.4284	0.6170
X_2^2	0.1361	0.7169	0.0153	0.8688	0.0373	0.8632	-0.6350	0.6390	-0.6903	0.6416
X_1^2	-0.0751	0.8413	-2.8572	<0.0001	0.0520	0.8101	-4.8378	0.0016	-0.1712	0.9079
X_1X_2	-0.2347	0.3801	-0.1374	0.0463	-0.3555	0.0284	-0.1093	0.9088	-0.1165	0.9113

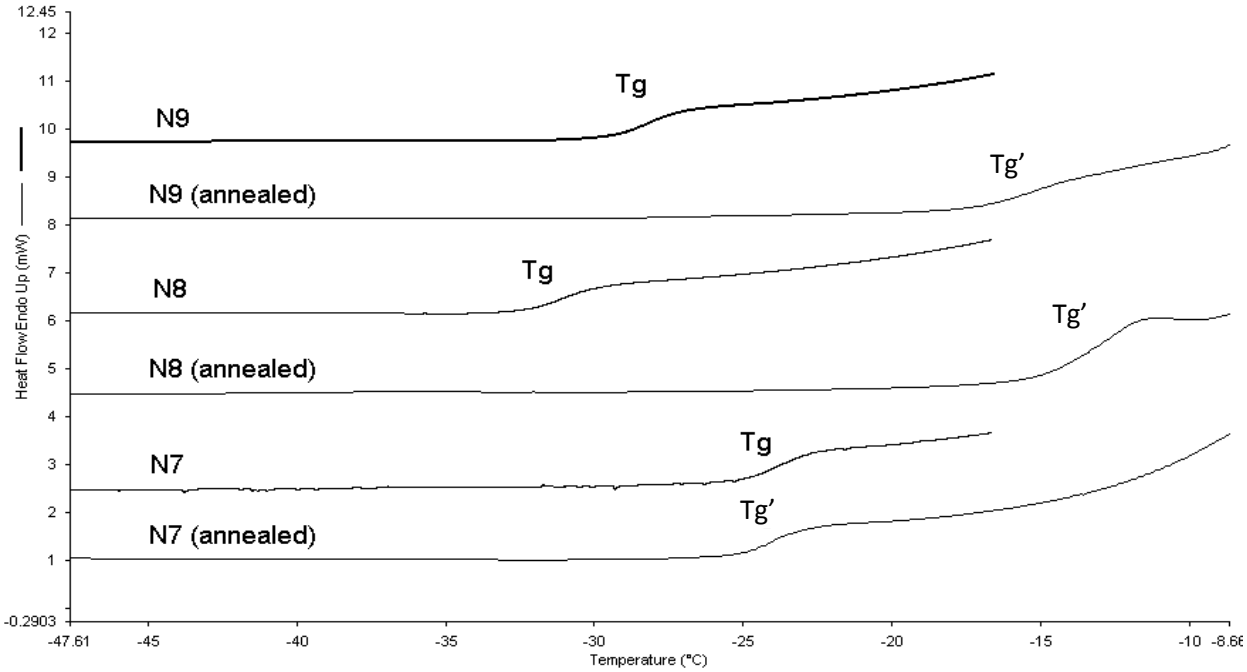


Figure 7.23 Overlaid DSC heating scans of frozen formulations based on high (N8), medium (N9) and low (N7) alanine concentrations with medium concentration of loperamide HCl.

For hardness (Y_3), linear terms of alanine concentration (X_1) and loperamide concentration (X_2) were identified as the only significant factors with p value < 0.0001 and 0.0062, respectively. The quantitative estimation (**Table 7.17**) of the significant terms indicated that increasing alanine concentration was an effective way to enhance the hardness of the ODTs as suggested by its positive coefficient (+ 0.7230), which confirms the role of alanine as a matrix supporting agent (chapter 3). This enhancement was dependent on the incorporated concentration of loperamide HCl, which was found to reduce the hardness significantly in a linear way (X_2), suggesting that increasing loperamide concentration in the formulation decreased that hardness of the tablets. However, the results suggested minimal quantitative deterioration in hardness as a results of incorporating loperamide HCl possibly due to its low concentration in the formulation and the small negative coefficient of its linear model (-0.3752).

For the fourth response Y_3 (friability), alanine concentration (X_1) was the only factor that showed significant effect. The results suggested (**Table 7.17**) that increasing alanine concentration was an efficient way to reduce the friability of the tablet, as X_1 had high negative coefficient (-3.3212) which confirms its role as matrix supporting agent. However, the

significant influence of X_1^2 (polynomial term of alanine concentration) with negative coefficient indicated that reducing the friability of the tablets can be achieved only at high concentrations of alanine (> 30% w/w).

The results for the mechanical properties (hardness and friability) can be explained by various factors that influence the hardness of the tablet, which include intermolecular bonding force and contact points between the excipients within the tablets. The improvement in the mechanical properties associated with increasing alanine concentration in the tablet might be a result of both the factors; the intermolecular bonding force, possibly through hydrogen bonds with the binder (gum arabic) as both contain hydrogen bond donors and acceptors, and contact points between the excipients, as a result of decrease in the porosity (chapter 3).

ANOVA results (**Table 7.17**) indicated that all the model terms including linear, interactive and polynomial, had no significant influences on the drug content ($p>0.05$) suggesting that the results were randomly distributed and the variation was only a matter of experimental errors in formulation and/or analysis.

7.4.5.3. Revised model and surface response plots

After analysing the influence of all the quadratic model terms on the dependent variables (responses), the insignificant terms were omitted to generate a revised model that only included model terms with significant influence. The resulting equations for all five responses, Y_1 (disintegration time), Y_2 (Tg'), Y_3 (hardness), Y_4 (friability) and Y_5 (drug content), are presented below:

$Y_1 = + 4.08942$

$Y_2 = - 15.1904 + 4.42822\ X_1 - 0.347082\ X_2 - 2.85724\ X_1^2$

$Y_3 = + 3.23817 + 0.723033 X_1 - 0.375224 X_2$

$Y_4 = + 22.1249 - 3.32121\ X_1 - 4.83781\ X_1^2$

$Y_5 = + 100.455$

The results for testing the validity of the model are summarised in **Table 7.18**. P values for all the simulated responses were below the significant level (<0.05) suggesting that all the revised

models were significant in predicting their response values. The high value of correlation coefficient (R^2) for Tg', hardness and friability indicated a good fit to the revised model.

Based on the revised equations, the software was used to generate response surface graphs that simulate the influence of the independent factors on each response individually. The graphs for disintegration time Tg', hardness and friability are presented in **Figures 7.24**, **7.25** and **7.26**, respectively.

Table 7.18 Summary of results for testing validity of the revised models. DF indicates: degrees of freedom; SS: sum of squares; MS: mean of square; F: Fischer's ratio; p: probability; R^2: regression coefficient.

Disintegration time						
	DF	SS	MS (variance)	F	p	R^2
Regression	4	1.8519	0.4630	0.5802	0.680	0.110
Lack of Fit	4	4.8889	1.2222	1.7368		

Tg'						
	DF	SS	MS (variance)	F	p	R^2
Regression	4	615.629	153.9070	3146.98	<0.0001	0.998
Lack of Fit	4	1.02367	0.2559	88.1353		

Hardness						
	DF	SS	MS (variance)	F	p	R^2
Regression	4	20.4683	5.1171	19.592	<0.0001	0.781
Lack of Fit	4	3.5365	0.8841	7.20245		

Friability						
	DF	SS	MS (variance)	F	p	R^2
Regression	4	581.169	145.292	13.8735	<0.0001	0.724
Lack of Fit	4	209.773	52.4432	45.7677		

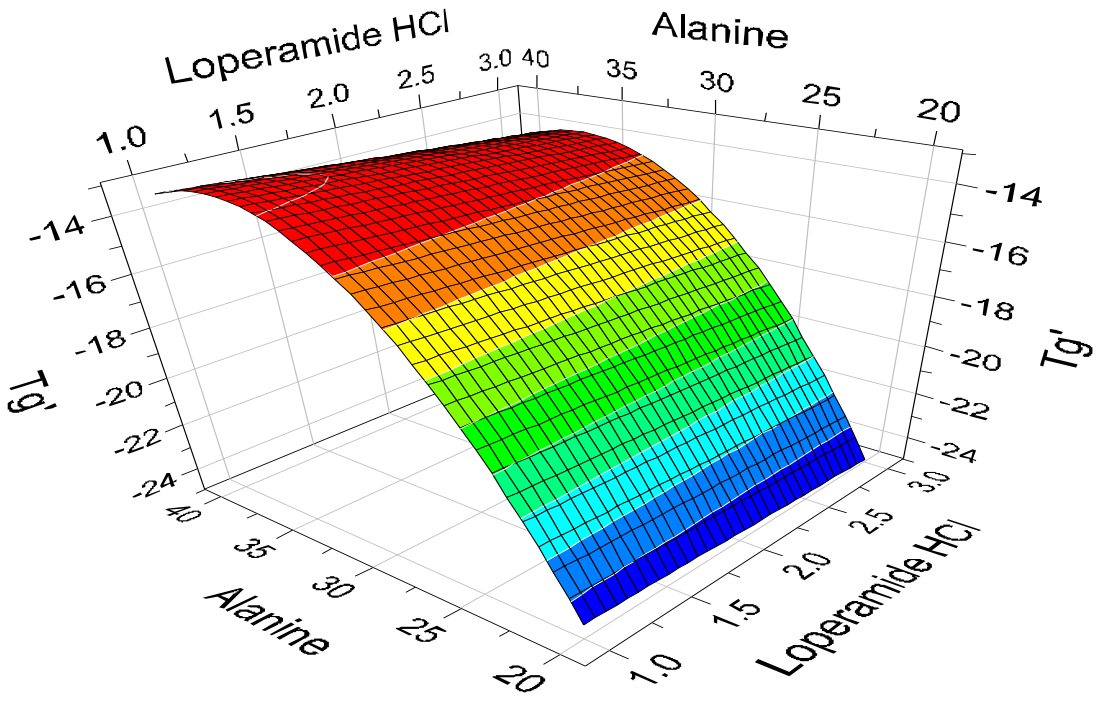


Figure 7.24 Surface response plot showing the influence of varying alanine and loperamide HCl concentration on the Tg'.

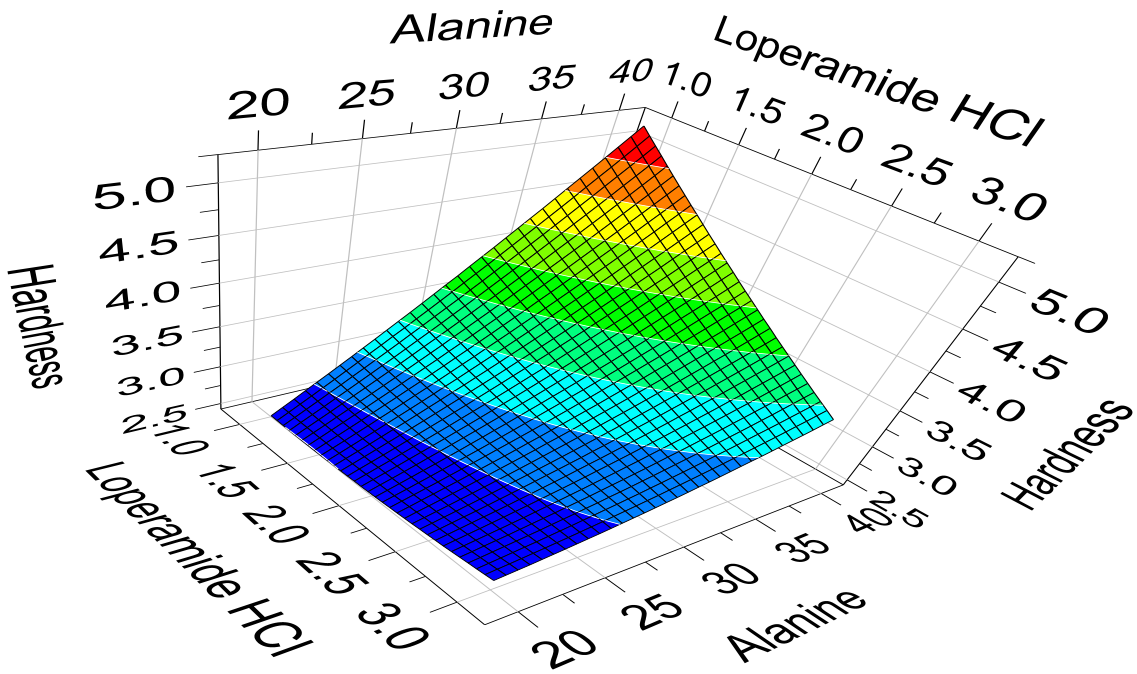


Figure 7.25 Surface response plot showing the influence of varying alanine and loperamide HCl concentration on the hardness of the ODT.

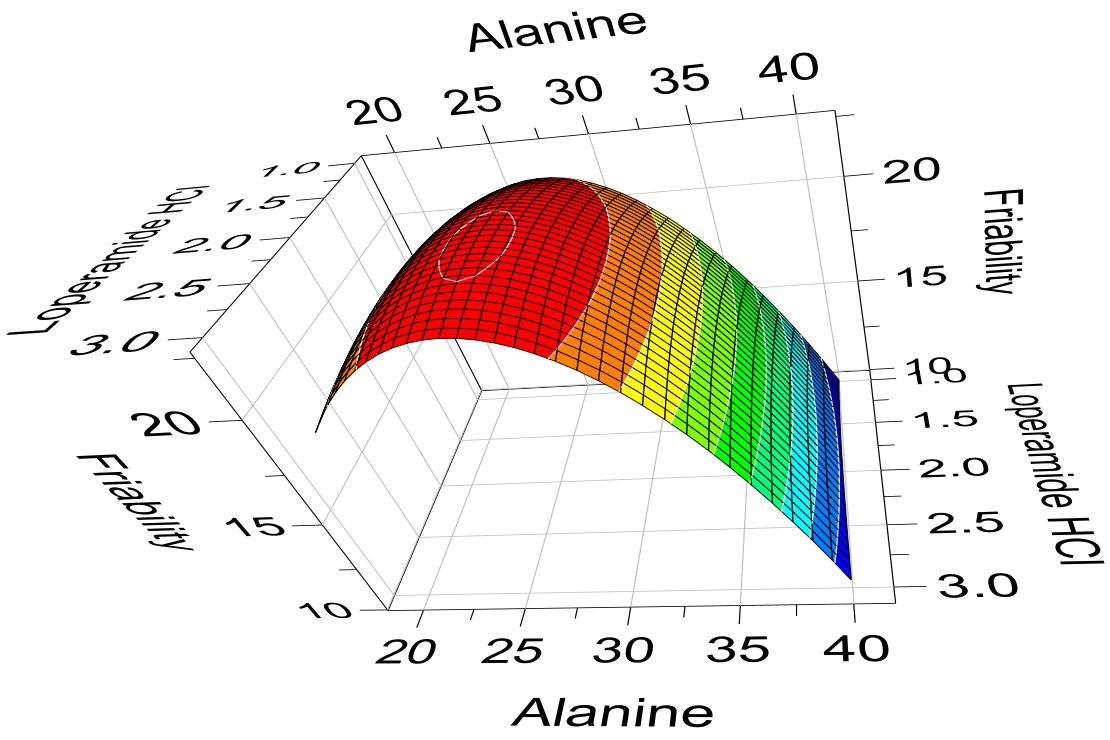


Figure 7.26 Surface response plot showing the influence of varying alanine and loperamide HCl concentration on the friability of the ODT.

7.4.5.4. Optimum ODTs formulation

Based on the response surface plots, the software was used to perform hot spot analysis to find the optimum formulation variables (alanine and loperamide HCl concentration s) for formulating ODTs with optimum characteristics such as high Tg', high hardness and low friability. The optimal formulation was determined as 40% (w/w) alanine and 1 % (w/w) loperamide HCl. The observed response values of the optimised formulation compared to the predicted values are presented in **Table 7.19**. The characterisation results verified, experimentally, the established statistical models, as only small differences were observed between the actual (observed) and calculated (predicted) values.

7.4.5.5. Formulation of 2 mg loperamide HCl lyophilised ODTs

Based on the optimised formulation, lyophilised ODTs containing 50 mg dose of loperamide HCl were prepared using 13.5 mm diameter mould. Characterisation summary of the tablets is presented in **Table 7.20**. As expected, the tablets showed instant disintegration (less than 4 seconds) without leaving any lumps or gritty particles in the disintegration vessel. The average thickness of the dried tablets was 7.95 mm (SD=0.27, n=6).

Table 7.19 Observed and predicted (from the revised model) responses and residual values for the optimised formulation. The observed results are means, n=3.

Response	Observed	Predicted	Residual
Disintegration time (s)	3	4	-1
Tg (˚C)	-13.15	-13.33	0.18
Hardness (N)	5.62	5.12	0.50
Friability (%)	11.41	10.50	0.91
Drug content (%)	98.75	100.46	-1.71

Table 7.20 Characterisation of the prepared lyophilised ODTs after 0 and 3 months at 40 ˚C and 75% RH. Results are means ± SD, n=3.

Parameters	Time interval (months)	
	0	3
Moisture content (%)	1.58 ± 0.34	1.87 ± 0.42
Disintegration time (s)	3 ± 1	3 ± 1
Hardness (N)	5.89 ± 0.49	5.68 ± 0.71
Friability (%)	11.94 ± 2.23	10.04 ± 2.07
Drug content (%)	98.78 ± 2.74	97.38 ± 3.32

7.4.5.6. Dissolution studies

The dissolution profiles of the prepared lyophilised tablets and commercial lyophilised ODTs (Imodium® Instant) that contain 2 mg loperamide HCl are presented in **Figure 7.27**. According to the US Pharmacopeia, not less than 80% of the dose should dissolve within 30 min under the prescribed dissolution conditions.

All the tested ODTs from both formulations showed instant disintegration in the dissolution medium. Our lyophilised ODTs showed faster dissolution rate with around 70% drug release in just 2 min, and achieved the mandatory drug release (80%) within 10 min. On the other hand, the commercial lyophilised ODTs showed huge variation in their dissolution rate, especially in the first 6 min, and required 25 min for 80% dose release. Moreover, the prepared ODTs displayed efficient dissolution with cumulative drug release of more than 90% at the end of the experiment compared to a maximum cumulative release of 80% for the commercial ODTs. The superior dissolution profile of the prepared ODTs might be attributed to the emulsifying properties of arabic gum that facilitate the dissolution of the drug and/or, simply, due to larger size of our tablets (13.50mm × 7.91 mm) compared to the commercial one (Imodium® Instant), which may allow better dispersion of the drug inside the dry tablets, and consequently provide faster and more consistent dissolution profile.

7.4.5.7. Stability studies

The short term stability studies of the prepared ODTs are summarised in **Table 7.20**. The results indicated that no significant change in appearance, moisture content, disintegration time, drug content and mechanical properties was observed suggesting that the formulation was chemically and physically stable.

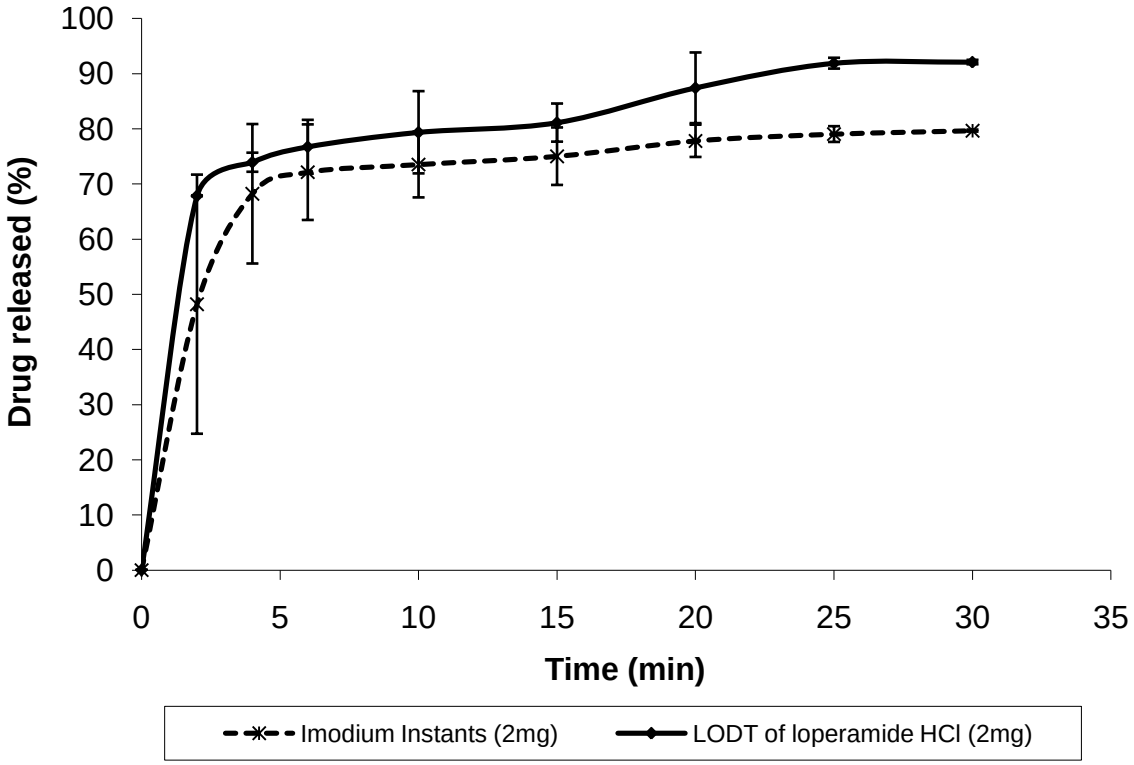


Figure 7.27 Dissolution profiles of commercially available lyophilised ODTs (Imodium Instant) and formulated lyophilised orally disintegrating tablets (LODT) of loperamide HCl. Results are means ± SD, n=3.

7.5. Conclusion

The application of 3^2 factorial design illustrated the influence of varying the selected formulation factors individually and simultaneously on the quality of ODTs. The results of analysis of variance (ANOVA) led to a statistical model and three dimensional plots that described adequately the relationship between the dependent and independent variables. The revised model showed high degree of reliability and therefore succeeded to generate ODT formulations with optimised properties. Compared to commercial products, our formulations showed faster, more efficient and reproducible dissolution profiles. The formulation of lyophilised ODTs based on gum arabic and alanine showed capability to deliver diverse range of drugs with advantages over commercial products.

Chapter Eight: Summary and Implications of Research Findings

Summary and Implications of Research Findings

8.1. Summary of research findings

This thesis describes a systematic development strategy for lyophilised orally disintegrating tablets as a novel solid oral dosage form that improves patient compliance along with providing pharmaceutical advantages for the active drug. Investigating the formulation factors that control the preparation process and performance of ODTs based on the common excipients revealed the need for alternative materials to be used in the formulation. Accordingly, the research was performed to explore and optimise new advantageous material as matrix forming agents. As a result of this research, two novel systems that can address limitations and widen the application of lyophilised ODTs were studied.

8.1.1. Formulation and optimisation of lyophilised ODTs based on gelatin and saccharide

Successful development of fast disintegrating tablets by lyophilisation technique requires careful optimisation of formulation parameters in order to obtain an optimal balance between the tablet properties, namely: thermal properties, primary drying time, mechanical properties and disintegration time.

The results showed that disintegration time of the tablets dramatically decreased by decreasing the concentration and bloom strength of gelatin in the stock solution, whereas the mechanical properties of the tablets were influenced by the concentration of gelatin rather than the bloom strength. Enhancing the mechanical properties of the freeze-dried tablets by increasing gelatin concentration inversely influenced their disintegration time. On the other hand, increasing gelatin concentration in the formulation decreased the sublimation rate significantly, which results in longer primary drying time to formulate ODT from high concentration of gelatin. Accordingly, low bloom strength gelatin with stock solution concentration between 2-5% (w/w) was most suitable for developing lyophilised orally disintegrating tablets.

Mannitol crystallised during freeze drying and consequently produced elegant tablets. Xylitol, glucose, trehalose and maltotriose were more resistant to crystallisation and therefore they may act as lyoprotectants in the formulation. The disintegration time profiles of gelatin/saccharide systems were parabolic with different dip values (shortest disintegration time) at distinctive concentrations for each saccharide. High concentration of trehalose, maltotriose and mannitol (equal or higher than 40% w/w) significantly enhanced the mechanical properties of the tablets. Mannitol at concentration between 30 to 40 % w/w (of total solid material) achieved the greatest balance between the disintegration time and hardness as demonstrated by the LTI value. The optimised ODT formulation in this study was able to deliver therapeutic dose of clonidine HCl efficiently.

8.1.2. Investigation of amino acids as matrix forming agent in the development of saccharide free ODTs

Formulation of saccharide free lyophilised ODTs will enable their use for the treatment of long term chronic conditions and also for multiple dose medications, especially for children, diabetic and obese patients who have limitations on daily intake of saccharides. Naturally occurring amino acids are prospective candidates because of their versatility in terms of physicochemical properties, high safety profile and availability. Replacement of saccharide requires excipients that fulfill stringent characteristics such as reasonable drying time, stability during and after freeze-drying process as well as formation of elegant tablets with short disintegration time and adequate mechanical properties.

The crystallisation behaviour of alanine, glycine, cysteine and serine in the frozen state at high concentration increased the stability of the formulation during the freeze drying process and, although, arginine, histidine, threonine, asparagine, phenylalanine and methionine did not show tendency to crystallise they displayed relatively high Tg', suggesting their suitability as freeze drying excipients. Proline formulations (≥ 30% w/w) were difficult to freeze dry due to their low glass transition temperature. The characterisation of the ODTs suggested that high concentration of amino acids is required to enhance the mechanical properties, whereas only optimum concentrations promote faster disintegration. The mechanisms of disintegration of the ODTs depend on the physicochemical properties of the amino acid. The highly wettable amino acids promote disintegration by enhancing the overall wettability of the ODT, which was highly dependent on the wetting time of the amino acid and the total porosity of the tablet.

Therefore, parabolic relationships between the disintegration time and the concentration of these amino acids were noticed. On the other hand, poorly wettable amino acids generate intermolecular repulsive forces within the hydrophilic matrix that encourage the disintegration. Depending on the lyophilised tablet index values, 30% alanine formulation achieved the best balance between the hardness and disintegration time.

8.1.3. Investigation of formulation and process of lyophilised ODTs using novel amino acid combinations

The use of amino acids individually to replace the saccharide in the formulation of lyophilised ODTs showed varied capability to fulfil all the required characteristics. For instance, proline showed complete wettability in water (disintegrating medium) with short wetting time, which is expected to improve the disintegration of ODTs; however, its inclusion in freeze dried formulations was limited due to the extremely low glass transition temperatures and consequently resulting in the collapse of the prepared formulations. On the other hand, serine based formulations displayed higher collapse temperature and produced elegant tablets even at high concentration, due to its tendency to crystallise in the frozen state, but was characterised by long disintegration time, which was explained by serine's partial wetting property, as the measured contact angle (θ) with water was $0^{\circ} < \theta < 90^{\circ}$.

Inclusion of optimised combinations of serine and proline in the formulation of lyophilised orally disintegrating tablets verified our hypothesis to combine the benefits of high wettability and stability of proline and serine, respectively, and resulted in the formation of tablets with superior properties over that of individual amino acids.

Studying the influence of freezing protocol revealed that annealing induces morphological changes in the ODTs that not only allow faster sublimation rate but also shorter disintegration time.

8.1.4. Formulation of multiparticulate systems as lyophilised ODTs

To formulate multiparticulate systems (pellets) as lyophilised ODTs, the stock solution of the matrix forming agents should be viscous enough to keep the pellets stable and suspended

throughout and after the formulation process, exhibit adequate mechanical strength in the dry state and disintegrate quickly upon hydration.

Gelatin, carrageenan and alanine as matrix forming agents was selected as independent variables and their influences on the crucial responses of the formulation (disintegration time, hardness, viscosity and pH) were studied by applying on a central composite face centered (**CCF**) design. The disintegration time and viscosity were controlled by the associative interaction between gelatine and carrageenan upon hydration which forms a strong complex that increases the viscosity of the stock solution and forms tablet with more resistant to disintegration in aqueous medium. Therefore, the levels of carrageenan, gelatin and their interaction in the formulation were the significant factors. In terms of hardness, increasing gelatin concentration was the most effective way to improve the hardness, due to the formation of extensive 3D networks of gelatin fibres. To lesser extent, increasing alanine concentration also enhanced the hardness significantly. Accordingly, optimum concentrations of these excipients were needed to find the best balance that fulfilled all formulation requirements. The revised model showed high degree of predictions and optimisation reliability and therefore succeeded to find an ODT formulation with optimised properties that were able deliver enteric coated multiparticulate of omeprazole without compromising their functionality.

8.1.5. Investigation of alternative binders for the formulation of lyophilised ODTs

Gum arabic was found to have an outstanding potential to be used as a binder in the formulation of lyophilised ODTs. Gum arabic showed immediate dispersion in either cold or hot water to form low viscosity solutions, which allowed the use of high concentrations and simplified the formulation process at the same time. Formulations with high concentration of gum arabic was found to provide elegant freeze dried tablets with rapid disintegration time and sufficient mechanical strength to withstand manual handling. Tablets based on 15% w/w gum arabic achieved the best balance between hardness the disintegration time. Compared to gelatin formulation, the tablets based on gum arabic showed superior performance in term of disintegration time and hardness. Moreover, tablets comprising of gum arabic were prepared using a shorter freeze drying cycles than those with Gelatin. Inclusion of matrix supporting/disintegrating enhancing agents further enhanced the tablet characteristics.

8.1.6. Formulation design and optimization of lyophilised ODTs incorporating hydrophilic and hydrophobic drugs using gum arabic as a binder

The application of gum arabic as a binder in the formulation of lyophilised ODTs showed an outstanding potential to improve the formulation, production process and performance of the tablet and consequently expand their use. However, the actual incorporation of active drugs can have multiple consequences on the manufacturing process as well as the quality and performance of the lyophilised ODTs. Accordingly, the feasibility of incorporating therapeutics doses of active drugs was investigated using full factorial design (3^2) studies that evaluated the influence two formulation variables, alanine and active drugs concentrations, on five crucial responses, disintegration time, Tg', hardness, friability and drug content. The design of experiment (DoE) and the range of formulation excipients were different for each drug depending on its therapeutic dose and hydrophilicity. Highly soluble drugs (ranitidine HCl) required a high concentration of alanine to conceal the low collapse temperature of the system at high concentration of the drug and consequently allow the production of intact tablets. In case of poorly and slightly soluble drugs (5,5diphenylhydantoin, ibuprofen and loperamide HCl), the level of alanine was decided mainly to allow the optimisation of the disintegration time and the mechanical properties due to the minimal influence of these drugs on the collapse temperature.

The application of 3^2 factorial design of experimental succeeded to reveal the influence of varying the selected formulation factors individually and simultaneously on the quality of the ODTs, which led to a statistical model and three dimensional plots that described adequately the relationship between the dependent and independent variables.

Optimisation results showed that a concentration of 40% w/w alanine achieved the required balance between the thermal properties, disintegration time and mechanical strength. Whereas, the optimised level of the active drug was different in each case, which can be attributed to their differences in the physicochemical properties.

The formulation of lyophilised ODTs based on gum arabic and alanine showed capability to deliver diverse range of drugs with advantages over commercial products in terms of providing faster, more efficient and reproducible dissolution profiles. Moreover, short term stability studies of the prepared lyophilised ODTs indicated no significant changes in appearance,

moisture content, disintegration time, drug content and mechanical properties, suggesting that the formulations were chemically and physically stable.

8.2. Future directions

The studies carried out in this thesis have introduced two platform technologies that showed excellent in-vitro potential to add significant advances to the field of lyophilised orally disintegrating tablets. Some extended work is underway to explore the clinical performance of these ODTs in terms of patient acceptance, manual handling, mouth feeling upon disintegration and other in vivo data such as sites of absorption, GIT residence time and blood level curve. In term of process development, determination the effect of the shape and size of the tablets on the freeze drying regime as well as ODT characteristics would be of interest.

The studies carried out in this thesis reveal that further advances in the development of lyophilised ODTs can be achieved by exploring new materials, innovative formulation processes and novel applications. The future prospects of this dosage form would rely on:

i. Development of a novel lyophilised ODT formulation with mechanical properties comparable to the conventional compressed tablet and accordingly avoids the need of specialised packaging. This is a challenging task because of the highly porous nature of the lyophilised ODTs which compromises the mechanical properties.

ii. Employment of excipients coprocessing technology to create multifunctional excipients that combined the benefits of the incorporated excipients and minimize their drawbacks (Saha & Shahiwala, 2009). Basically, the coprocessed excipients should dissolve quickly in water to allow easy formulation, possess high wettability in aqueous medium to allow fast disintegrating in the mouth and form elegant tablet with adequate mechanical strength in short freeze drying cycle. Other advantageous characteristics can be added such as suspending, bioadhesive and emulsifying properties.

iii. Development of a new disintegration test method to assess the disintegration time, texture and taste of the ODTs which can simulate the disintegration nature in the oral cavity and provide reasonable in vitro in vivo correlation (IVIVC). Although several methods have been proposed using texture analyser (Abdelbary et al., 2005; El-Arini

and Clas, 2002) rotary shaft (Narazaki et al., 2004) or E-tongue (Murray et al., 2004), none of them has been officially recognized by the regulatory authorities.

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Appendix I

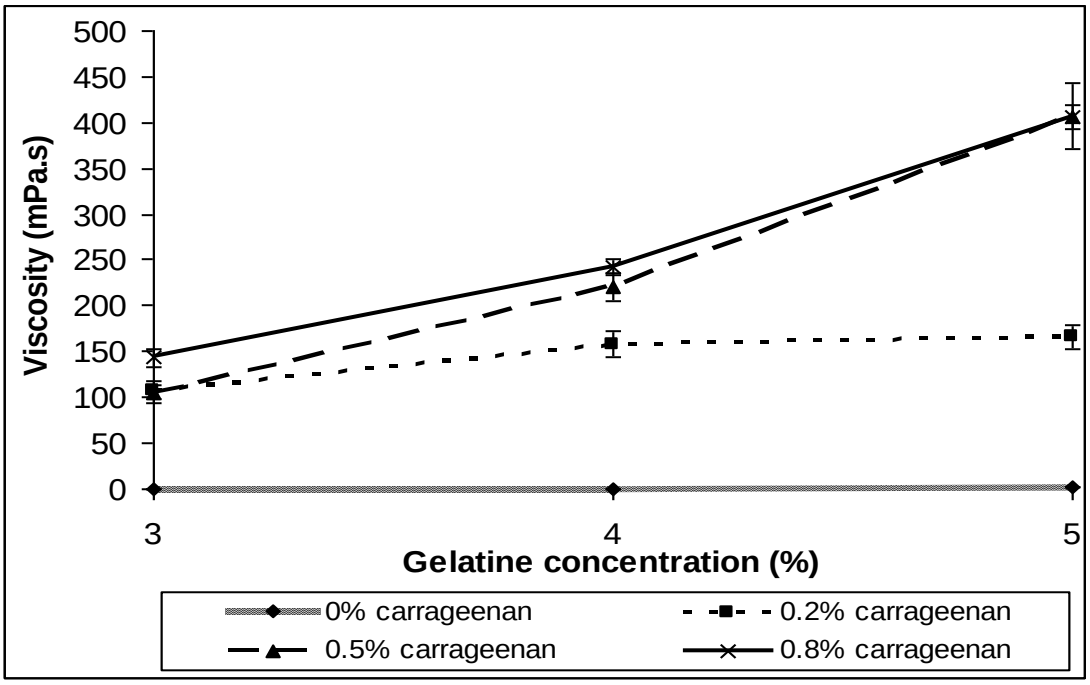

Figure: Preliminary experiments to study the viscosity of different solution of gelatine (3, 4 and 5%) before and after the addition of 0.2, 0.5 and 0.8% carrageenan. The results show substantial increase in the solution viscosity after addition of small concentration of carrageenan as a result of their associative interaction. Addition of 0.2% carrageenan to for 5% gelatine solution increased the viscosity by more than 100 fold, from 1.5 ± 0.1 mPa.s for 5% gelatine alone to 165.8 ± 13.7 mPa.s upon addition of 0.2% carrageenan. Results are mean ± SD, n=3.