DOCTOR OF PHILOSOPHY

Formulation and process engineering of freeze-dried orally disintegrating tablets

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Aston University

FORMULATION AND PROCESS ENGINEERING OF FREEZE-DRIED ORALLY DISINTEGRATING TABLETS

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Thesis Summary

Orally disintegrating tablets (ODTs) which are also referred to as orodispersible and fast disintegrating tablets, are solid oral dosage forms which upon placing on the tongue, disperse/disintegrate rapidly before being swallowed as a suspension or solution. ODTs are therefore easier and more convenient to administer than conventional tablets and are particularly beneficial for paediatric and geriatric patients, who generally have difficulty swallowing their medication.

The work presented in this thesis involved the formulation and process development of ODTs, prepared using freeze-drying. Gelatin is one of the principal excipients used in the formulation of freeze-dried ODTs. One of the studies presented in this thesis investigated the potential modification of the properties of this excipient, in order to improve the performance of the tablets. As gelatin is derived from animal sources, a number of ethical issues surround its use as an excipient in pharmaceutical preparations. This was one of the motivations, Methocel™ and Kollicoat® IR were evaluated as binders as alternative materials to gelatin. Polyox™ was also evaluated as a binder together with its potential uses as a viscosity increasing and mucoadhesive agent to increase the retention of tablets in the mouth to encourage pre-gastric absorption of active pharmaceutical ingredients (APIs). The *in vitro* oral retention of freeze-dried ODT formulations was one property which was assessed in a design of experiments – factorial design study, which was carried out to further understand the role that formulation excipients have on the properties of the tablets. Finally, the novel approach of incorporating polymeric nanoparticles in freeze-dried ODTs was investigated, to study if the release profile of APIs could be modified, which could improve their therapeutic effect.

The results from these studies demonstrated that the properties of gelatin-based formulations can be modified by adjusting pH and ionic strength. Adjustment of formulation pH has shown to significantly reduce tablet disintegration time. Evaluating Methocel™, in particular low viscosity grades, and Kollicoat® IR as binders has shown that these polymers can form tablets of satisfactory hardness and disintegration time. Investigating Polyox™ as an excipient in freeze-dried ODT formulations revealed that low viscosity grades appear suitable as binders whilst higher viscosity grades could potentially be utilised as viscosity increasing and mucoadhesive agents. The design of experiments – factorial design study revealed the influence of individual excipients in a formulation mix on resultant tablet properties and *in vitro* oral retention of APIs. Novel methods have been developed, which allows the incorporation of polymeric nanoparticles *in situ* in freeze-dried ODT formulations, which allows the modification of the release profile of APIs.

Key words/phrases: lyophilisation, excipients, oral retention, nanoparticles, sustained release.

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Chapter One: Introduction

1.1 Oral Drug Delivery

The oral route of drug administration is the most common route of delivering active pharmaceutical ingredients (APIs) to the human body (York, 2007). In comparison with other routes of drug administration, the oral route is considered the safest, simplest and most convenient (York, 2007). Additionally, delivering APIs via the oral route receives a high level of patient compliance, as well as being non-invasive (Mohammed et al. 2011). There are also some disadvantages related to the oral drug delivery route such as slower API absorption compared with APIs delivered parenterally and the destruction of certain APIs due to the harsh conditions of the stomach (acidic nature) and gastrointestinal enzymes (Ansel et al. 2011).

Solutions, suspensions, tablets and capsules are examples of the most common oral drug delivery dosage forms. The vast majority of these dosage forms are swallowed which allows the APIs to be absorbed in the gastrointestinal tract (GIT) and enter systemic circulation, whilst a small minority exert a local therapeutic effect in the GIT (Ansel et al. 2011).

1.1.1 Dosage Form Factors Which Influence API Bioavailability

As mentioned, there are a wide range of delivery systems available for oral drug delivery and the dosage forms in which APIs are formulated have a tremendous influence on the bioavailability of the APIs (Mohammed et al. 2011) (Figure 1.1). Formulation of an API in the form of a suspension or capsule for example, influences the rate and extent that the API is absorbed and becomes available at the site of therapeutic action (Ashford, 2002a).

APIs formulated as aqueous dosage forms display the highest bioavailability compared to other oral dosage forms (Mohammed et al. 2011). In an aqueous solution, the API is readily available for dissolution into the GIT contents which allows for swift absorption into systemic circulation (Mohammed et al. 2011).

APIs formulated as aqueous suspensions are generally second in efficiency in terms of absorption, as the rate limiting factor is the dissolution of the API (Mohammed et al. 2011). Following oral administration of APIs in the form of aqueous suspensions, the large surface area of the APIs in the gastrointestinal fluids allows efficient dissolution and subsequently absorption into systemic circulation (Mohammed et al. 2011). In terms of suspensions, a number of factors relating to the formulation can have a significant influence on the rate and extent of API absorption in the GIT including the

particle size of the API and the viscosity of the suspension formulation in the GIT (Ashford, 2002a).

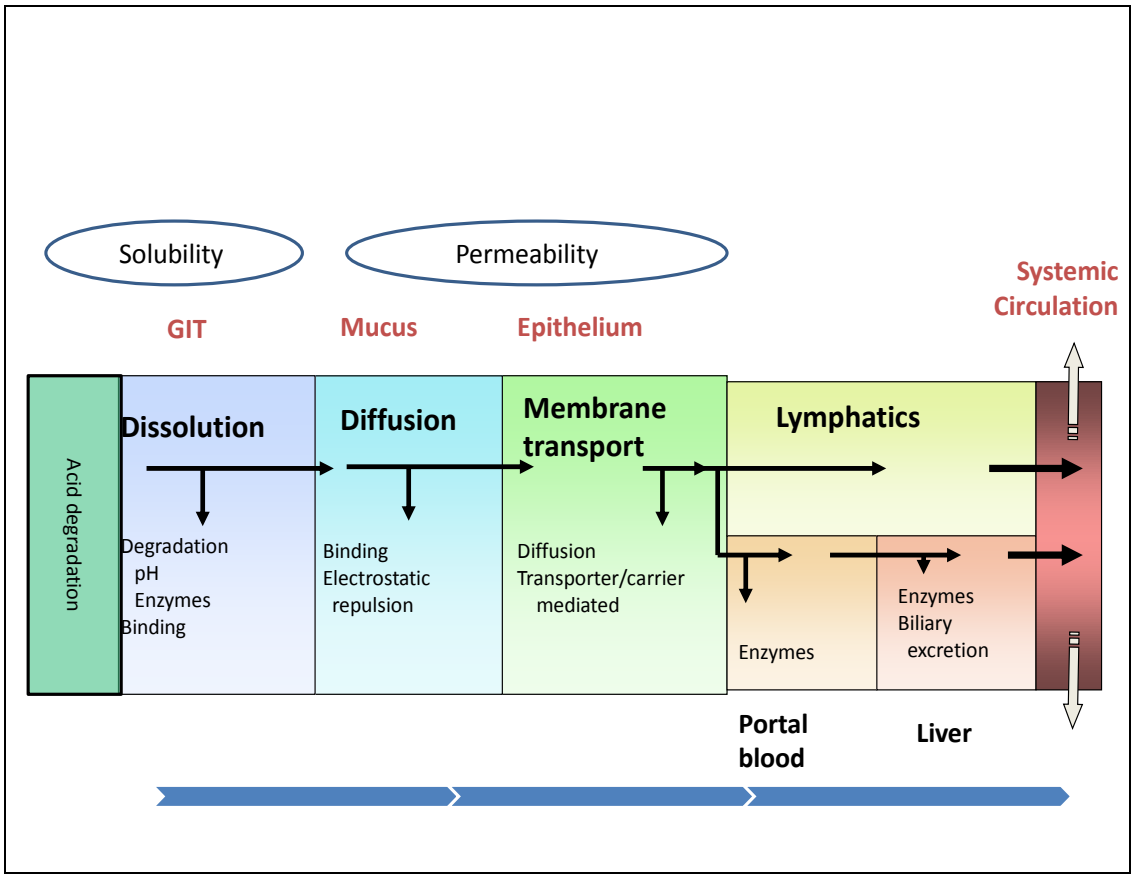


Figure 1.1 Schematic illustration representing the different processes and the barriers encountered in oral drug delivery.

Following solutions and suspensions dosage forms, APIs formulated as hard gelatin capsules are considered third in terms of efficiency of absorption of APIs. Following the administration of a hard gelatin capsule and its subsequent disintegration, as long as the formulation disperses sufficiently, the API will exhibit a large surface area in the GIT for dissolution (Mohammed et al. 2011). However, the surface area of the API is considered less than is the case encountered with suspension dosage forms, and hence why suspensions are considered second in efficiency behind solutions in terms of the efficiency of absorption of APIs (Ashford, 2002a).

One formulation strategy to encourage the API to exhibit a large surface area following the administration and disintegration of a hard gelatin capsule is to include a hydrophilic excipient in the formulation (Mohammed et al. 2011). As encountered with suspension dosage forms, a number of formulation factors play a role on the rate and

extent of API absorption in the GIT, *e.g.* drug-excipient interactions and the packing density of the capsule formulation (Ashford, 2002a).

Behind solutions, suspensions and hard gelatin capsules, tablets are considered fourth in terms of the efficiency of API absorption, as tablets undergo compression at high forces during preparation which results in a considerable decrease in surface area of the API (Mohammed et al. 2011). This has a significant influence on the bioavailability of an API, due to the challenging task of presenting a suspension of fine API particles in the GIT, following administration of a tablet (Ashford, 2002a).

Following the oral administration of a tablet, it disintegrates into aggregates/granules, followed by deaggregation in order to form a suspension of fine API particles (Mohammed et al. 2011). Only then can the API dissolve before being absorbed systemically (Ashford, 2002a). Taking this into consideration it becomes apparent why tablets are considered fourth in terms of the efficiency of API absorption, as tablets need to undergo a number of steps/processes for the API to be able to be absorbed. As with suspensions and hard gelatin capsules dosage forms, a number of formulation factors are associated with tablets which influence API bioavailability, such as the nature and quantities of the formulation ingredients and the compaction pressures used to prepare the tablets (Ashford, 2002a).

1.1.2 Physiological Factors of the Gastrointestinal Tract Which Influence API Bioavailability

As discussed, the dosage form in which an API is formulated, together with the associated formulation factors play a tremendous role on the bioavailability of an API. Another group of factors which have a significant influence on the rate and extent of API absorption in the GIT is the physiology of the GIT.

The surface areas of the gastrointestinal absorption sites exhibit substantial variability along the GIT (Mohammed et al. 2011). The regions of the small intestine generally possess the largest surface area and subsequently this is where maximum absorption of APIs occurs, in contrast, minimal API absorption occurs in the stomach and colon due to small surface areas (Mohammed et al. 2011).

The pH of the GIT also influences API absorption and similarly to the surface areas of the gastrointestinal absorption sites, varies considerably along the GIT (pH 1.8 – 7.8) (Mohammed et al. 2011). pH influences API absorption as it effects the chemical

stability of APIs (Ashford, 2002b). The most acidic location is found in the stomach (due to the secretion of hydrochloric acid from parietal cells (Widmaier et al. 2011)) whilst more alkaline environments are encountered in the small and large intestines as a result of the secretion of bicarbonate into the lumen of these areas of the GIT (Ashford, 2002b).

The fed or fast state can have a tremendous effect on the bioavailability of an API. When a person is in a fed condition, a reduction in gastric emptying rate can take place, which delays the therapeutic action of an API (Mohammed et al. 2011). Additionally, following the consumption of food, the secretion of gastric fluids and enzymes can be stimulated which can degrade some APIs or in some cases increase the bioavailability depending on the nature of the API and the food consumed (Ashford, 2002b).

The consumption of food can lead to a reduction in API bioavailability due to the complexation between an API and a component in the diet (Mohammed et al. 2011). A well-documented example is the complexation between tetracycline and calcium, which forms a non-absorbable complex (Ashford, 2002b). The consumption of food can also influence the viscosity of gastrointestinal contents and the blood flow to the liver, which can affect the absorption and bioavailability of APIs (Mohammed et al. 2011).

Aging is another physiological factor which can affect API absorption. Aging is generally linked to a reduction in gastric emptying rate along with influencing postprandial pH response, and a reduction in gastrointestinal transit times (Mohammed et al. 2011).

Other physiological factors which can influence API absorption are gastric emptying rate, defined as the rate at which an API in solution leaves the stomach and enters the duodenum, and intestinal motility (Mohammed et al. 2011). There are two types of intestinal movements including propulsive and mixing forces. Propulsive forces allow for the movement of materials through the GIT and determine the residence time of the API/formulation in the small intestine before absorption. They have a direct relationship with the amount of drug absorbed as greater the intestinal motility, the shorter the time that an active drug ingredient or dosage form is present in the small intestine, and is available for absorption. Mixing movements in the GIT is significant in fed conditions when compared to fast conditions as the presence of food in the GIT dictates the rate

and extent of mixing of the dosage form with the contents. Mixing movements can increase active drug ingredient absorption and consequently bioavailability by increasing the interaction of active drug ingredients with the gastrointestinal membrane. They can also increase the rate of dissolution of active drug ingredients from solid oral dosage forms, such as tablets.

As discussed, a number of parameters need to be taken into consideration when developing an oral drug delivery product (Table 1.1). An understanding of these factors will aid the development of a pharmaceutical product, which is effective and displays the required absorption/bioavailability. With an aging population, and the subsequent influence this has on the physiology of the GIT, which directly impacts API absorption, the development of future pharmaceutical products lies with formulations which will utilise the changes in GIT physiology in order to maximise the absorption of APIs.

Table 1.1 Variations in physiological environment encountered throughout the entire length of the GIT. Table taken from Mohammed et al. (2011)

1.1.3 Mechanisms and Transport Routes of API Absorption in the Gastrointestinal Tract

When an active drug ingredient has undergone dissolution in the small intestine of the GIT, and is in a form suitable for absorption across the intestinal mucosa, a number of different routes and mechanisms exist for the drug candidate to be absorbed from the apical side of the intestinal mucosa into the systemic circulation for hepatic metabolism. These routes include; transcellular, paracellular, efflux and metabolism, as shown in Figure 1.2.

The majority of active drug molecules follow the transcellular route, which is an example of a passive diffusion mechanism, in which drug molecules move down the concentration gradient, from a region of high concentration to a region of lower

concentration without the use of energy. The paracellular route, where the drug permeates between the tight junctions of the epithelial cells is another example of a passive diffusion mechanism, in which molecules transport between the epithelial cells of the intestinal mucosa, through water-filled pores.

For API's to undergo passive diffusion via the transcellular route, they require certain properties which relate to lipophilicity, polar surface area, hydrogen bonding potential, non-polar surface area and number of rotatable bonds. In contrast, for active drug ingredient molecules to undergo passive diffusion via the paracellular route, the molecules require certain properties which relate to molecular weight/volume, flexibility and charge, and are generally restricted to small polar compounds (molecular weight < 250 g/mol) (Mohammed et al., 2011).

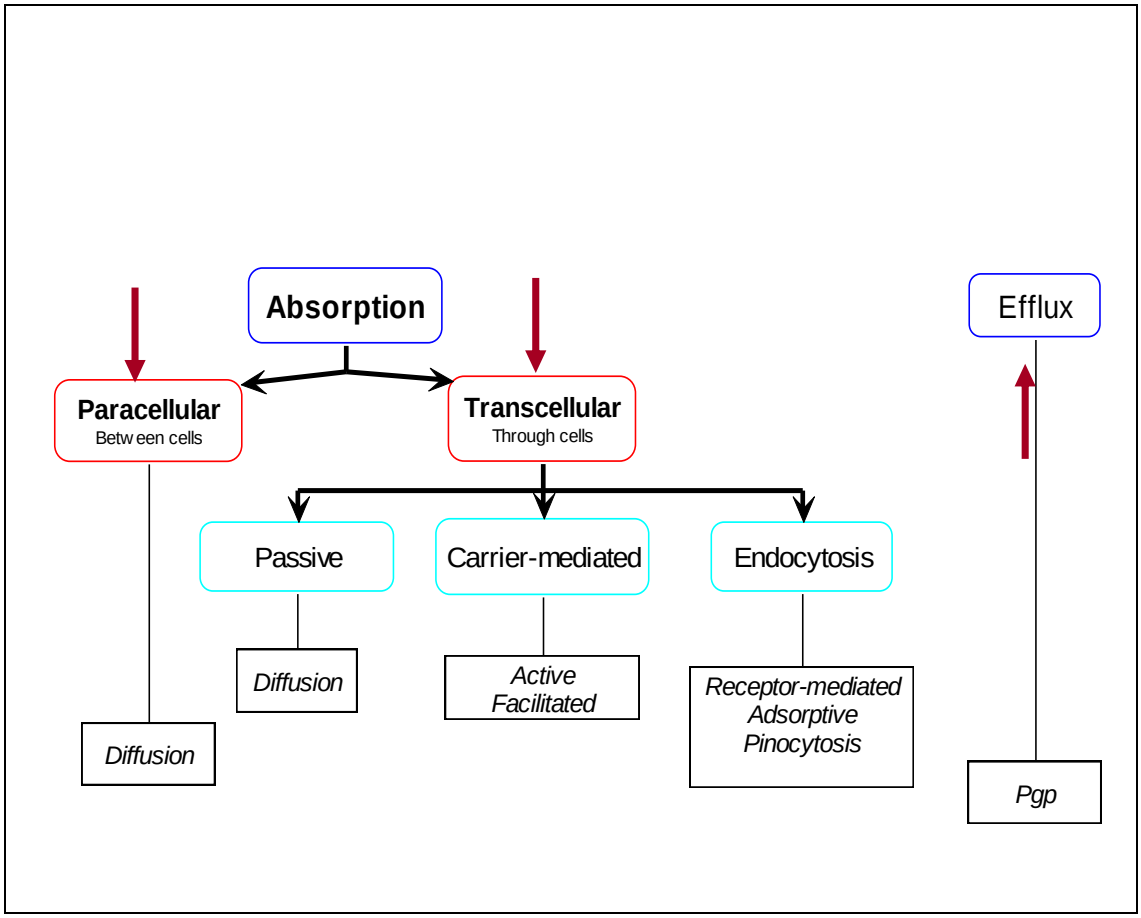


Figure 1.2 Flow chart summarising the various processes encountered in drug absorption from the GIT.

Drug molecules can also cross the intestinal mucosa via active transport absorption routes and mechanisms, as shown in Figure 1.2. The drug molecules move against their concentration gradient, which is driven by adenosine triphosphate (ATP). In terms

of carrier mediated routes and mechanisms, the drug molecules require specific binding with the transporter proteins for permeation across the cells. Efflux and influx routes follow a similar principle to carrier mediated routes, in which an active drug ingredient molecule binds to a transporter/carrier. In the case of efflux, an active drug ingredient molecule enters the epithelial cell via the apical side, and binds to an efflux transporter, which transports the drug molecule back to the lumen of the small intestine. Whilst in the case of influx, an active drug ingredient molecule enters the epithelial cell via the apical side, and binds to an influx transporter, which transports the drug molecules to the basolateral side of the epithelial cell, for absorption into systemic circulation (Mohammed et al., 2011).

Active drug ingredient molecules can be transported across the intestinal mucosa membrane via endocytosis, in which an active drug ingredient molecule fuses with the lipid membrane on the apical side of the membrane, and a membrane forms around the molecule. The molecule then travels through the cell and exits the cell by fusing with the lipid membrane on the basolateral side of the membrane.

1.2 Swallowing Issues and Dysphagia related to Conventional Solid Oral Dosage Forms

Of the various oral dosage forms available, tablets are considered the most common (Alderborn, 2007). Some of the reasons for the high acceptability of tablets include:

- tablets exhibit superior chemical and physical stability, compared to liquid dosage forms
- dose uniformity
- low production cost
- easy to handle, and can be modified in terms of their use and delivery of the API (Alderborn, 2007).

Despite the numerous advantages of tablets as solid dosage forms, and the reasons why they are considered the most common form currently in use, a significant issue/disadvantage with tablets and other solid oral dosage forms exists. A number of people have difficulty swallowing their medications, *e.g.* it has been reported that around one third of patients in long term care facilities experience difficulties with swallowing solid oral dosage forms, and in general, it has been reported that up to one

third of people in all age groups experience swallowing difficulties during their lifetime (Stegemann et al. 2012).

Additionally, it is expected that swallowing issues and dysphagia, which have a direct impact on oral medication administration and patient compliance, are becoming an increasing problem due to the aging population (Stegemann et al. 2012).

Swallowing difficulties are not only related to aging (presbyphagia) but also to pathological conditions (dysphagia) (Stegemann et al. 2012). Examples of pathological conditions causing dysphagia include; central-nervous diseases such as Parkinson's disease, musculoskeletal diseases such as osteoarthritis, metabolic diseases such as diabetes and finally oncological diseases such as oropharyngeal tumours (Stegemann et al. 2012). Additionally, certain APIs can induce dysphagia, such as the antipsychotic; haloperidol (Stegemann et al. 2012).

In terms of the impact of the formulation of solid oral dosage forms on dysphagia, it has been identified that the; size, shape and surface texture of the dosage forms are the principle factors influencing swallowability (Kelly et al. 2010). In particular, it has been reported that patients report that bigger sized solid oral dosage forms and poorer quality of tablet coatings have led to non-compliance and possibly discontinuation of medication programs (Payot et al. 2011).

To overcome the issues of dysphagia and difficulty in swallowing, a survey has shown that two thirds of the patients, who participated, dealt with this by opening capsules or crushing tablets before administering their medication, whilst two thirds of patients ceased their medication programs (Strachan and Greener, 2005). Modifying the solid oral dosage form, by opening capsules or crushing tablets can result in possible API instability and an alteration of the performance of the solid oral dosage form which has not been tested or authorised (Stegemann et al. 2012). These modifications of the stability of the API and the performance of the solid oral dosage form can possibly result in adverse effects or intoxication to/of the patients (Cornish, 2005). Additionally, another administration issue can become apparent, such as patients being exposed to poor tasting formulation ingredients (Kelly et al. 2009 and Kelly et al. 2010).

1.3 Orally Disintegrating Tablets (ODTs) and Their Advantages

To overcome the issues of patients having difficulty swallowing their medication, in particular, medications in the form of solid oral dosage forms, a potential solution to this problem is orally disintegrating tablets (ODTs). ODTs which are also referred to as orodispersible and fast disintegrating tablets, are tablets which when placed in the mouth, disperse/disintegrate rapidly before being swallowed, due to the action of saliva (Council of Europe, 2002).

ODTs are characterised as solid oral preparations that disintegrate rapidly in the oral cavity, with an *in vitro* disintegration time of approximately 30 seconds or less when based on the United States Pharmacopoeia (USP) disintegration test method (Food and Drug Administration (FDA), 2008). The distinct advantage of ODTs is that they are products which are designed to disintegrate or dissolve rapidly on contact with saliva, thus eliminating the need to chew the tablet, swallow an intact tablet, or take the tablet with liquids (FDA, 2008).

ODTs are solid oral dosage forms, but as they disintegrate in the oral cavity, they perform as suspensions or solutions (based on the solubility of the API), which as discussed earlier, exhibit the most effective absorption of APIs. Consequently ODTs can provide a more rapid onset of therapeutic action, compared to tablets and capsules.

One of the several advantages/benefits of ODTs as solid oral dosage form is that ODTs aid the administration of APIs to patients who experience swallowing difficulties or to those patients who have no access to water (Seager, 1998). ODTs therefore are a convenient dosage form not only as an alternative to conventional tablets/capsules, which some people find difficult to swallow, but also to patients who have no access to water/liquids, which is used to aid the administration of conventional tablets/capsules.

Schwartz et al. (1995) reported in a study, which investigated the preference of patients to administer freeze-dried ODTs or conventional tablets of famotidine, that 75% of the sample population revealed a preference for freeze-dried ODTs and the results from the study were consistent for younger (< 60 years old) and older (> 60 years old) subjects.

Clarke et al. (2003) reported in a study which investigated patient preference for administering either a lyophilised ODT or conventional tablet of selegiline (used in the

treatment of Parkinson's disease), that 78% of the subjects preferred the ODT to their normal/conventional tablet, with 98% of the subjects reporting that they found the ODT easy to administer. Meanwhile, Clarke et al. (2003) highlighted the potential application of ODTs to patients suffering from certain conditions/diseases, by reporting that due to the ease of administration of ODTs, they can be particularly be applicable in conditions such as Parkinson's disease, where the incidence of dysphagia is a common occurrence with patients (reportedly up to 82% of Parkinson's disease patients may suffer from dysphagia) (Edwards et al. 1991, Kurihara et al. 1993, Leopold and Kagel 1996 and Singer et al. 1992).

Another advantage/benefit of ODT's, is that they can improve the overall clinical performance of medications by reducing the incidence of medication non-compliance (Seager, 1998). As already discussed, a significant number of people who suffer with issues of dysphagia and swallowing difficulties cease to adhere to their medication programs (Strachan and Greener, 2005). ODTs therefore potentially have a significant application in addressing the issues of dysphagia and subsequent poor compliance with medication programs.

Schwartz et al. (1995) reported that freeze-dried ODTs have the potential of improving compliance, when used as an alternative to tablets and other conventional dosage forms. In fact, in a recent study which compared the compliance rates of two oral delivery methods for the delivery of selegiline (used in the treatment of Parkinson's disease); standard pill and an ODT formulation that achieved pre-gastric absorption, results showed a 98.5% compliance rate with the ODT formulation compared to 81% with the standard oral treatment in US Medicare patients (Hamlen and MacGregor, 2011).

Clarke et al. (2003) reported the potential for an ODT product to improve patient compliance for patients suffering from Parkinson's disease, due to the ease of administration of ODTs, in particular for patients suffering from dysphagia.

A third advantage/benefit of ODTs is that they can enhance the clinical effects of some APIs through pre-gastric absorption from the mouth, pharynx and/or oesophagus, which can lead to an increase in bioavailability and reduction in adverse drug reactions (if these reactions are caused by first-pass hepatic metabolism) (Seager, 1998). An example of a freeze-dried ODT product which demonstrates pre-gastric absorption (predominantly buccal) is Zelapar™, in which selegiline is the core therapeutic

candidate administered for the treatment of Parkinson's disease (Kearney, 2003 and Clarke et al. 2003). Due to pre-gastric absorption, a 1.25 mg dose ODT has equivalent efficacy to a 10 mg dose conventional tablet, with the added advantage of a reduction in adverse drug reactions (Kearney, 2003 and Clarke et al. 2003). The 1.25 mg dose ODT showed greater than 90% reduction in plasma levels of the primary metabolites of selegiline (Kearney, 2003 and Clarke et al. 2003), which are pharmacologically active and associated with neurotoxicity (Brust, 1993) and cardiovascular toxicity (Pickar et al. 1981 and Churchyard et al. 1997).

ODTs are also associated with certain drawbacks and disadvantages, *e.g.* generally, ODTs exhibit poorer mechanical properties than conventional tablets. Another disadvantage of ODTs is that taste-masking of poor-tasting excipients and/or APIs needs to be considered with ODT formulations, as these tablets disintegrate in the oral cavity. In addition, other factors which are applicable for consideration in the development of conventional tablets also apply to ODT. These include: physicochemical properties of the active drug ingredient in the gastrointestinal fluids, *e.g.* particles size, crystal form and chemical stability, nature and quantities of the diluent, binder and other excipients and conditions of storage.

1.4 Technologies Used to Manufacture ODTs

A number of technologies are used to manufacture ODTs, namely; freeze-drying (lyophilisation), moulding and conventional compression methods (Fu et al. 2004). More recently, novel technologies have emerged, which include tablet loading (Holm and Slot 2009), compression of pulverized components (Bauer and Rohrer 2007) and sublimation (Lee et al. 2002).

In a review carried out by AlHusban et al. (2010a), which involved evaluating patents based on ODTs, published from 1999 to 2010, techniques used to prepare the ODTs were quantitatively analysed. Figure 1.3 shows the proportion of each technology applied to make the ODTs, which were detailed in the patents evaluated. Figure 1.3 shows that 85% of the patents related to conventional tablet compression technologies (direct compression and granulation-compression) (AlHusban et al. 2010a). This could possibly be attributed to the low costs of performing conventional compression technologies, as standard equipment and materials are used. 2% of the patents were attributed to tablet loading and compression of pulverised components technologies, respectively, which are both new technologies used in the preparation of ODTs

(AlHusban et al. 2010a). Only 4% of the patents related to freeze-drying technologies, whilst 9% of the patents were attributed to tablet moulding technologies (AlHusban et al. 2010a).

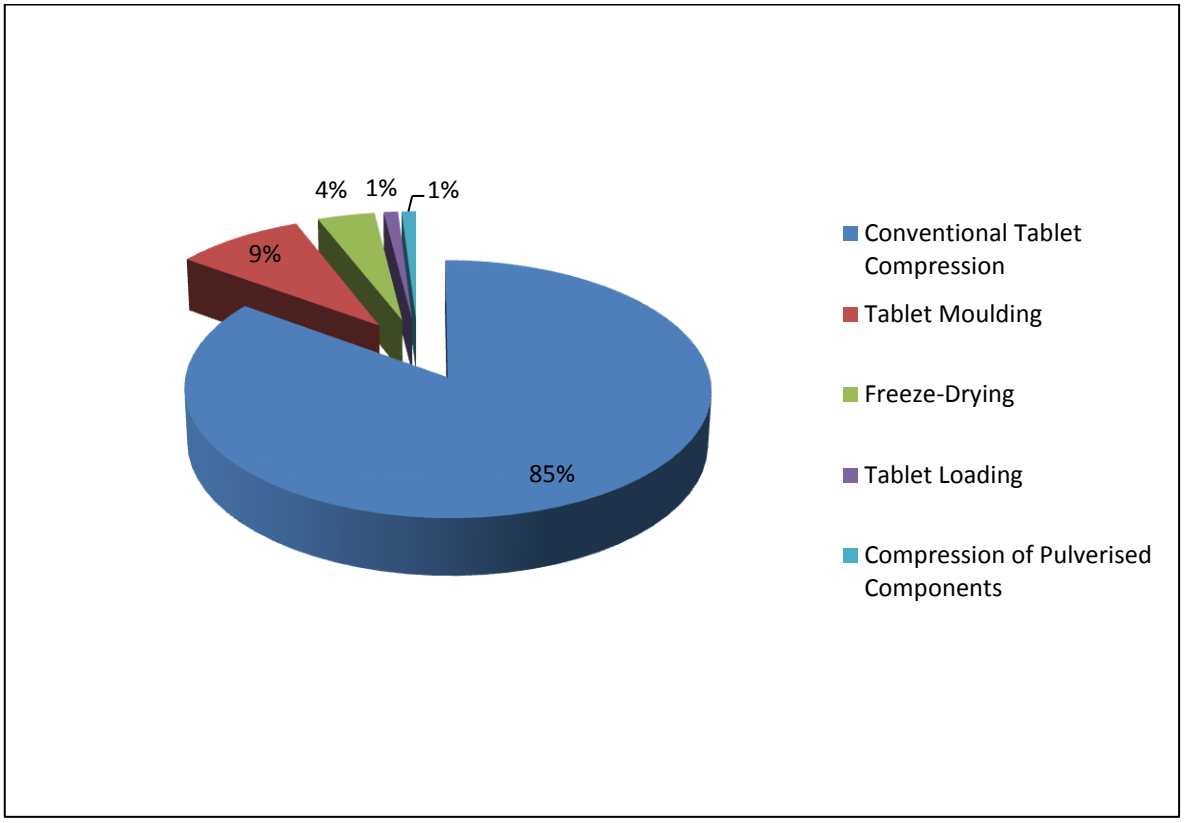


Figure 1.3 The various technologies used in the manufacture of ODTs in the period 1999 to 2010. The results shown are the percentage of patents published from 1999 to 2010, detailing the technology used to prepare ODTs. Figure adapted from AlHusban et al. (2010a).

1.4.1 Conventional Compression Methods – Direct Compression and Granulation-Compression

The use of direct compression to form ODTs, involves the mixing of carefully chosen excipients followed by compression at low forces in order to maximise the porosity of the tablets to encourage rapid disintegration of the tablets in the mouth.

As mentioned, systematic investigation of the excipients combinations needs to be carried out for the successful development of ODTs, to ensure that the tablets display rapid disintegration, possess a pleasant mouth-feel and exhibit satisfactory mechanical properties (AlHusban et al. 2010a).

The excipients used to make directly compressed ODTs generally include a disintegrant *e.g.* starch or a combination of disintegrants, which promotes the swift disintegration of the tablets. In addition to this, a sugar alcohol, (mannitol is commonly used) is used due to its high water-soluble nature which with the disintegrants promotes the rapid disintegration of the tablets.

Interestingly it has been reported that some formulations consist of water-insoluble excipients, such as microcrystalline cellulose (AlHusban et al. 2010a). The rationale for this was to utilise the repulsive forces between these excipients and the water-soluble excipients, as a means of promoting tablet disintegration (AlHusban et al. 2010a). In addition to the two principle excipients; disintegrants and highly water-soluble excipients, directly compressed ODT formulations also include supplementary excipients such as binders, glidants and flavouring agents.

The use of conventional compression methods is not only limited to direct compression, but also extends to granulation, and the subsequent compression of the granules. The general approach of formulating these types of ODTs involves the mixing of highly water-soluble excipients and disintegrants (as discussed with the direct compression formulations), and the API, followed by granulation to form rapidly dispersible granules which are subsequently compressed to form ODTs (AlHusban et al. 2010a). Examples of other excipients used in these types of formulations, that differ from the standard excipients, are other hydrophilic materials such as amino acids (Ohta et al. 2004). Meanwhile, the use of effervescent agents such as sodium carbonate and malic acid (used in combination) has also been reported in order to encourage tablet disintegration (Ouali, 1998).

In addition to the careful selection of excipients, another approach which has been reported with these types of ODTs is the development of innovative production methods in order to prepare ODTs via granulation (AlHusban et al. 2010a). *E.g.* Ramalho and Mulchande (2006) reported a method of mixing the API with pre-formed granules, which consisted of mannitol and maize starch gum, therefore omitting the API from the granulation process.

The use of granulation to prepare ODTs has also been used to compress multiparticulates into ODTs, with the aim of masking bitter tasting APIs and protecting the APIs. An example of this approach has been reported by Mimura et al. (2009) in order to mask the taste of the bitter-tasting mitiglinide calcium hydrate, by coating the

API granules with the water insoluble polymer; ethyl acrylate-methyl-methacrylate copolymer.

The method of coating APIs has also been applied to deliver acid-labile APIs in the form of ODTs, through the use of enteric coating. *E.g.* Shimizu et al. (2001) reported a multi-step method to prepare enteric coated pellets of lansoprazole. This involved coating a neutral core with the API and basic inorganic salt, followed by coating with a water-soluble polymer (such as hydroxypropyl methylcellulose), enteric coating and finally the application of a sugar-alcohol (mannitol). This approach allows the pellets to reach the small intestine, dissolve, and release the API.

It has also been reported that ODTs prepared by conventional compression methods, undergo post-compression treatment in a bid to improve the physical and disintegration properties of the tablets. An example of this approach was reported by Uemura et al. (2009), in which an alcohol solvent was applied to the surface of the tablets, to dissolve the binder, and consequently form bridges between the granules. Following the evaporation of the alcohol, the binder-bridges solidified, which improved the physical properties of the tablets (Uemura et al. 2009).

1.4.2 Tablet Moulding

The use of moulding to manufacture ODTs, principally involves the use of water-soluble ingredients (Fu et al. 2004). The manufacturing method involves moistening the powder mixture with a solvent, in which ethanol or water are commonly used, and then moulding the mixture into tablets under pressures which are lower than those used in conventional tablet compression methods (Fu et al. 2004). The solvent used during tablet manufacture, is ultimately removed by air-drying, and as the tablets are manufactured using pressures which are lower than those used in conventional tablet compression methods, the tablets have a high porosity, which subsequently enhances dissolution (Fu et al. 2004) and results in tablets which exhibit rapid disintegration. More recently, variations/modifications in the moulding manufacturing method have emerged, such as heat moulding and no-vacuum lyophilisation.

An example of the use of heat moulding has been reported by Takaishi et al. (2005). The authors investigated the use of mannitol and erythritol (saccharides) which melt following heating, and form bridges between the formulation ingredients, with an ultimate objective of improving the physical properties of the tablets.

A significant development in the field of ODTs prepared by moulding was reported by Myers et al. (1999) who reported an apparatus which combined each step of the tablet preparation process; mixing, filling, tamping and curing. This development therefore improved the efficiency and convenience of preparing ODTs using the moulding method.

1.4.3 Tablet Loading

Holm and Slot (2009) recently reported a new method of preparing ODTs. The tablets prepared using this method usually consists of at least 60% of a sorbent material such as magnesium oxide and metal silicate such as sodium silicate. In addition a hydrophilic substance (*e.g.* glucose) and superdisintegrant (such as sodium carboxymethyl cellulose) were included in the formulation, together with a filler and binder.

The formulation was compressed, followed by loading of the tablet with the API (in liquid form), which involved spraying the liquid onto the tablet, or placing the tablet in an excess of the liquid (which can be in the form of a suspension). The tablet loading method of preparing ODTs was claimed to be applicable for poorly water-soluble APIs. (Holm and Slot, 2009).

1.4.4 Compression of Pulverised Components

In addition to the tablet loading method, a novel new method of manufacturing ODTs has also been reported which involves compressing components which are in a pulverised form. It has been reported that tablets prepared using this method display a similar porous structure to the tablets prepared using freeze-drying.

The formulation of tablets prepared using this method usually consist of a dry mixture of a binder such as acacia, API, filler, and other supplementary excipients such as a lubricant. Under high pressure, liquefied or compressed gases or gas mixtures, optionally in the presence of a low-boiling point solvent (*e.g.* methanol) is used to moisten the dry mixture. This is followed by the preparation of a mouldable plasticised mass, following the stirring and homogenisation of the mixture in an autoclave. Tablets are prepared by filling the wetted mixture in a mould under pressure, followed by decompression to produce the highly porous ODTs. (Bauer and Rohrer, 2007).

1.4.5 Freeze-Drying

Freeze-drying, which is also known as lyophilisation, is the method which produces tablets that exhibit the most rapid disintegration and dissolution, due to their highly porous nature, that allows the rapid penetration of saliva into the porous matrix of the tablets. The proposed desired properties of freeze-dried ODTs are shown in Table 1.2.

Table 1.2 Proposed desired properties for freeze-dried ODTs.

Tablet Property	Desired Value
Tablet Hardness	12 N
Tablet Disintegration Time	10 s
***In Vitro* Oral Retention Time**	100 s

The process of freeze-drying generally consists of three steps, which include; freezing, primary drying and secondary drying. The first step, freezing, involves the transition of water from a liquid state to a frozen (solid) state, consequently, the solutes are separated from the ice.

The primary drying step, which is also referred to as ice sublimation, involves the application of vacuum (a reduction in pressure) and an increase in shelf temperature. During this step, ice is transferred from a solid state to a vapour/gaseous state, due to sublimation. The final step of the freeze-drying process, secondary drying, is performed at an increased temperature and under vacuum, in order to remove water from the freeze concentrate. (Tang and Pikal, 2004) (Figure 1.4).

Figure 1.4 Graph demonstrating the triple point below which sublimation occurs. S in the diagram denotes solid phase, L represents liquid phase and G gaseous phase. The point at which all the three phases converge is referred as the triple point. (Polyparadigm, 2006).

The particular advantages of freeze-drying are that the initial solution is frozen such that the final dry product is a network of solid occupying the same volume as the original solution. Thus the product is light and porous and readily soluble, which subsequently exhibits rapid disintegration in the oral cavity when in contact with saliva. (Aulton, 2002).

Zydis® is a freeze-dried tablet-shaped ODT dosage form product, which has been in commercial production since 1986. Zydis® tablets are unique, as they spontaneously disintegrate in the mouth in seconds, due to their characteristic high porosity, produced by the freeze-drying process (Figure 1.5). The highly porous structure of the tablets, allows the rapid ingress of saliva, which subsequently quickly dissolves the soluble excipients, thus releasing the drug particles as a suspension or solution on the tongue. The suspension or solution is then swallowed, and the drug is absorbed in the conventional way. (Kearney 2003). Interestingly, human *in vivo* studies, using gamma scintigraphy, have shown that even when taken without water, the component materials of the formulation uniformly disperse over the mucosa and are subsequently

cleared efficiently from the buccal and oesophageal regions (Washington et al. 1989). This demonstrates the potential application of these dosage forms to encourage pre-gastric absorption of APIs.

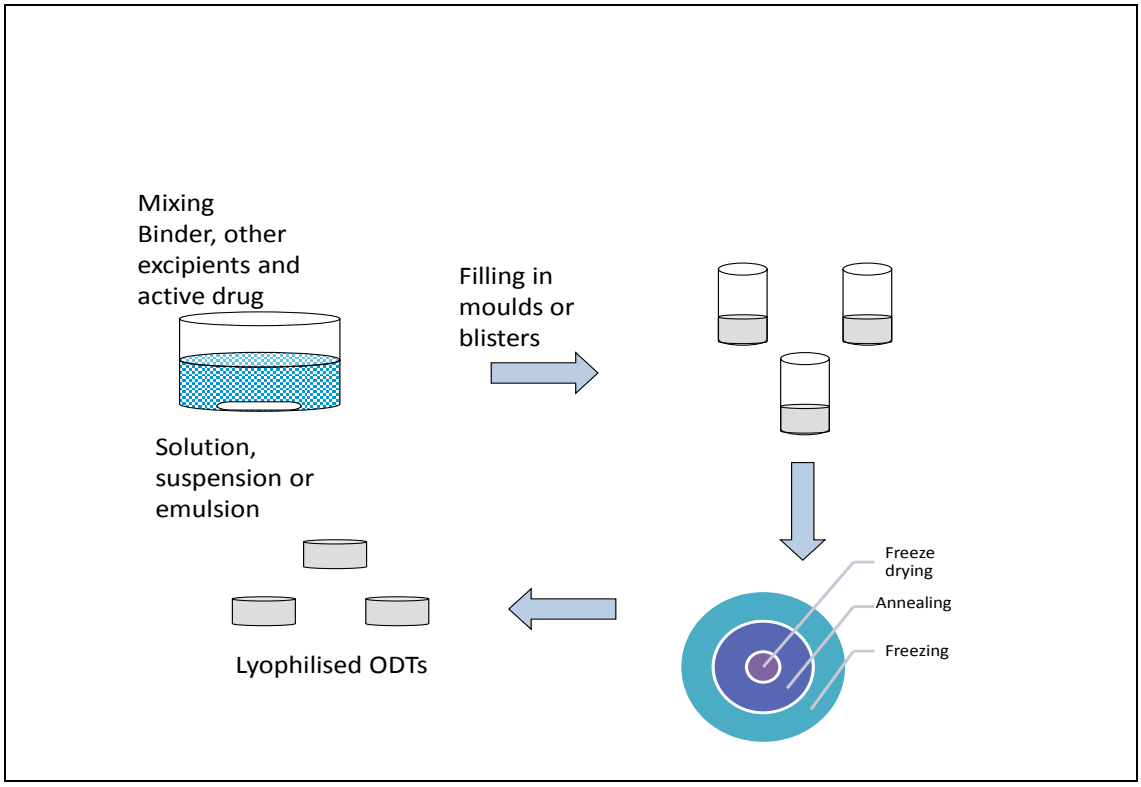


Figure 1.5 Schematic illustration representing the various stages involved in the formulation of lyophilised ODTs.

The formulation of Zydis® products, consist of a tablet matrix composed of materials that can be readily freeze-dried and also impart sufficient strength to allow easy removal from the packaging. The selected materials also have to be compatible with a wide range of APIs. Kearney (2003) reported that no single excipient had the ideal characteristics, but a suitable matrix structure was achieved by using the combination of a water-soluble polymer (*e.g.* gelatin) and a sugar alcohol (*e.g.* mannitol).

The Zydis® formulating process requires an API to be dissolved or suspended in an aqueous solution of water-soluble structure formers. Gelatin and mannitol are the two most commonly used structural excipients, whilst other suitable structure formers such as starch and gums can also be used, depending on the properties of the APIs. A general rule with Zydis® products is that the best physical characteristics are achieved by using a mixture of a water-soluble polymer and a crystalline sugar-alcohol or amino acid, at a typical combined concentration of 10% w/w in the matrix solution. The

polymer component provides the strength and resilience, whilst the crystalline component provides the hardness and texture. (Kearney 2003).

Lyoc™ is another freeze-dried ODT technology that involves preparing an oil-in-water emulsion which is subsequently freeze-dried in its final packaging (Lafon, 1986). It has been reported that Lyoc™ formulations require a large amount of an undissolved inert filler to increase the viscosity of the formulations to prevent in-homogeneity by sedimentation during freeze-drying. It was further reported that the incorporation of a large amount of an undissolved inert filler in the formulations, results in reducing tablet porosity and subsequently increasing tablet disintegration time. (Fu et al. 2004).

QuickSolv® is an ODT technology, which involves dispersing or dissolving matrix forming agents and an API in water that is consequently accurately dosed into pre-formed blisters. The formulation is then frozen, followed by immersion in a water miscible alcohol. The solidified/frozen matrix formulation is therefore exposed to the water miscible alcohol, to which the matrix components are insoluble in, and as a result water is removed yielding the matrix components and API. Residual water miscible alcohol may then be removed by placing the product in a vacuum chamber under reduced pressure. (Gole et al. 1993).

LyoPan® is an additional freeze-drying technology used to manufacture ODTs. LyoPan® differs from other freeze-drying technologies, as the LyoPan® process utilises partial freeze-drying as not all of the formulation undergoes freeze-drying. The LyoPan® process involves the formulation existing as two components; a powdered component and an aqueous component. The powdered component is firstly dosed into the tablet mould followed by the aqueous component and as a result only a small volume of the formulation is frozen and dried in this partial or reduced freeze-drying process. The LyoPan® freeze-drying process has several distinct advantages, in particular; as the amount of water used by this process is considerably less than completely freeze-dried formulations, which consequently results in corresponding savings in production time and energy consumed, the freeze-drying time is significantly reduced. Additional advantages include; high doses of API can be incorporated in the tablets, the absorption properties of APIs can be controlled or adjusted by using pre-processed pellets, *e.g.* pellets with intestinal soluble coatings, and finally taste-improving components and/or sweeteners can be added to the formulations. (Bauer, 2007).

NanomeIt®, a Nanocrystal™ technology, is another application used with freeze-dried ODTs, which is claimed to improve compound activity and final product characteristics. The fundamental principle, on which the technology is based, involves decreasing the particle size of the API, which increases its surface area that subsequently results in an increase in the dissolution rate of the API. The technology is used for poorly water-soluble APIs. Nanocrystal™ particles are nano-sized drug substances, typically less than 1000 nm in diameter, which are produced by milling using a proprietary wet milling technique and are stabilized against agglomeration to create a suspension that behaves like a solution. In order to formulate the freeze-dried ODTs, Nanocrystal™ colloidal dispersions of API are combined with water soluble ingredients, which are filled into blisters and lyophilized. It is claimed that this approach is mainly used when formulating highly potent or hazardous materials, as it avoids a number of manufacturing steps, commonly encountered when formulating conventional tablets, such as granulation, blending and tableting which generate large amounts of aerosolised powder and possess a greater risk of toxicity through exposure. (Badgujar and Mundada, 2011).

As discussed above, extensive research has been focussed on freeze-drying technology in order to develop ODT products. In addition to this, widespread research has focussed on the selection of excipients, in particular the polymeric binder and matrix supporting/disintegration enhancing agents used in freeze-dried ODT formulations, as identified by AlHusban et al. (2010a) in a review of recent research trends in the field of ODTs.

As reported earlier, gelatin is one of the most commonly used excipients in the formulation of freeze-dried ODTs (Kearney, 2003). However, a significant disadvantage of using gelatin as an excipient in pharmaceutical products is that non-adherence to medication programs can occur due to religious beliefs and/or dietary preferences (Sattar et al. 2004).

Sattar et al. (2004) reported that patients stopped taking their medication when they discovered that gelatin was used as an excipient in their medication. The consequences of patients ceasing their medication programs can include worsening of clinical symptoms and relapse of their illness'. The long term effects of medication nonadherence due to religious beliefs can include increasing hospitalisation rates and healthcare costs, and possibly poor healthcare provider-patient relationships. (Sattar et al. 2004).

In a study reported by Vissamsetti et al. (2012) which involved investigating patient preferences to the origin of excipients in formulations, 40% of the patients responded that they had a dietary restriction which requires avoiding consumption of animal-derived excipients.

Other findings from the study by Vissamsetti et al. (2012), indicated that 150 (85.2%) of the 176 patients who preferred vegetarian only treatment stated that they would not take any oral medication which comprised of animal-derived excipients. However, 100 (56.8%) of these would consume medications consisting of animal-derived excipients if no alternatives were available. Interestingly, only 36 (20.5%) of patients who preferred vegetarian medication would have specifically asked their doctor or pharmacist regarding the composition of their medication, to see if it conflicted with their dietetic restrictions. It was also reported in the study that 20% of the UK population might practice dietetic restriction due to religious reasons. (Vissamsetti et al. 2012).

It has been reported that one approach for decreasing the inadvertent prescription of medications consisting of animal-derived excipients, is to modify pharmaceutical formulations so that only non-animal-derived excipients are used (Vissamsetti et al. 2012).

The ethical and formulation issues related to gelatin, were the rationale identified by Li et al. (2007) in order to identify alternatives to gelatin in the formulation of freeze-dried ODTs. Li et al. (2007) identified pullulan as an alternative to gelatin, with the added advantages of simpler formulation process steps and shorter freeze-drying time. Murray et al. (2003) also reported a gelatin-free freeze-dried ODT formulation using modified starches as alternatives to gelatin. Murray et al. (2003) claimed that higher concentrations of API could be used with formulations consisting of modified starches than with formulations consisting of gelatin.

The use of alternative materials to gelatin has also been reported by Remon and Corveleyn (2000) and Johnson et al. (2002). Remon and Corveleyn (2000) reported the use of maltodextrin as matrix forming agents and water-soluble polymers such as xanthan gum, methylcellulose and hydroxypropyl methylcellulose as binding agents. Johnson et al. (2002) meanwhile reported the use of a wide range of polymeric materials derived from plant and synthetic origins as binding agents, *e.g.* acacia, agar and dextran. Johnson et al. (2002) additionally reported the use of sugar alcohols

and/or amino acids as matrix supporting/disintegration enhancing agents in the formulation of freeze-dried ODTs.

Subsequent chapters of this thesis investigated other materials as alternatives to gelatin in the formulation of freeze-dried ODTs including Methocel™ (hydrophilic cellulose ethers) and Kollicoat® IR (polyvinyl alcohol-polyethylene glycol co-polymer). Additionally, Chapter 7 of this thesis investigated the novel approach of incorporating polymeric biodegradable nanoparticles in freeze-dried ODTs to develop sustained release formulations comprising of non-animal-derived excipients.

1.5 Recent Trends and the Need for Further Developments in the Field of ODTs

The field of ODTs has witnessed extensive developments over recent years. In relation to ODTs prepared by conventional compression methods (direct compression and granulation-compression), these technologies appear most extensively developed. This can possibly be attributed to the standard equipment used, low costs of preparing the tablets and the application of a wide range of excipients together with the ease at which these technologies can be adapted in terms of the methods used to prepare the tablets (AlHusban et al. 2010a).

These technologies have seen recent developments in the selection of excipients used in the formulations. For instance, the use of water-insoluble excipients to promote tablet disintegration by utilising the repulsive forces between water-soluble and insoluble components is an innovative approach. There have also been developments relating to the granulation methods used to prepare the tablets. Additionally, developments in the taste-masking of APIs have enabled commercial exploitation of these technologies. This has allowed ODTs to be used to deliver acid-labile APIs, to maximise absorption in the small intestines and subsequently increase bioavailability. (AlHusban et al. 2010a).

There have also been developments regarding improving the physical and disintegration properties of ODTs, through the application of post-compression treatments to the tablets. This demonstrates that development opportunities with ODTs not only exist with the formulation of the tablets but also post formulation compression of tablets. (AlHusban et al. 2010a).

The emergence of tablet loading and compression of pulverised components technologies, as novel methods of preparing ODTs, highlights the efforts made in this field to continue to develop the properties of the tablets. The tablet loading method claims to be applicable for poorly water-soluble APIs, whilst the compression of pulverised components method, is reported to prepare tablets which exhibit a similar porous structure to the tablets prepared using freeze-drying. (AlHusban et al. 2010a).

Recent developments in ODTs prepared by freeze-drying have involved advancements with the technology itself, such as the LyoPan® technology, as discussed earlier. Additionally, developments have focussed on the selection of excipients which are suitable for being applied to freeze-dried ODT formulations, with particular emphasis on uncovering water-soluble polymeric binders which can be used as an alternative to gelatin. (AlHusban et al. 2010a).

Despite the recent developments in the field of ODTs, one of the principal motivations for further work into this area is the aging population. It is widely acknowledged that the population in both developed and developing nations is aging, and the number of people aged 60 years or older, is projected to increase significantly over the next 38 years, as shown in Figure 1.6. As discussed earlier aging is one factor which is related to difficulty in swallowing (Stegemann et al. 2012). Consequently, ODTs are particularly beneficial for geriatric patients, as they are convenient to administer.

Figure 1.6 Projection of the number of people aged 60 years or over, in both developed and developing countries, from 1950-2050. Figure sourced from: United Nations Department of Economic and Social Affairs (2011).

The ability for elderly patients to administer their medication easily and conveniently is particularly important, as a significant number of elderly patients are prescribed multiple medications for a range of medical conditions. Consequently, these patients are expected to administer several medications, a number of times during a day. As a result, the potential for medication errors and nonadherence to medication programs is high. As discussed earlier, the consequences of medication nonadherence includes; worsening of clinical symptoms and increasing healthcare costs (Sattar et al. 2004). Additionally, it has been shown that patients comply better with an ODT tablet than with a conventional tablet (Hamlen and MacGregor, 2011), therefore ODTs appear as a logical method of improving patient compliance and reducing nonadherence to medication programs, especially in chronic long term medical conditions.

1.6 Aims and Objectives of the Thesis

Due to the aging population and the anticipated increase in the emergence, development and use of convenient dosage forms, such as ODTs, the research in this thesis aims to contribute to the formulation of ODTs prepared by freeze-drying. The principle aim in this thesis is to evaluate the application of novel commercially available polymers in the development of freeze dried ODTs together with investigating techniques to formulate APIs in a sustained release system that would disintegrate rapidly. It is anticipated that success of the above two strategies will have significant impact on the availability of ODT dosage forms that will have wider appeal to patient populations from diverse social and religious beliefs, improve bioavailability through application of simple techniques such as viscosity enhancement and increase of pre-gastric residence time and reduce the frequency of administration of dosage forms with the overarching accomplishment of enhancing patient healthcare through novel formulation excipients and developmental strategies.

As discussed above, there is both a societal and a scientific requisite to further develop ODT formulations through the testing of existing new materials and novel formulation strategies. The proposed work in the thesis has been investigated to develop a necessary framework to illustrate the properties of material suitability in the formulation of freeze-dried ODTs. Together with studying and developing novel product development methodologies with the ultimate aim of engineering novel ODTs based on composition and the dosage form being accepted by a wide range of patient populations from diverse backgrounds.

The objectives of the work can be classified into three critical domains:

1. Evaluation and understanding of formulation development and characterisation of widely studied gelatin-based ODT preparations, to extract the key parameters in formulation and process development.

2. Investigation of the suitability of various materials including cellulose derivatives, and synthetic polymers such as polyethylene oxide and polyvinyl alcohol-polyethylene glycol co-polymer through a systematic and comprehensive evaluation of material properties.

3. Development and optimisation of novel sustained release ODTs through extensive studies on integration of multiple formulation methods into a simple and scalable technology.

Chapter Two: The Influence of Formulation and Manufacturing Process Parameters on the Characteristics of Lyophilised Orally Disintegrating Tablets

Publication Related to This Chapter: JONES, R.J., RAJABI-SIAHBOOMI, A., LEVINA, M., PERRIE, Y. and MOHAMMED, A.R. (2011) The Influence of Formulation and Manufacturing Process Parameters of the Characteristics of Lyophilised Orally Disintegrating Tablets. *Pharmaceutics*, 3 (3), pp. 440-457.

2.1 Introduction

Orally disintegrating tablets (ODTs), which are also referred to as orodispersible and fast disintegrating tablets, are tablets which when placed in the mouth, disperse/disintegrate rapidly before being swallowed, due to the action of saliva (Council of Europe, 2002). The Food and Drug Administration recommends that ODTs be considered as solid oral preparations that disintegrate rapidly in the oral cavity, with an *in vitro* disintegration time of approximately 30 seconds or less, when based on the United States Pharmacopeia disintegration test method or alternative (Food and Drug Administration, 2008). This form of solid dosage form is therefore highly applicable for groups of the population who commonly have difficulty in swallowing conventional solid dosage forms (*e.g.* conventional tablets and capsules), such as pediatric and geriatric patients (Abdelbary et al. 2005).

A number of technologies have been used to manufacture ODTs including freeze-drying (lyophilization), molding and conventional compression methods (Fu et al. 2004). More recently new technologies such as tablet loading (Holm and Slot, 2009), compression of pulverized components (Bauer and Rohrer, 2007) and sublimation (Lee et al. 2002) have also been reported.

However, ODTs manufactured using freeze-drying have been the most successful commercially. Tablets manufactured using this technology, generally exhibit rapid disintegration and dissolution due to their highly porous nature, which allows penetration of saliva into the matrix of the tablets, resulting in disintegration. The freeze-drying process involves the transition of water from liquid to solid during freezing, and then solid to vapor during sublimation (Aulton, 2002). A particular advantage of freeze-drying is that the solution is frozen such that the final dry product is a network of solid occupying the same volume as the original solution, resulting in a light and porous product which is readily soluble (Aulton, 2002).

Gelatin and mannitol are both excipients which are used in the formulation of freeze-dried ODTs. These materials are responsible for forming the highly porous matrix structure of the dosage form. Gelatin, a protein, which acts as a glassy amorphous compound, provides structural strength, whilst mannitol (a sugar alcohol) provides crystallinity, hardness and elegance. Water is used as a manufacturing process media, which induces the porous structure upon sublimation during the freeze-drying stage. (Sastry et al. 2000).

Interestingly, studies investigating molecular variations in gelatin configuration have shown that various factors such as pH and salt concentration influence its packing and solubility. A pH-swelling curve for lime processed gelatin (type B) has been reported (Sheppard, 1942). The results showed that adjusting the pH away from the isoelectric point resulted in a significant swelling of the material. Subsequently, the swelling properties of gelatin could potentially be utilized to increase the porosity of the freeze-dried tablet matrix, and could lead to a reduction in disintegration time. Another study investigating the solubility of gelatin has shown that it exhibits its lowest solubility at pH 5 (isoelectric point), with improvements in solubility above the isoelectric point (Cortesi et al. 1999 and Benjakul et al. 2009). Other factors that have shown to influence gelatin swelling and solubility include the addition of neutral salts and variations in ionic strength of the formulation (Benjakul et al. 2009 and Xiao et al. 2004). Despite the availability of literature on gelatin behavior, there has been no work reported in exploiting gelatin properties under different conditions in the formulation of ODTs.

Besides varying parameters such as pH and ionic strength that will potentially influence the physicochemical properties of gelatin, another factor which has received very little attention is particle size reduction. Ball milling is a widely used technique to reduce particle size (Zahrani and Fathi, 2009) and has been shown to influence the transition of materials from crystalline to amorphous form (Lefort et al. 2004 and Mallick et al. 2008); to change the performance of a variety of dosage forms by improving their solubility (Huang et al. 2008), dissolution (Van Eerdenbrugh et al. 2007), and bioavailability (Liversidge and Cundy, 1995).

The aim of the current study was to exploit the various process parameters such as adjustment of pH, ionic strength of the formulation and ball milling to study their influence on tablet properties with the aim of reducing disintegration time without compromising tablet hardness. The formulated tablets used in this study consisted of 9% w/w gelatin and 30% w/w (of the dried tablet weight) mannitol, as excipients. The choice of formulation was influenced by the preliminary results which showed that the above combination exhibited high tablet hardness and long disintegration time (around two minutes).

2.2 Materials

Gelatin (type B, 60 and 75 bloom) and mannitol were supplied by Sigma-Aldrich Chemicals (Poole, UK). HPLC grade methanol was supplied by Fisher Scientific (Loughborough, UK). Ibuprofen, sodium chloride and sodium hydroxide were supplied by Sigma-Aldrich Chemicals (Poole, UK). Hydrochloric acid was supplied by Fisher Scientific (Loughborough, UK). All chemicals were used without further purification.

2.3 Methods

2.3.1 Preparation of Freeze-Dried Tablets

Gelatin was dissolved in double-distilled water at about 40°C, followed by the addition of mannitol to form a solution. 1.5 g of the resulting solution was dosed into a tablet mould, frozen at –70°C for a minimum of sixty minutes and freeze-dried (ADVANTAGE, Freeze-Dryer, VIRTIS) according to the following regime; primary drying for forty eight hours at a shelf temperature of –40°C, secondary drying for ten hours at a shelf temperature of 20°C and vacuum pressure of 6.67 Pa (50 mTorr). A minimum of ten tablets were prepared for each formulation.

2.3.2 Ball Milling

Mixtures of gelatin and mannitol were milled using a planetary micro mill (FRITSCH Pulverisette 7, Germany), with 45 mL agate grinding bowls and 10 mm diameter agate balls at room temperature. The milling process was performed under various conditions, as shown in Table 2.1, in order to investigate the effect of the milling parameters, i.e., milling time, rotation speed, and ball: powder weight ratio, on the; wettability, porosity of the milled samples and glass transition of the formulations in their frozen state prior to freeze-drying. Ultimately the effect of milling on the properties of the freeze-dried tablets, namely; disintegration time, porosity, hardness and fracturability were investigated. The weight of the gelatin-mannitol mixture was 3 g for all of the milling conditions. The milling parameters were determined using MODDE factorial-experimental design software.

Table 2.1 Ball milling conditions of the various formulations.

Formulation	Milling Time (minutes)	Rotation Speed (rpm)	Ball: Powder Weight Ratio
N1	15	100	5
N2	60	100	5
N3	15	400	5
N4	60	400	5
N5	15	100	15
N6	60	100	15
N7	15	400	15
N8	60	400	15
N9	37.5	250	10
N10	37.5	250	10
N11	37.5	250	10

2.3.3 Differential Scanning Calorimetry (DSC)

DSC (Pyris Diamond DSC and Intercooler 2P: Perkin Elmer, Wellessey, USA) was used to determine the glass transition temperature (T_g) of the formulations in their frozen state (before freeze-drying). 10–15 mg of the liquid samples were loaded into aluminum pans, cooled to –65°C at a rate of 5°C/min with a nitrogen purge of 20 mL/min, an empty aluminum pan was used as a reference for all measurements.

The resulting thermograms were analyzed by Pyris manager software. T_g values were determined from the intersection of relative tangents to the baseline. Three samples/measurements were taken for each formulation, and the mean values ± standard error were reported.

2.3.4 Wettability Analysis

The wettability of the milled and non-milled (control formulation) samples were analyzed using the Wilhelmy method, to determine their contact angle. A Camtel© (Hertfordshire, UK) QCT-100 surface tensiometer was used to determine the contact angle and subsequent wettability of the samples.

Glass slides measuring 24 × 24 mm in size were covered with 12 × 24 mm double-sided adhesive tape. The glass slides were then placed in the various formulations, to coat them. Excess formulation was removed by gentle tapping to ensure a uniform coat. Glass slides were then securely attached to the microbalance of the apparatus, and during wettability analysis, a glass beaker containing the test liquid (80 mL of double distilled water) was raised and lowered at a rate of 0.200 mm/s, to immerse the glass slides. During this period, contact angles were determined automatically at regular intervals.

Each formulation was analyzed in triplicate for their greatest contact angle, and mean ± standard error is reported. The test liquid was replaced for analyzing each formulation.

A linear relationship between wetting time (wettability) and disintegration time of rapidly disintegrating tablets has been reported (Sunada and Bi, 2002). Therefore, analysis of the wettability of excipients is a useful tool in understanding the performance (disintegration time) of fast disintegrating tablets.

2.3.5 Powder Porosity Analysis

The porosity of the milled and non-milled (control formulation) samples were measured using helium pycnometry (MULTIPYCNOMETER, Quantachrome Instruments, Hampshire, UK). 1 mL of sample was placed in a suitably sized sample cup and subjected to helium pycnometry, to determine the true density of the sample. The true density value was then used in the following equation (Equation 2.1) to determine the porosity of the sample:

$$\text{Porosity} = (1 - \text{bulk density/true density} \times 100\%) \quad \text{(Equation 2.1)}$$

Bulk density was determined by considering, the mass and volume of the sample. Three porosity measurements were taken for each formulation, and the mean ± standard error is reported. The porosity of the samples was expressed as a percentage.

2.3.6 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 245.17 N load cell. The instrument was calibrated by standard weights of 4.90 N and 49.03 N. The tablets were placed in a holder with a cylindrical hole. The hardness was taken as the peak force after 1mm penetration of a 5 mm diameter probe at a rate of 6 mm/min. Three measurements were taken for both hardness and fracturability, for each formulation, and the mean ± standard error is reported.

2.3.7 Disintegration Time of the Tablets

The disintegration time of the tablets was determined using a USP disintegration tester (Erweka, ZT3). 800 mL of double distilled water, which was kept at 37 ± 2°C, was used as the medium and the basket was raised and lowered at a fixed rate of 30 per minute.

Three tablets were evaluated from each formulation, and the mean values ± standard error is reported.

2.3.8 Tablet Porosity

The porosity of the tablets was measured using helium pycnometry (MULTIPYCNOMETER, Quantachrome Instruments, Hampshire, UK). Two freeze-dried tablets were placed in a suitably sized sample cup and subjected to helium pycnometry, to determine the true density of the tablets. The true density value was then used in the equation, as reported above (Equation 2.1), to determine the porosity of the tablets.

Bulk density was determined by considering tablet weight, diameter and thickness. The diameter and thickness of the tablets were determined using a screw gauge (LINEAR Farnell). Three porosity measurements were taken for each formulation, and the mean values ± standard error is reported. The porosity of the tablets is expressed as a percentage.

2.3.9 Statistical Analysis and Factorial-Experimental Design

The effect of milling on the performance and properties of freeze-dried tablets was compared to those of the control (non-milled formulation); using one-way analysis of variance (ANOVA) with the Dunnett multiple comparisons test. The significant effect of treatment/level of statistical significance was judged as being $p < 0.05$, with a confidence limit of 95%. This statistical analysis test was also used for the ionic strength study.

In terms of factorial-experimental design, the milling factors consisted of; milling time (ranging from 15–60 minutes), rotation speed (ranging from 100–400 rpm) and ball:powder weight ratio (ranging from 5–15). Eleven formulations were proposed, which underwent different milling conditions, according to the three factors, as shown in Table 2.1.

The responses measured included excipient properties; wettability, powder porosity and glass transition (of the formulations in their frozen state, prior to freeze-drying). The responses measured also included tablet properties; disintegration time, porosity, hardness and fracturability.

Statistical analysis of the dissolution of ibuprofen from tablets prepared from both non-milled and milled excipients was performed using the unpaired t-test with Welch correction. This statistical analysis test was also used for the pH study. Ibuprofen was used as a model active pharmaceutical ingredient (API), as it is a readily available API. Also, the majority of API's used in freeze-dried ODTs are insoluble or poorly soluble in water, and as ibuprofen is poorly soluble in water, it was deemed as a suitable model API to use in the dissolution study.

2.3.10 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 and vertical samples of the tablets were prepared by cutting them with a surgical blade. The samples were placed onto double-sided adhesive strips on aluminum stubs and coated with a thin layer of gold using a sputter coater (Polaron SC500, Polaron Equipment, Watford, UK) at 20 mA for three minutes (this was performed twice for each set of samples) and then examined by the SEM. The acceleration voltage (kV) and the magnification can be seen on each micrograph. The pore size of the freeze-dried tablets was measured by using the scale which was visible on each micrograph. The average pore size was measured by measuring the pore diameter of around 10 randomly selected individual pores, from these values the average pore diameter was calculated.

2.3.11 Dissolution Study and HPLC Conditions

The dissolution rate of ibuprofen from the two formulations (milled and non-milled tablet excipients), was examined using a Caleva 8ST dissolution bath. The two formulations were analyzed in triplicate. The dissolution system employed was USP dissolution apparatus 2 (paddle at 50 rpm rotation speed), for a test time of 60 minutes. The dissolution medium consisted of 900 mL of pH 7.2 phosphate buffer at 37°C. Dissolution samples were filtered through a 0.45 µm Nylon syringe filter, to remove undissolved ibuprofen. 10 µL of the samples were analyzed by HPLC (Dionex), with UV detection performed at 230 nm, on a Thermo Scientific Hypersil Gold C18 250 × 4.6 mm 5 micron column, with methanol:water (80:20) as the mobile phase and a flow rate of 1.00 mL/min.

2.4 Results and Discussion

2.4.1 Influence of Changes in pH on Tablet Formation

The first phase of the study investigated the influence of changes in pH of formulation solution comprising of gelatin and mannitol. Three pH values of 3, 5 and 8 were chosen to determine the effect of pH variation below, at and above the isoelectric point of gelatin. The results showed that tablets prepared from solutions with pH adjusted to 3 resulted in severely denatured/degraded tablets when compared to formulations prepared at pH 5 and 8.

The instability of the formulation at pH 3, which included the detection of no T_g, can be attributed to various reasons including acid hydrolysis of gelatin, maximum stability of gelatin at pH values between 5 and 8 and incompatibility of mannitol in strongly acidic solutions (Lund, 1994, Northrop, 1921 and Kramer and Inglott, 1971). The lack of intact tablet formation resulted in no further characterization of formulations prepared at pH 3.

2.4.1.1 Glass Transition and Tablet Mechanical Properties

DSC analysis of the formulation adjusted to pH 8, in its frozen state (prior to freeze-drying), indicated that this formulation exhibited a mean onset T_g of –29.1 ± 0.4 °C, whilst the control formulation (pH 5) exhibited a mean onset T_g of –29.8 ± 0.5 °C. The results have shown that pH adjustment does not have any plasticization effect or reduced the physical stability of the formulations in their frozen state (prior to freeze-drying).

Measurement of hardness of the resultant tablets prepared upon pH adjustment to 8 indicated a mean hardness of 60.0 ± 1.7 N, compared to 52.4 ± 8.8 N, the mean hardness of the tablets prepared at pH 5. Adjustment of the pH to 8 did not significantly vary the hardness when compared to pH 5 ($p > 0.05$). Similar results were obtained for fracturability studies.

2.4.1.2 Tablet Porosity, SEM and Disintegration Time Analysis

Porosity analysis of the formulation adjusted to pH 8, as shown in Figure 2.1, indicated that there were significant differences between tablet porosity upon adjustment of pH. The mean porosity value of formulations prepared at pH 8 was 93.7 ± 0.1%, whilst the formulations prepared at pH 5 exhibited a mean porosity value of 87.7 ± 0.2% ($p <$

0.0001). The differences in porosity upon pH adjustment of the formulation can possibly be due to differences in swelling properties of gelatin upon change in pH. Previous studies have shown that increasing the pH above isoelectric point of gelatin resulted in greater swelling of strands due to variations in molecular chains of gelatin molecules thereby resulting in larger pore sizes within the tablet matrix (Rowe et al. 2009 and Lin et al. 2002).

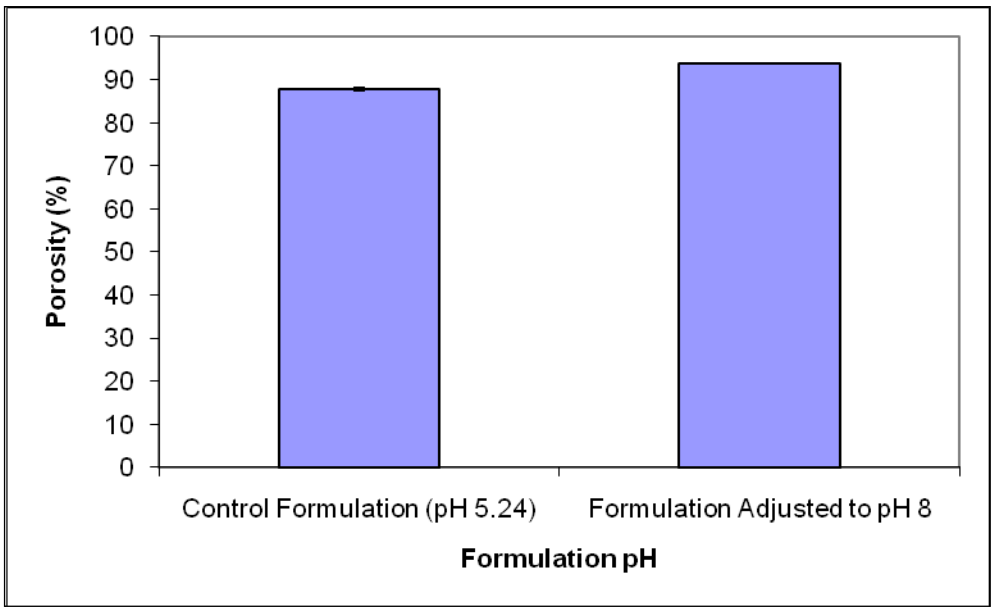
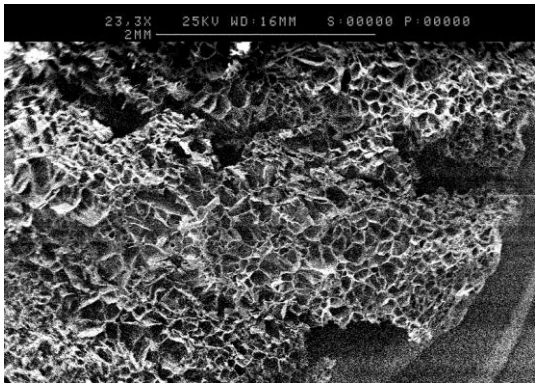
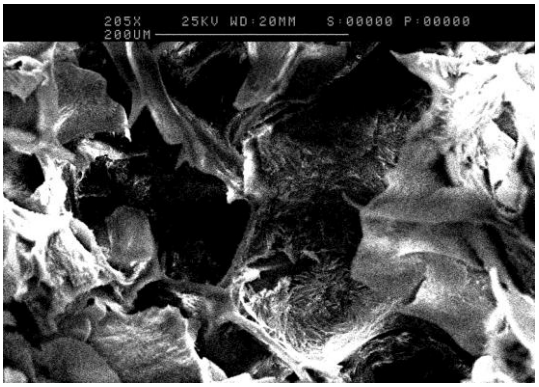


Figure 2.1 The effect of formulation pH on the porosity of the freeze-dried tablets (mean ± S.E., n = 3).

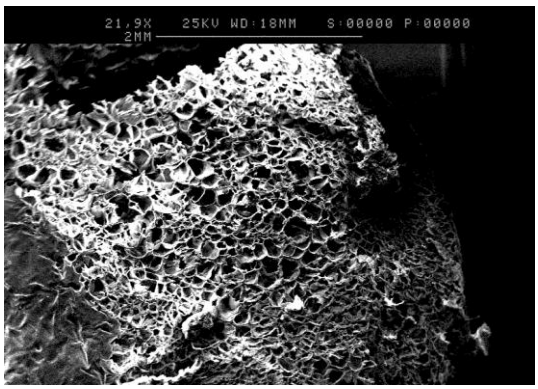
To further study the differences in porosity, anatomical studies using scanning electron microscopy were carried out. SEM analysis of the formulation adjusted to pH 8, as shown in Figures 2.2a and 2.2b, further supports the swelling behavior of gelatin upon pH variations. The 2-dimensional porous structure of the freeze-dried tablets of the formulation adjusted to pH 8, appeared to exhibit greater average pore diameter (100–140 µm) and thinner average pore wall thickness (20 µm) (Figures 2.2a and 2.2b), compared to the freeze-dried tablets of the control formulation (average pore diameter of 90 µm, and average pore wall thickness of 40 µm), as shown in Figures 2.3a and 2.3b. This observation can be attributed to the swelling behavior of gelatin at pH 8 (Sheppard et al. 1942) that results in the formation of larger pores with thinner walls.

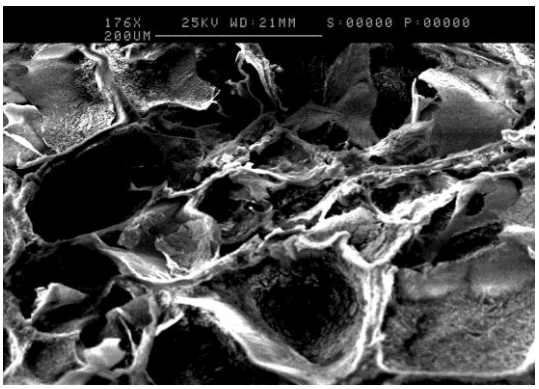




(a) (b)

Figure 2.2 (**a**) SEM image of the tablet matrix of the formulation adjusted to pH 8. Horizontal Sample. Low Magnification, ×23; (**b**) SEM image of the tablet matrix of the formulation adjusted to pH 8. Horizontal Sample. High Magnification, ×205.





(a) (b)

Figure 2.3 (**a**) SEM image of the tablet matrix of the control formulation. Horizontal Sample. Low Magnification, ×22; (**b**) SEM image of the tablet matrix of the control formulation. Horizontal Sample. High Magnification, ×176.

Analysis of the disintegration time of the formulation adjusted to pH 8, as shown in Figure 2.4, indicated a mean disintegration time of 54 ± 1.7 s, whereas the formulation prepared at pH 5 exhibited a mean disintegration time of 132 ± 25.4 s. Adjustment of pH resulted in the reduction of disintegration time by over a half ($p < 0.05$), as shown in Figure 2.4 This observation can be attributed to two factors. Firstly, porosity and SEM studies revealed that the tablets prepared at pH 8 had higher porosity and thinner pore walls which could result in an increase in water uptake and subsequent better wetting/dispersibility (He et al. 2008). Secondly, previously published reports have shown that aqueous solubility of gelatin is influenced by variations in pH with values above the isoelectric point of the material exhibiting an increase in its solubility (Cortesi et al. 1999 and Benjakul et al. 2009).

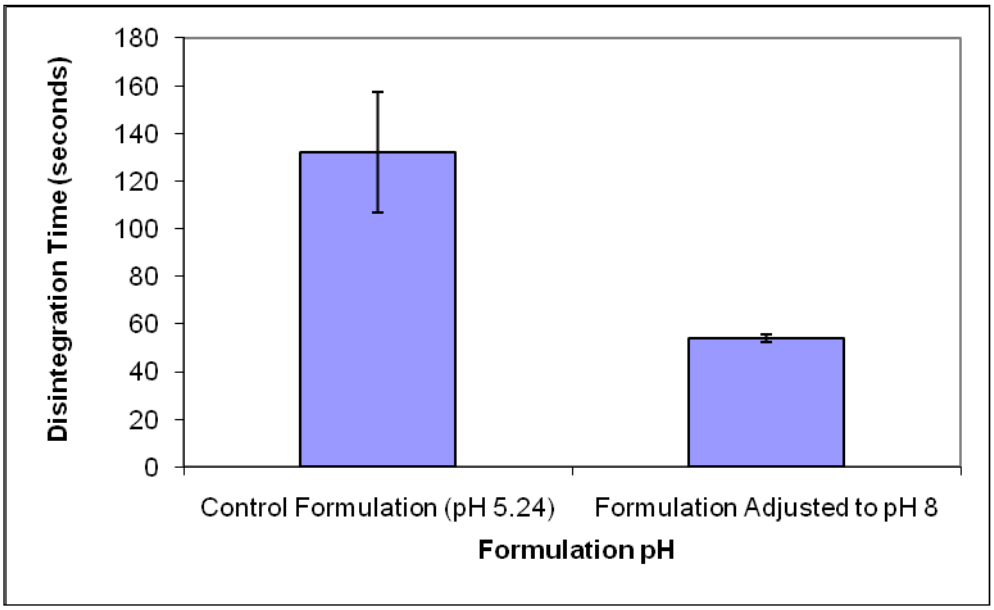


Figure 2.4 The effect of formulation pH on the disintegration time of the freeze-dried tablets (mean ± S.E., n = 3).

As reported previously, the Food and Drug Administration recommends that ODTs be considered as solid oral preparations that disintegrate rapidly in the oral cavity, with an *in vitro* disintegration time of approximately 30 seconds or less, when based on the United States Pharmacopeia disintegration test method or alternative (Food and Drug Administration, 2008). As Figure 2.4 shows, despite disintegration time decreasing from 132 ± 25.4 s to 54 ± 1.7 s, this value is greater than the 30 s value recommended by the Food and Drug Administration.

2.4.2 Influence of Ionic Strength

To investigate the influence of ionic strength, various ratios of sodium chloride were incorporated in the formulation with gelatin and mannitol. The results revealed that sodium chloride had a concentration dependant influence. Formulations comprising of a 1:40 molar ratio of gelatin:sodium chloride resulted in a collapse of the final product with no tablet formation. Lower molar ratios (1:5, 1:10, 1:20 and 1:30) produced intact tablets and were characterized further for mechanical as well as thermal properties.

2.4.2.1 Glass Transition and Tablet Mechanical Properties Analysis

DSC analyses of formulations with 1:5–1:30 molar ratios of gelatin:sodium chloride, as shown in Table 2.2, indicated mean onset T_g values comparable to the control formulation. The mean onset T_g values were –29.3 ± 0.1 °C, –30.5 ± 0.1 °C, –31.2 ± 0.2 °C and –31.7 ± 0.2°C, for 1:5, 1:10, 1:20 and 1:30 ratios, respectively, whilst the control formulation exhibited a mean onset T_g value of

–29.8 ± 0.5 °C. Statistical analysis of the results indicated that the formulations consisting of gelatin:sodium chloride in molar ratios of 1:20 and 1:30, exhibited mean onset T_g values which were significantly different ($p < 0.01$) from the control formulation.

Table 2.2 Glass transition data of the formulations consisting of various ratios of gelatin:sodium chloride.

Formulation	Mean Onset T_g (°C)	Standard Error
Control	–29.8	0.5
1:5	–29.3	0.1
1:10	–30.5	0.1
1:20	–31.2	0.2
1:30	–31.7	0.2

These results are in coherence with previous research investigating the effect of cations and anions of various electrolytes on the glass transition temperature of frozen solutions of excipients commonly used in freeze-drying, resulting in a decrease in glass transition temperature upon increasing ion concentration (Nesarikar and Nassar, 2007). Formulations consisting of 1:20 and 1:30 gelatin:sodium chloride molar ratios, did exhibit significantly different mean onset T_g values relative to the control formulation ($p < 0.01$). However, as the difference was only around 2°C, the structural collapse/shrinkage seen with the tablets of the formulation consisting of a gelatin:sodium chloride molar ratio of 1:40 was considered a physical rather than a thermal stability issue.

The inclusion of sodium chloride in the formulations with gelatin:sodium chloride molar ratios of 1:5–1:30 did not result in a significant increase in tablet hardness when compared to the control ($p > 0.05$), as shown in Table 2.3. Formulations with gelatin:sodium chloride molar ratios of 1:5, 1:10, 1:20 and 1:30, exhibited hardness values of 63.6 ± 5.6 N, 63.2 ± 8.0 N, 70.1 ± 2.1 N and 67.8 ± 8.3 N, respectively, whilst the control formulation had a mean value of 52.4 ± 8.8 N.

Table 2.3 Tablet hardness data of the formulations consisting of various ratios of gelatin:sodium chloride.

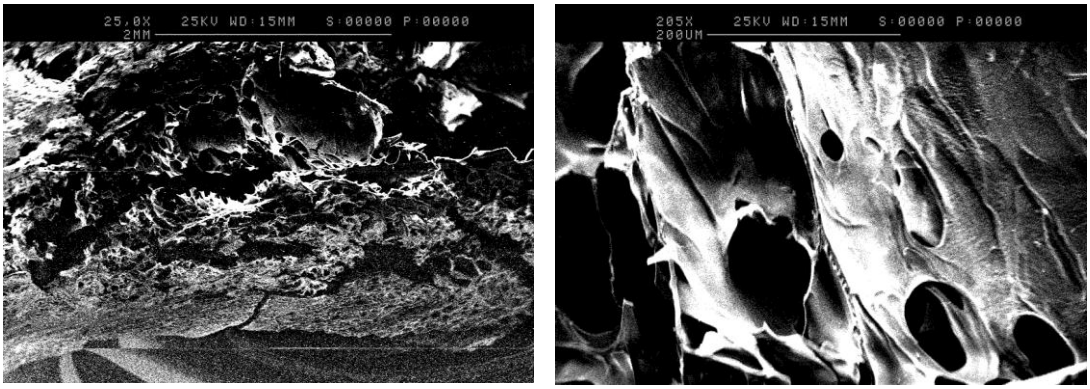
Formulation	Mean Tablet Hardness (N)	Standard Error
Control	52.4	8.8
1:5	63.6	5.6
1:10	63.2	8.0
1:20	70.1	2.1
1:30	67.8	8.3

Analysis of the fracturability of the tablets of formulations with gelatin:sodium chloride molar ratios of 1:5–1:30 indicated that there was no statistical difference in the fracturability values of the various ratios of sodium chloride when compared to the control.

2.4.2.2 Tablet Porosity, SEM and Disintegration Time Analysis

The inclusion of sodium chloride in the formulations resulted in differences in porosity of the tablets. Statistical analysis of the results indicated that the formulations consisting of gelatin:sodium chloride molar ratios of 1:5, 1:20 and 1:30, showed tablet porosities which were statistically significant ($p < 0.01$), when compared to the tablet porosity of the control formulation. There appeared to be a general trend that increasing the molar ratio of gelatin:sodium chloride from 1:5 to 1:20, produced a general increase in tablet porosity (1:5, 1:10 and 1:20, exhibited tablet porosity values of 88.70 ± 0.08%, 87.70 ± 0.08% and 89.50 ± 0.10%, respectively). The differences in porosity upon inclusion of sodium chloride can potentially be attributed to the differences in swelling behavior of gelatin in the presence of monovalent ions. Previous research has highlighted that the presence of sodium chloride had a bearing on the cross-linking of gelatin strands (Collett and Moreton, 2002). It is possible that inclusion of sodium chloride reduced the cross-linking during gelation which subsequently influenced tablet porosity.

These results were further confirmed by SEM. SEM micrographs showed that the inclusion of sodium chloride in the formulations produced porous structures which generally exhibited the formation of larger pores and thinner pore walls (Figures 2.5a and 2.5b), compared to the porous structure of the control formulation (Figures 2.3a and 2.3b).



(a) (b)

Figure 2.5 (**a**) SEM image of the tablet matrix of the 1:30 gelatin:soluble salt molar ratio formulation. Vertical Sample. Low Magnification, ×25; (**b**) SEM image of the tablet matrix of the 1:30 gelatin:soluble salt molar ratio formulation. Vertical Sample. High Magnification, ×205.

Horizontal sections of the tablets with gelatin:sodium chloride molar ratios of 1:10, 1:20 and 1:30, revealed average pore diameters of 100, 210 and 120 µm, respectively. Interestingly, tablets with a 1:20 gelatin:sodium chloride molar ratio, exhibited the greatest tablet porosity and shortest disintegration time of the four gelatin-soluble salt formulations. SEM analysis of the tablets revealed that increasing gelatin:soluble salt molar ratio appeared to increase disruption/damage to the porous matrix structures. The formulation with gelatin:sodium chloride molar ratio of 1:30, in particular, exhibited structural instability (as it appeared that pores had collapsed, forming cavities in the matrix) as shown in Figures 2.5a and 2.5b. This was likely due to the higher gelatin:soluble salt molar ratio, which appeared to weaken the structure, which led to the collapse of pores.

Analysis of the tablet disintegration times of formulations with gelatin:sodium chloride molar ratios of 1:5–1:30, showed that the inclusion of sodium chloride in the formulations with gelatin:sodium chloride molar ratios of 1:10 (126.7 ± 4.0 s) and 1:20 (102.3 ± 10.1 s), did not produce a significant reduction in disintegration time compared to the control formulation (132.0 ± 25.6 s mean disintegration time) ($p > 0.05$). The formulation with a gelatin:sodium chloride molar ratio of 1:5 produced a disintegration time (134.7 ± 12.0 s) comparable to the control formulation.

2.4.3 Milling Study

Ball milling has several pharmaceutical applications, which rely on a number of milling factors/parameters, such as; milling time, number of milling balls and milling jar volume, to fulfil their applications. These milling factors/parameters have a large range of operation, *e.g.* milling time can range from a few minutes to several hours, thus

making the possible number of milled sample formulations very large. Hence, factorial-experimental design software was used in order to propose a more suitable/manageable number of formulations (as shown in Table 2.1), which underwent various milling conditions based on three parameters; milling time, rotation speed and ball:powder weight ratio.

2.4.3.1 Wettability Analysis

The wettability analysis results are shown in Figure 2.6. Formulations N7 (milling conditions; milling time of 15 minutes, at a rotation speed of 400 rpm, with a ball:powder weight ratio of 15) and N11 (milling conditions; milling time of 37.5 minutes, at a rotation speed of 250 rpm, with a ball:powder weight ratio of 10) showed the lowest contact angles, 94.0 ± 0.3° and 94.1 ± 1.0° respectively, and thus the greatest wettability ($p < 0.05$), whilst the control formulation exhibited a contact angle of 120.7 ± 11.0°. Milling is regularly used for the reduction of particle size (Mallick et al. 2008), the observed improvement in wettability as a result of ball milling, can be attributed to a reduction in particle size and subsequent increase in surface area of the formulations (Kim et al. 2010).

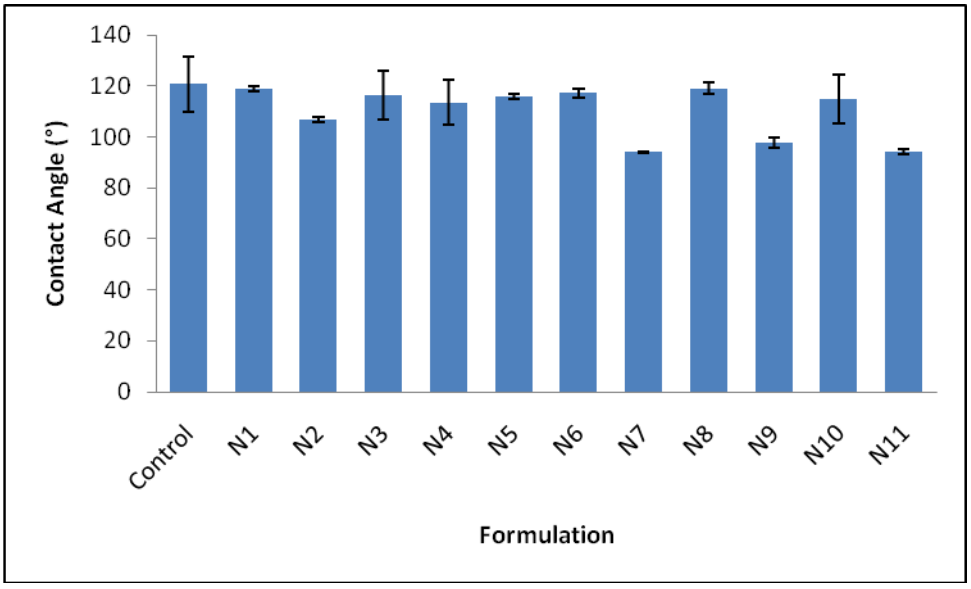


Figure 2.6 Analysis of the wettability of the milled formulations, expressed through their determined contact angle (mean ± S.E., n = 3).

2.4.3.2 Powder Porosity Analysis

A significant variation in powder porosity between the formulations was recorded, as some of the mixtures exhibited greater porosity than the control (non-milled, 62.1 ± 0.3%), whilst others showed lower porosity. Formulation N8 showed the greatest porosity of 69.55 ± 0.1%, whilst formulation N6 produced the lowest value of 49.19 ± 0.2%. The porosity of the studied formulations is associated with their bulk density (Staniforth, 2002). Therefore, as porosity varies between the formulations, so do their bulk density, which is related to the way in which the particles of the formulations are packed, during sample porosity analysis (Staniforth, 2002). As milling is associated with particle size reduction, the various milling conditions are expected to produce a range of differing particle sizes. It is therefore expected that the way these particles pack during porosity analysis varies greatly, which results in differences in inter-particulate void spaces and subsequent variation in porosity between the formulations. All eleven of the formulations exhibited statistical significance, which indicates that ball milling has a significant effect on the porosity of the powders. Formulation N10 had a p value of < 0.05, whilst all the other formulations had a p value of < 0.01.

2.4.3.3 Glass Transition and Tablet Mechanical Properties Analysis

DSC analysis of freeze-dried products is essential in order to fully appreciate and understand critical formulation properties such as the collapse temperature of the formulation (Tang and Pikal, 2004). The macroscopic collapse temperature of a formulation is defined as the temperature above which the freeze-dried product loses macroscopic structure and collapses during freeze-drying (Mackenzie, 1966). The macroscopic collapse temperature is closely related to the glass transition temperature of the formulation in its frozen state (Pikal and Shah, 1990). Therefore, in order to produce an acceptable freeze-dried product, it is always required to freeze dry a formulation at a temperature lower than the macroscopic collapse temperature (Pikal, 1990a and Pikal, 1990b).

Six of the eleven formulations exhibited glass transition temperatures which were considered not statistically significant ($p > 0.05$), when compared to the mean glass transition temperature of the control (non-milled) formulation. It can therefore be concluded that ball milling does not adversely affect the physical stability of the formulations in their frozen state or induce a plasticization effect, as comparable glass transition temperatures were observed.

All eleven of the formulations produced tablet hardness values which were not statistically significant ($p > 0.05$), when compared to the value of the control (non-milled) formulation (62.2 ± 3.8 N). Formulation N5 had the tablet hardness value of 55.0 ± 5.0 N, whilst formulation N10 produced the tablets which had hardness of 65.3 ± 1.0 N.

Similar results were obtained for fracturability analysis as no significant differences were observed when compared to the non-milled control formulation.

2.4.3.4 Tablet Porosity, SEM and Disintegration Time Analysis

Tablet porosity is a critical property of ODTs, as highly porous tablets allow the rapid penetration of saliva into the tablet, which results in rapid oral disintegration. Tablet porosity significantly impacts the initial wetting and dispersion of active pharmaceutical ingredients (He et al. 2008). It is therefore advisable to make tablets as porous as possible in order to achieve rapid disintegration. However, it is important to note that the physical/mechanical properties of the tablets such as hardness, should be maintained (He et al. 2008). In general it is considered that increasing tablet porosity leads to an increase in water uptake and subsequent better wetting/dispersibility of active pharmaceutical ingredients (He et al. 2008), and consequently tablets exhibit shorter disintegration times.

The control formulation had porosity of 93.4 ± 0.4%. Formulation N6 produced tablets with the highest porosity of 94.4 ± 0.3%, whilst formulation N5 resulted in tablets with the lowest porosity of 92.9 ± 0.1%.

All studied formulations exhibited tablet porosities which were not statistically significant ($p > 0.05$), when compared to the tablet porosity of the control formulation.

These data were further confirmed with the SEM analysis as no microscopic differences were observed between milled and non-milled formulations.

The disintegration time was slightly different between the formulations. The control (non-milled) had a mean disintegration time of 23 ± 1 s. Tablets from formulation N5 exhibited the greatest disintegration time of 28 ± 2 s, whilst formulations N2, N10 and N11 all had the shortest disintegration time of 21 s.

2.4.3.5 Dissolution Study Analysis

Figure 2.7 illustrates the mean dissolution results for ibuprofen tablets, prepared from non-milled and milled tablet excipients. Although the time required for 80% ibuprofen dissolution from the tablets prepared from non-milled and milled materials, were 20 and 10 minutes, respectively, statistical analysis of the results indicated that there was no significant difference ($p > 0.05$) in the dissolution behavior of ibuprofen from the two tablet formulations. The results have therefore shown that milling the excipients did not influence ibuprofen dissolution from the lyophilized ODTs.

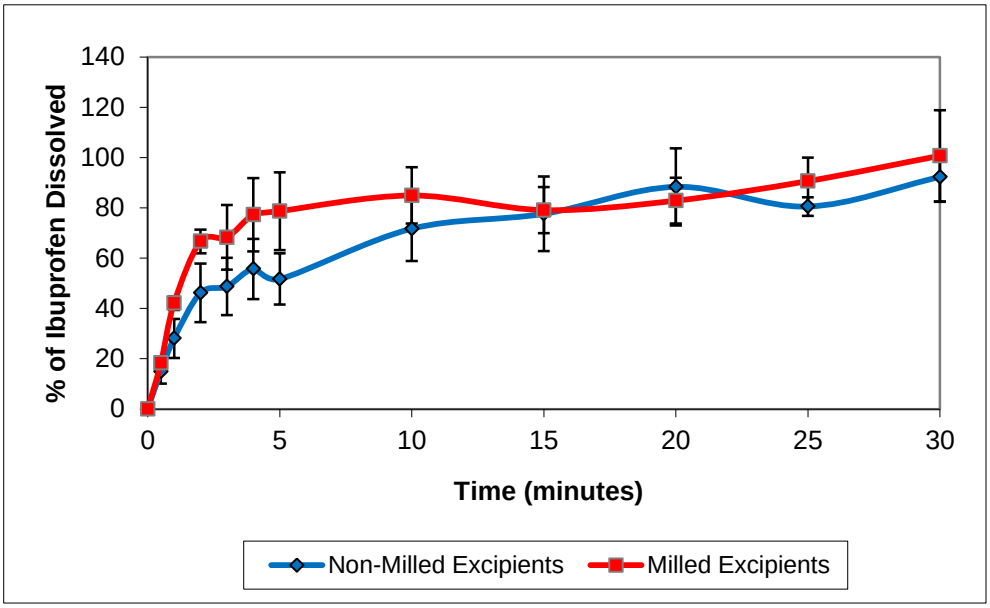


Figure 2.7 Dissolution profile of ibuprofen from lyophilized ODTs, prepared from non-milled and milled tablet excipients (mean ± S.E., n = 3).

Tablet porosity and disintegration time are both critical properties in determining active pharmaceutical ingredient dissolution, as tablet porosity in particular, significantly impacts the initial wetting and dispersion of active pharmaceutical ingredients (He et al. 2008). Initial results from this milling study, indicated that ball milling did not significantly affect tablet porosity and tablet disintegration time, when compared with tablets prepared from non-milled excipients. Therefore, comparable dissolution profiles of ibuprofen from tablets prepared from non-milled and milled materials was expected.

2.5 Conclusions

The study has shown that process parameters such as pH adjustment can have a significant influence on the disintegration time of gelatin based orally disintegrating tablets. The reduction in disintegration time did not compromise tablet hardness, which

is a key parameter to measure ODT performance. The reduction in disintegration time can be attributed to an increase in tablet porosity, which allows the more rapid penetration of saliva or disintegrating medium into the tablet matrix, and an increase in gelatin solubility. The inclusion of sodium chloride in the formulations, to modify the ionic strength of the formulations, had an effect on tablet porosity and the glass transition of the formulations. However, inclusion of sodium ions is concentration dependent, with tablets comprising of higher salt concentration resulting in structural collapse/shrinkage. The study has also shown that ball milling influences formulation characteristics, such as powder porosity, and improves powder wettability.

Chapter Three: Investigating the Application of Methocel™ (Hydrophilic Cellulose Ethers) as Binders in Freeze-Dried ODTs

3.1 Introduction

Methocel™ cellulose ethers are cellulose-derived water-soluble polymers (DOW Chemical Company, 2002). The majority of Methocel™ products are based on hydroxypropyl methylcellulose (HPMC), whilst the remaining products are based on methylcellulose (DOW Chemical Company, 2002).

HPMC, which is also referred to as hypromellose, is defined as a partly *O*-methylated and *O*-(2-hydroxypropylated) cellulose (British Pharmacopoeia Commission, 2011), for which the structural formula is shown in Figure 3.1. In terms of its appearance, it is a white or creamy-white fibrous or granular powder that is odourless and tasteless (Rogers, 2009).

Figure 3.1 Structural formula of HPMC, where R is H, CH_3, or $CH_3CH(OH)CH_2$. Figure obtained from Rogers (2009).

HPMC is practically insoluble in hot water, acetone, anhydrous ethanol and toluene, whilst it is soluble in cold water, in which it forms a colloidal solution (British Pharmacopoeia Commission, 2011).

In order to form an aqueous solution of HPMC, a method called the “hot/cold” technique is used, which utilises the insolubility of HPMC in hot water (DOW Chemical Company, 2002). The technique involves firstly dispersing the HPMC powder in 1/5 to 1/3 of the total volume of water, that has been heated to above 90ºC (DOW Chemical Company, 2002). To ensure complete solubilisation of the HPMC powder, the remaining volume of water is then added as cold water to reduce the temperature of the dispersion (DOW Chemical Company, 2002). Once the dispersion reaches critical

temperature, HPMC becomes water soluble which results in hydration of the powder and an increase in solution viscosity (DOW Chemical Company, 2002).

HPMC has a broad spectrum of applications in pharmaceutical formulations, in which it is used widely in oral, nasal, topical and ophthalmic formulations (Rogers, 2009). In terms of oral products, it is principally used as a tablet binder (Chowhan, 1980), in film-coating (Rowe, 1980) and as a matrix-former in sustained-release tablet formulations (Dahl et al. 1990). Another application of HPMC in oral formulations is its use in liquid formulations as a suspending and/or thickening agent (Food and Drug Administration, 2011), whilst it has the same application/use in topical and ophthalmic formulations (Rogers, 2009).

The broad application spectrum of HPMC in pharmaceutical formulations is credited to its versatility as a molecule. HPMC is adaptable in terms of molecular weight, which has a bearing on solution viscosity, and is versatile in terms of degree of substitution, which influences its water solubility.

As already mentioned, some Methocel™ products are based on methylcellulose (DOW Chemical Company, 2002), which is defined as a partly *O*-methylated cellulose (British Pharmacopoeia Commission, 2012), for which the structural formula is shown in Figure 3.2.

Figure 3.2 Structural formula of methylcellulose, where the structure shown is with complete substitution of the available hydroxyl units with methoxyl units. Figure obtained from Allen Jr (2012).

Methylcellulose has a similar appearance to HPMC; white, fibrous powder or granules, which is practically odourless and tasteless (Allen Jr, 2012). As with HPMC,

methylcellulose is practically insoluble in hot water, and also in acetone, anhydrous ethanol and toluene, whilst it is soluble in cold water, in which it forms a colloidal dispersion (British Pharmacopoeia Commission, 2012), which is prepared by the already discussed “hot/cold” technique (DOW Chemical Company, 2002).

Similar to HPMC, methylcellulose has a wide range of applications in pharmaceutical formulations, in which it is widely used in oral, topical and ophthalmic preparations (Allen Jr, 2012). Methylcellulose is used as a binding agent (Wan and Prasad, 1989 and Itiola and Pilpel, 1991), disintegrant (Esezobo, 1989) and as a matrix-forming/sustained-releasing agent (Sanghavi et al. 1990) in tablet formulations. It also has applications as a suspending or thickening agent in oral liquid preparations (Dalal and Narurkar, 1991).

In terms of topical products, methylcellulose is used as a thickening agent (Allen Jr, 2012), whilst for ophthalmic preparations, it is used as a vehicle for eye-drops (Gerbino, 2005).

HPMC and methylcellulose have both been investigated in the formulation of lyophilised ODTs. Corveleyn and Remon (1998 and 2000) reported the use of HPMC and methylcellulose as binding and emulsifier-tablet binding agents. Corveleyn and Remon (1998) reported in a study, in which HPMC was evaluated as an emulsifier-tablet binding agent, in the preparation of dry emulsion tablets and that HPMC concentration had a significant influence on tablet strength and disintegration time. In the study reported by Corveleyn and Remon (1998), two HPMC grades were evaluated; Methocel™ E15LV and K100LV.

The difference between Methocel™ E15LV and K100LV grades, is that the E15LV grade has a methoxyl degree of substitution of 1.9 and a hydroxypropyl molar substitution of 0.23, whilst the K100LV grade has a methoxyl degree of substitution of 1.4 and a hydroxypropyl molar substitution of 0.21 (DOW Chemical Company, 2002). The E15LV grade has a viscosity of 15 mPa s, whilst the K100LV grade has a viscosity of 100 mPa s (typical viscosity values for 2% (w/v) aqueous solutions, measured at 20ºC) (DOW Chemical Company, 2002). The “LV” suffix found in the names of both grades, refers to “low viscosity” grades (DOW Chemical Company, 2002).

Ahmed et al. (2011) reported a study which evaluated the use of maltodextrin as a sugar-matrix former along with several cellulosic binders in the preparation of

nimesulide freeze-dried ODTs. The study investigated the different grades including Methocel™ E5LV, E15LV and A15LV. The grades differ in that both E5LV and E15LV are HPMC grades, whilst the A15LV is a grade of methylcellulose. In terms of the two HPMC grades, both have a methoxyl degree of substitution of 1.9 and a hydroxypropyl molar substitution of 0.23 (DOW Chemical Company, 2002). The E5LV grade has a nominal viscosity of 5 mPa s, whilst the E15LV grade has a nominal viscosity of 15 mPa s (DOW Chemical Company, 2002).

Tablet characteristics, *in vitro/in vivo* disintegration time and *in vitro* dissolution of nimesulide were investigated using full factorial design (Ahmed et al. 2011). The optimized ODT formulation consisted of 5% w/v maltodextrin DE 29, 2% w/v Methocel™ E15LV and 5% w/v nimesulide, which exhibited a disintegration time of less than 10s, and 70% dissolution of nimesulide within 2 minutes, in comparison to 1.52% of the drug alone and 7.25% of an immediate release commercial tablet (Ahmed et al. 2011). The study suggested that the optimized ODT formulation maybe an alternative to conventional nimesulide formulations such as formulations administered via intramuscular or rectal routes, which are inconvenient to patients (Ahmed et al. 2011).

The studies carried out by Ahmed et al. (2011) and Corveleyn and Remon (1998) evaluated two grades of HPMC as binders in freeze-dried ODTs. Ahmed et al. (2011) studied Methocel™ E5LV and E15LV, whilst Corveleyn and Remon (1998) investigated Methocel™ E15LV and K100LV. The three different grades of HPMC which were evaluated (both Ahmed et al. (2011) and Corveleyn and Remon (1998) evaluated Methocel™ E15LV), are all low viscosity grades of HPMC.

Due to the considerable versatility of Methocel™, the aim of this chapter was to carry out a more extensive, systematic and comprehensive evaluation of its application as a binder in lyophilised ODTs, in terms of evaluating both low and high viscosity grades, as well as investigating grades which possess different methoxyl and hydroxypropyl degrees of substitution.

Table 3.1 shows the grades of Methocel™ which were investigated in this study, for their application as binders in freeze-dried ODTs. Several formulations were prepared based on each of the seven grades of Methocel™ evaluated in this study. Each formulation prepared was assessed for its ability to form intact tablets, which were subsequently characterised for hardness and disintegration time. The viscosity and thermal properties (determined by DSC) of the formulations were also assessed.

Table 3.1 Information detailing the different grades of Methocel™ evaluated in this study.

Methocel Grade	Substitution Type (First two digits refer to the approximate percentage content of the methoxy group (OCH_3). Second two digits refer to the approximate percentage content of the hydroxypropoxy group ($OCH_2CH(OH)CH_3$)).	Nominal Viscosity at 20°C (mPa s)	Methoxyl Degree of Substitution	Methoxyl %	Hydroxypropyl Molar Substitution	Hydroxypropyl %
A15LV	N/A	15	1.8	30	N/A	N/A
A4M	N/A	4,000	1.8	30	N/A	N/A
E3LV	2910	3	1.9	29	0.23	8.5
K3LV	2208	3	1.4	22	0.21	8.1
K100LV	2208	100	1.4	22	0.21	8.1
K4M	2208	4,000	1.4	22	0.21	8.1
K100M	2208	100,000	1.4	22	0.21	8.1

Note: The initial letter “A” of the Methocel™ grade refers to methylcellulose products, whilst the initial letters “E” and “K” refer to HPMC products. The number that follows the initial letter refers to the viscosity of the products. Relating to the viscosity of the products, the letter “M” refers to 1,000. The suffix “LV” refers to low viscosity products. Information included in this table was obtained from Rogers (2009) and DOW Chemical Company (2002).

3.2 Materials

Methylcellulose (Methocel™ A15LV (Lot No. DT292864) and A4M (Lot No. DT241464)), HPMC (Methocel™ E3LV (Lot No. DT220342), K3LV (Lot No. DT232237), K100LV (Lot No. DT199337), K4M (Lot No. DT243235) and K100M (Lot No. DT259593)) were supplied by Colorcon Ltd. (Dartford, UK). Mannitol was supplied by Sigma-Aldrich Chemicals (Poole, UK). All the chemicals were used without further purification.

3.3 Methods

3.3.1 Preparation of Freeze-Dried Tablets

For Methocel™ A and K based formulations, respectively, the required amount of polymer was mixed with half of the desired volume of water at 70°C and 80-90ºC, respectively. The remaining half of the desired volume of water at ambient temperature was then added to the hot polymer suspension, in order to form a clear aqueous solution. This was followed by the addition of mannitol, to form the final solution.

1.5 g of the resulting solutions were dosed into a tablet mould, frozen at -70°C for a minimum of sixty minutes and freeze-dried (ADVANTAGE, Freeze-Dryer, VIRTIS), according to the following regime; primary drying for forty eight hours at a shelf temperature of -40°C, secondary drying for ten hours at a shelf temperature of 20°C, and vacuum pressure of 6.67 Pa (50 mTorr). A minimum of ten tablets were prepared for each formulation.

3.3.2 Formulation Viscosity Analysis

For all formulations, the viscosities of 100 mL samples were measured using a DV-I+ Brookfield digital viscometer (Harlow, UK). The viscosities of the formulations were measured at 37 ± 2°C, in order to replicate the viscosity of the formulations in the oral cavity, at physiological temperature. The viscometer spindles were selected based on the viscosity of the individual formulations, as each spindle is suitable for analysing a certain viscosity range. The rotational speed of the spindle was set at 100 rpm. Each formulation was analysed in triplicate, and the mean values ± standard deviation is reported.

3.3.3 Concentration Profiling and Macroscopic Evaluation of the Formulations

During the preparation of the formulations, several visual assessments were carried out in order to evaluate the polymers as binders. The solubility of the material was assessed by observing the ease at which the polymer went into solution. The ease of

dosing of the formulations was also assessed, in terms of the viscosity of the prepared formulations.

In terms of the macroscopic evaluation of the tablets, several assessments were carried out in order to evaluate the ability of the polymers to form robust tablets with satisfactory mechanical strength. The first assessment involved the evaluation of the ability of the formulations to form intact tablet shaped dosage forms following freeze-drying. The physical appearance of the tablets was assessed, classifying them as either soft or hard, and having either elastic or a more robust/solid consistency. The ability of the tablets to be removed from the moulds was also assessed. Finally, the performance of the tablets when handled was assessed, to observe if they deformed permanently or retained their shape.

The first phase of the investigation was focussed on concentration profiling of the low viscosity grades of Methocel™ (A15LV, E3LV, K3LV and K100LV) to determine the optimum binder concentrations and understand their function as binders. Tables 3.2-3.5 represent the various formulations that were prepared with the four low viscosity grades of Methocel™.

Table 3.2 Formulation details of Methocel™ A15LV formulations, detailing the concentrations of Methocel™ and mannitol used in each formulation.

Methocel™ Concentration (% w/w)	**Mannitol Concentration (% w/w of dried tablet weight)**
1	30
1	70
2	30
2	70
3	30
3	70
5	30
5	70

Table 3.3 Formulation details of Methocel™ E3LV formulations, detailing the concentrations of Methocel™ and mannitol used in each formulation.

Methocel™ Concentration (% w/w)	Mannitol Concentration (% w/w of dried tablet weight)
1	0
1	30
5	0
5	30
10	0
10	30
11	0
11	30

Table 3.4 Formulation details of Methocel™ K3LV formulations, detailing the concentrations of Methocel™ and mannitol used in each formulation.

Methocel™ Concentration (% w/w)	Mannitol Concentration (% w/w of dried tablet weight)
1	0
1	30
5	0
5	30
10	0
10	30

Table 3.5 Formulation details of Methocel™ K100LV formulations, detailing the concentrations of Methocel™ and mannitol used in each formulation.

Methocel™ Concentration (% w/w)	Mannitol Concentration (% w/w of dried tablet weight)
1	30
1	70
3	30
3	70

The second phase of the investigation was focussed on concentration profiling of the high viscosity grades of Methocel™ (A4M, K4M and K100M) to determine the optimum binder concentrations and understand their role as binders. Tables 3.6-3.8 represent the various formulations that were prepared with the three high viscosity grades of Methocel™.

Table 3.6 Formulation details of Methocel™ A4M formulations, detailing the concentrations of Methocel™ and mannitol used in each formulation.

Methocel™ Concentration (% w/w)	Mannitol Concentration (% w/w of dried tablet weight)
1	30
1	70
2	30
2	70

Table 3.7 Formulation details of Methocel™ K4M formulations, detailing the concentrations of Methocel™ and mannitol used in each formulation.

Methocel™ Concentration (% w/w)	Mannitol Concentration (% w/w of dried tablet weight)
1	30
1	70
2	30
2	70

Table 3.8 Formulation details of Methocel™ K100M formulations, detailing the concentrations of Methocel™ and mannitol used in each formulation.

Methocel™ Concentration (% w/w)	Mannitol Concentration (% w/w of dried tablet weight)
1	30
1	70
2	30
2	70

3.3.4 Differential Scanning Calorimetry (DSC)

DSC (Pyris Diamond DSC and Intercooler 2P: Perkin Elmer, Wellessey, USA) was used to determine the glass transition temperature (T_g) and thermal properties of the formulations in their frozen state (before freeze-drying). 10-15 mg of liquid samples of the formulations were loaded into aluminium pans, cooled from 25ºC to -65ºC at a rate of 5ºC/min, and then heated to 20ºC at a rate of 5ºC/min, with a nitrogen purge of 20 mL/min. An empty aluminium pan was used as a reference for all measurements.

The resulting thermograms were analysed by Pyris manager software. T_g values were determined from the intersection of relative tangents to the baseline, and other thermal properties of the formulations were also assessed. Three measurements were taken for each formulation, and the mean values ± standard deviation were reported.

3.3.5 Tablet Hardness and Fracturability

The hardness and fracturability of the tablets was investigated using a texture analyzer (QTS 25: Brookfield, Essex, UK) equipped with a 25 kg load cell. The instrument was calibrated by standard weights of 500 g and 5 kg. The tablets were placed in a holder with a cylindrical hole. The hardness was taken as the peak force after 1 mm penetration of a 5 mm diameter probe at a rate of 6 mm/min. The fracturability was taken as the peak force after 3 mm penetration of a 1 mm diameter probe at a rate of 6 mm/min. Three measurements were taken for each formulation and the mean ± standard deviation is reported.

3.3.6 Disintegration Time of the Tablets

The disintegration time of the tablets was determined using a USP disintegration tester (Erweka, ZT3). 800 mL of double distilled water, which was kept at 37 ± 2ºC, was used as the medium and the basket was raised and lowered at a fixed rate of 30 rpm. Three tablets were evaluated from each formulation, and the mean values ± standard deviation is reported.

3.3.7 Investigation of Tablet Porosity

The porosity of the tablets was measured using helium pycnometry (MULTIPYCNOMETER, Quantachrome Instruments, Hampshire, UK). Two freeze-dried tablets were placed in a suitably sized sample cup and subjected to helium pycnometry, to determine the true density of the tablets. The true density value was then inserted into the equation, as shown below, to determine the porosity of the tablets.

$$\text{Porosity} = 1 - \left[\frac{\text{bulk density}}{\text{true density}}\right] \times 100\%$$

(Equation 3.1)

Bulk density was determined by considering; the mass of the tablets, the diameter of the tablets, and the height/depth of the tablets. The diameter and height/depth of the tablets were determined using a screw gauge (LINEAR Farnell). Three porosity measurements were taken for each formulation, and the mean values ± standard deviation is reported. The porosity of the tablets is expressed as a percentage.

3.3.8 Statistical Analysis

Statistical analyses of the formulation and tablet characterisation results were performed using GraphPad Prism 5 statistical analysis software. One-way analysis of variance (ANOVA) was the statistical analysis test performed, using the Tukey post test. A significance level of $p < 0.05$ (95% confidence interval) was judged as being statistically significant.

3.4 Results and Discussion

3.4.1 Low Viscosity Grades of Methocel™

3.4.1.1 Concentration Profiling and Macroscopic Evaluation of the Formulations of Low Viscosity Grades of Methocel™

Tables 3.9-3.12 represent the macroscopic evaluation of the tablets prepared from the various formulations that were prepared with the four low viscosity grades of Methocel™.

Table 3.9 Concentration profiling and macroscopic evaluation of the various Methocel™ A15LV formulations prepared.

Formulation	Comments
1% w/w Methocel A15LV with 30% w/w (of dried tablet weight) mannitol	Intact tablets were formed. The tablets were soft with a "spongy/foamy" consistency. The tablets were easy to remove from the moulds, and showed a very elastic nature following handling (returned to their original shape following compression).
1% w/w Methocel A15LV with 70% w/w (of dried tablet weight) mannitol	Intact tablets were formed. The tablets were very soft/fragile, and broke/disintegrated when handled. The tablets could not be removed from the moulds.
2% w/w Methocel A15LV with 30% w/w (of dried tablet weight) mannitol	Not all the tablets dried satisfactorily and formed intact compacts. Those that did dry satisfactorily were relatively solid in nature, with a "spongy/foamy" consistency. The tablets were easy to remove from the bijou tubes, and were difficult to deform following handling (as tablets returned to their original shape following compression).
2% w/w Methocel A15LV with 70% w/w (of dried tablet weight) mannitol	Intact tablets were formed. However the tablets were soft/fragile, and broke/disintegrated when handled. The tablets could not be removed from the moulds.
3% w/w Methocel A15LV with 30% w/w (of dried tablet weight) mannitol	Intact tablets not formed.
3% w/w Methocel A15LV with 70% w/w (of dried tablet weight) mannitol	Intact tablets were formed. However the tablets were fragile and disintegrated when handled. The tablets were easily removed from the moulds.
5% w/w Methocel A15LV with 30% w/w (of dried tablet weight) mannitol	Intact tablets not formed.
5% w/w Methocel A15LV with 70% w/w (of dried tablet weight) mannitol	Intact tablets were formed. The tablets were very hard/solid, and did not deform/break when handled. The tablets were easily removed from the moulds.

Table 3.10 Concentration profiling and macroscopic evaluation of the various Methocel™ E3LV formulations prepared.

Formulation	Comments
1% w/w Methocel E3LV	Intact tablets formed. Tablets were soft with a "spongy/foamy" consistency. Tablets did not deform when handled, they retained their shape. Tablets easily removed from moulds.
1% w/w Methocel E3LV with 30% w/w (of dried tablet weight) Mannitol	
5% w/w Methocel E3LV	Intact tablets formed. Tablets were hard and robust. Tablets did not deform when handled, they retained their shape. Tablets easily removed from moulds. The 5% w/w Methocel E3LV tablets (mannitol-free), had a noticeable "spongy/foamy" consistency.
5% w/w Methocel E3LV with 30% w/w (of dried tablet weight) Mannitol	
10% w/w Methocel E3LV	Intact tablets formed. Tablets were very hard and robust. Tablets did not deform when handled, they retained their shape. Tablets easily removed from moulds.
10% w/w Methocel E3LV with 30% w/w (of dried tablet weight) Mannitol	
11% w/w Methocel E3LV	
11% w/w Methocel E3LV with 30% w/w (of dried tablet weight) Mannitol	

Table 3.11 Concentration profiling and macroscopic evaluation of the various Methocel™ K3LV formulations prepared.

Formulation	Comments
1% w/w Methocel K3LV	Intact tablets formed. Tablets were soft with a "spongy/foamy" consistency. Tablets did not deform when handled, they retained their shape. Tablets easily removed from the moulds.
1% w/w Methocel K3LV with 30% w/w (of dried tablet weight) Mannitol	
5% w/w Methocel K3LV	Intact tablets formed. Tablets were hard and robust. Tablets did not deform when handled, they retained their shape. Tablets easily removed from moulds.
5% w/w Methocel K3LV with 30% w/w (of dried tablet weight) Mannitol	
10% w/w Methocel K3LV	Intact tablets formed. Tablets were very hard and robust. Tablets did not deform when handled, they retained their shape. Tablets easily removed from moulds.
10% w/w Methocel K3LV with 30% w/w (of dried tablet weight) Mannitol	

Table 3.12 Concentration profiling and macroscopic evaluation of the various Methocel™ K100LV formulations prepared.

Formulation	Comments
1% w/w Methocel K100LV with 30% w/w (of dried tablet weight) mannitol	Intact tablets formed. Tablets were soft with a "spongy/foamy" consistency. Tablets retained shape when handled, and did not deform permanently.
1% w/w Methocel K100LV with 70% w/w (of dried tablet weight) mannitol	Intact tablets formed. However, very soft/fragile, and the tablets broke/disintegrated easily when handled. Tablets could not be removed from the moulds intact.
3% w/w Methocel K100LV with 30% w/w (of dried tablet weight) mannitol	Intact tablets formed. Tablets were hard with a noticeable "spongy" consistency. Tablets retained shape when handled, and did not deform permanently.
3% w/w Methocel K100LV with 70% w/w (of dried tablet weight) mannitol	Intact tablets formed. Tablets were hard and physically stable, and did not deform when handled. The tablets were relatively easily removed from the moulds.

Observations made during the formulation of Methocel™ A15LV, revealed that this grade could be used in concentrations up to 5% w/w with higher concentrations proving difficult to dose due to their high viscosities. The viscosity results (Figure 3.3) revealed that the workable viscosity range for this grade of Methocel™, in order to prepare tablets was 0.77 ± 0.15 to 16.40 ± 9.76 mPa s. Macroscopic evaluation of the tablets of formulations made from this grade of Methocel™ indicated that this grade of the polymer did produce intact tablets from most of the formulations. However, it was not possible to make tablets from formulations containing 3 and 5% w/w Methocel™ with 30% w/w (of dried tablet weight) mannitol, as intact tablets were not formed following freeze-drying. Interestingly, the corresponding formulations consisting of 70% w/w (of dried tablet weight) mannitol, did form intact tablets suggesting that the ability of mannitol to function as a matrix supporting agent with this particular grade of Methocel™ is concentration dependent, as mannitol concentration of 30% w/w (of dried tablet weight) was not sufficient to provide the necessary matrix support to form intact tablets.

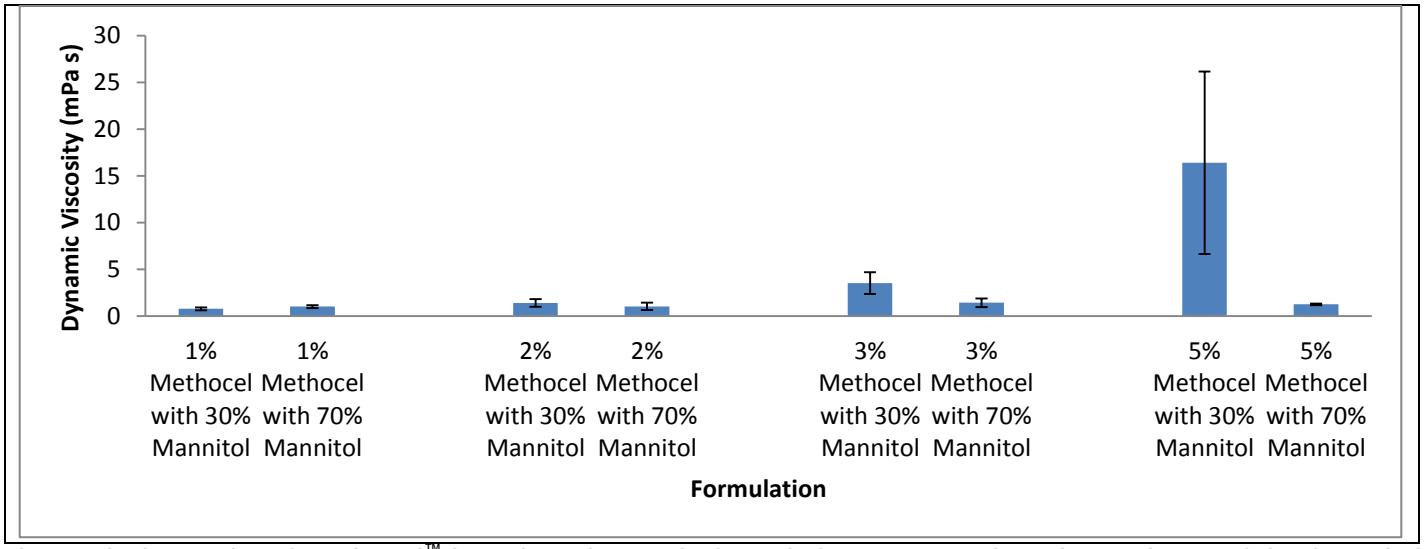


Figure 3.3 Viscosity analysis results of Methocel™ low viscosity grade formulation A15LV. Viscosity analyses of the formulations were carried out in triplicate and the mean values ± standard deviation are reported.

Formulations of Methocel™ A15LV at 1 and 2% w/w, resulted in intact "elastic" tablets which were easily removable from the moulds. Tablets containing 70% w/w (of dried tablet weight) mannitol, were softer and fragile compared to tablets made of 30% w/w mannitol. A possible explanation of this phenomenon could be based on matrix disruption of binder network upon inclusion of higher concentration of supporting agents. This can be related to the way that plasticisers disrupt and weaken the intermolecular interactions between polymer chains used in tablet coatings which subsequently alters the mechanical properties of the polymer (Wen and Park, 2010). The formulation containing 5% w/w Methocel™ and 70% w/w (of dried tablet weight) of mannitol produced the most satisfactory intact tablets that were hard/solid in nature and did not deform or break when handled. Those tablets were also easily removed from the moulds.

Studies investigating the optimisation of the concentration of matrix supporting agents in the formulation of freeze-dried ODTs have been previously reported. Chandrasekhar et al. (2009) investigated the influence of mannitol, sorbitol and sucrose concentrations on the hardness and disintegration times of the tablets. AlHusban et al. (2010c) additionally reported a study investigating the optimisation of the concentration of matrix supporting agents. AlHusban et al. (2010c) investigated the influence of amino acid (proline and serine used in combination) concentrations on the properties of the formulations and tablets.

Concentration profiling and macroscopic evaluation of Methocel™ E3LV formulations tablets, are shown in Table 3.7 Methocel™ E3LV polymer could be formulated up to concentration of 11% w/w and further increase in concentration was limited by poor solubility of the binder. Macroscopic evaluation of the tablets of Methocel™ E3LV formulations revealed that this low viscosity grade polymer did produce intact tablets. At a polymer concentration of 1% w/w, the tablets were soft with a noticeable "spongy/foamy" consistency. They did not deform when handled and retained their original shape. The tablets were also easily removed from their moulds.

Increasing polymer concentration to 5, 10 and 11% w/w, resulted in tablets which were hard and robust. The tablets of these formulations were also easily removed from their moulds.

Evaluation of Methocel™ K3LV formulations and tablets are shown in Table 3.8 In terms of the observations made for Methocel™ K3LV formulations, it was possible to

use Methocel™ K3LV up to 10% w/w in the formulations. Higher concentrations of the polymer solution could not be prepared due to limitations in solubility. Macroscopic evaluation of the tablets of formulations made from Methocel™ K3LV indicated that this low viscosity grade polymer did produce intact tablets. With a polymer concentration of 1% w/w, the formed tablets were very soft and "spongy/foamy" in consistency. Whilst with polymer concentrations of 5 and 10% w/w, the formed tablets were hard and robust. The tablets also would withstand manual handling, and retain their shape upon removal from their moulds. Macroscopic evaluation of the tablets of formulations made from Methocel™ K3LV indicated that this low viscosity grade polymer can be used as a binder in freeze-dried ODTs, due to the formation of hard and robust tablets.

Analysis for Methocel™ K100LV based formulations are shown in Table 3.12. During manufacture of Methocel™ K100LV based formulations, a number of observations were made. Concentrations of polymer above 3% w/w could not be formulated due to difficulties in dosing the solution into tablet moulds. Analysis of viscosity results (Figure 3.4D) provide the evidence for the workable range of 1.79 ± 0.06 to 32.97 ± 1.27 mPa s.

Macroscopic evaluation of the tablets of formulations made from the K100LV grade indicated that this grade of polymer produced intact tablets. The formulation which consisted of 1% w/w Methocel™ K100LV and 30% w/w (of dried tablet weight) mannitol, produced soft tablets which had a "spongy/foamy" consistency. The tablets retained their shape when handled, and did not deform permanently. Increasing mannitol concentration to 70% w/w (of dried tablet weight), resulted in tablets which were fragile and broke/disintegrated easily when handled. Increasing Methocel™ K100LV concentration to 3% w/w with both 30 and 70% w/w (of dried tablet weight) mannitol, resulted in tablets which were hard and did not deform when handled. Increasing mannitol concentration from 30 to 70% w/w (of dried tablet weight) appeared to produce tablets which were harder and more robust.

Concentration profiling and macroscopic evaluation of the Methocel™ low viscosity grades formulations and tablets indicated that formulation viscosity is a critical parameter, which influences ease of dosing and the successful formation of intact and robust tablets. *E.g.* Methocel™ E3LV and K3LV which were the two lowest viscosity grades (as shown in Table 3.1), could be formulated up to 10 and 11% w/w, respectively. Whilst Methocel™ K100LV, which had the highest viscosity grade out of the four grades that were investigated could only be formulated up to 3% w/w.

It was also observed that although Methocel™ E3LV and K3LV exhibit the same viscosity (as shown in Table 3.1), the E3LV grade could be formulated up to a higher concentration. This is proposed to be attributed to the greater percentage content of methoxyl and hydroxypropyl groups within E3LV grade relative to the K3LV, as shown in Table 3.1, which increases the hydrophilic nature of HPMC. In relation to this observation, Ahmed et al. (2011) reported that Methocel™ E grades are more hydrophilic than Methocel™ A grades, due to the presence of more polar (hydroxyl) groups. This study indicates that chemical structure is also a critical parameter which determines the suitability of a material to function as a binder with freeze-dried ODTs, as it governs at which concentrations the material can be used.

3.4.1.2 Evaluation of the Thermal Properties of the Formulations of Low Viscosity Grades of Methocel™

Freeze-dried products and formulations possess a number of significant formulation properties which are related to the freeze-drying method used, such as the collapse temperature of the formulation, API stability and excipient properties (Tang and Pikal, 2004). The collapse temperature of the formulation is defined as the temperature above which the freeze-dried product loses its structure and collapses during freeze-drying (Mackenzie, 1966), which is generally around 2ºC greater than the glass transition temperature of the formulation in its frozen state (Pikal and Shah, 1990). To produce a freeze-dried product or formulation of high quality, it is essential therefore to freeze-dry the formulation at a lower temperature than the collapse temperature (Pikal, 1990a and Pikal, 1990b).

DSC is a useful tool which can provide useful information on the important formulation properties of freeze-dried products/formulations, as discussed above. This information can subsequently be used to design suitable freeze-drying methods which will produce formulations of high quality.

DSC analysis of Methocel™ A15LV formulations indicated that formulations containing 1 and 2% w/w methylcellulose and 30% w/w (of dried tablet weight) mannitol, produced no clear/detectable thermal transitions, which were attributable to Methocel™ or mannitol (Figure 3.4 A). Formulations consisting of 3% w/w Methocel™ and 30% w/w (of dried tablet weight) mannitol produced thermograms which exhibited several thermal transitions which were attributed to mannitol. Glass transitions were observed

at around -26 and -22ºC, respectively, whilst exothermic crystallisation peaks were observed at around -16ºC.

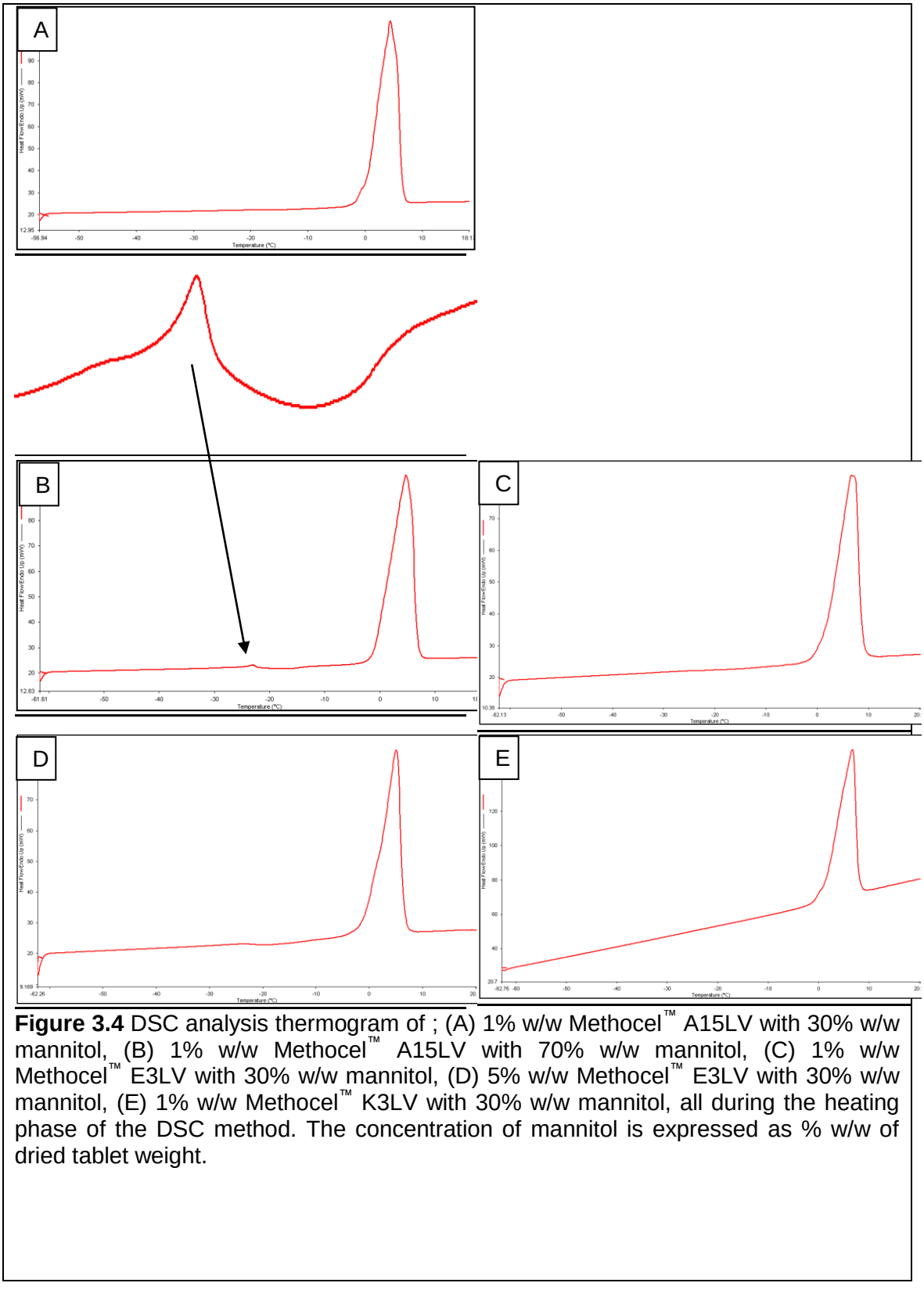


Figure 3.4 DSC analysis thermogram of ; (A) 1% w/w Methocel™ A15LV with 30% w/w mannitol, (B) 1% w/w Methocel™ A15LV with 70% w/w mannitol, (C) 1% w/w Methocel™ E3LV with 30% w/w mannitol, (D) 5% w/w Methocel™ E3LV with 30% w/w mannitol, (E) 1% w/w Methocel™ K3LV with 30% w/w mannitol, all during the heating phase of the DSC method. The concentration of mannitol is expressed as % w/w of dried tablet weight.

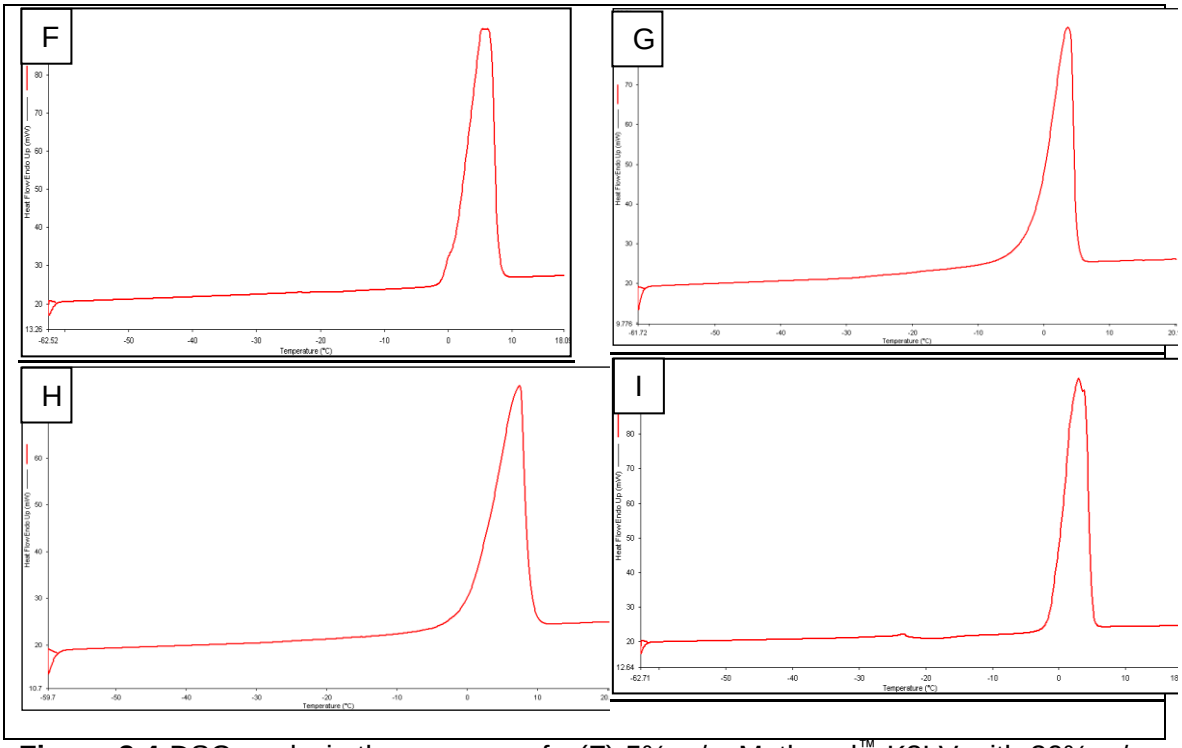


Figure 3.4 DSC analysis thermogram of ; (F) 5% w/w Methocel™ K3LV with 30% w/w mannitol, (G) 10% w/w Methocel™ K3LV with 30% w/w mannitol, (H) 1% w/w Methocel™ K100LV with 30% mannitol and (I) 1% w/w Methocel™ K100LV with 70% w/w mannitol formulations samples, all during the heating phase of the DSC method. The concentration of mannitol is expressed as % w/w of dried tablet weight.

The glass transition observed at around -26ºC was attributed to the mannitol phase plasticised by unfrozen water (Cavatur et al. 2002). The transition at around -22ºC was attributed to the amorphous freeze-concentrate of mannitol, whilst the crystallisation peaks observed at around -16ºC correspond to mannitol hydrate and the unfrozen water associated with the amorphous phase of mannitol (Cavatur et al. 2002).

Interestingly, the 5% w/w Methocel™ A15LV formulation with 30% w/w (of dried tablet weight) mannitol exhibited glass transitions at around -28ºC, which was attributed to the amorphous freeze-concentrate of mannitol (Cavatur et al. 2002). It is assumed that the reason for a single thermal transition with this formulation, in comparison to the three events observed with the 3% w/w Methocel™ formulation, is possibly to do with the increase in Methocel™ concentration which alters the composition of the formulation. Thermal events for water soluble components vary depending on the type and concentration of the added excipients.

All of the Methocel™ A15LV formulations which consisted of 70% w/w (of dried tablet weight) mannitol, produced several thermal transitions, as shown in Figure 3.4B. The DSC thermograms of the formulations indicated a glass transition at around -27 to -

30°C, followed by an endothermic melt at ~ -23°C, which has been postulated to being a glass transition (Cavatur et al. 2002). These two thermal transitions were followed by an exothermic crystallisation transition at ~ -18°C, which was attributable to the crystallisation of both mannitol hydrate and unfrozen water associated with the amorphous phase (Cavatur et al. 2002).

It therefore appears that mannitol concentration is a critical factor in promoting thermal transitions such as crystallisation and glass transition, as thermal transitions were more clearly evident with formulations consisting of 70% w/w (of dried tablet weight) mannitol.

The influence of mannitol concentration on the observed thermal transitions has been reported by AlHusban et al. (2010d). AlHusban et al. (2010d) investigated mannitol concentrations of 10, 30, 50 and 70% (of total solid material), and observed that mannitol maintained an amorphous state at 10 and 30% (of total solid material), whilst at 50 and 70% (of total solid material) mannitol underwent crystallisation. AlHusban et al. (2010d) reported that increasing the concentration of mannitol promotes its crystallisation.

Evaluation of the thermal properties of Methocel™ E3LV and K3LV formulations, respectively, revealed that the DSC thermograms of the mannitol-free formulations exhibited no thermal transitions which were attributable to Methocel™ or mannitol. The same was observed with the formulations which included 1% w/w Methocel™ and 30% w/w (of dried tablet weight) mannitol, as shown in Figures 3.4C and 3.4E, respectively.

The 5% w/w Methocel™ E3LV formulation with 30% w/w (of dried tablet weight) mannitol, produced thermograms which exhibited a single thermal transition, as shown in Figure 3.4D. Exothermic crystallisation peaks were observed at around -17ºC which were attributable to the crystallisation of mannitol hydrate and unfrozen water (Cavatur et al. 2002). Despite the presence of the crystallisation peak, it was interesting to note that the glass transition of the amorphous freeze-concentrate of mannitol was not observed. It has been previously reported that the crystallisation of mannitol hydrate and unfrozen water occurs immediately after the glass transition of the freeze-concentrate of mannitol (Cavatur et al. 2002), therefore this glass transition was expected to be observed.

5 and 10% w/w Methocel™ K3LV formulations with 30% w/w (of dried tablet weight) mannitol displayed thermograms which revealed a single thermal transition which corresponds to mannitol, as shown in Figures 3.4F and 3.4G. Glass transitions were observed at around -27ºC, which were postulated to be attributable to the glass transition of the amorphous freeze-concentrate of mannitol (Cavatur et al. 2002).

On the other hand, the 10 and 11% w/w Methocel™ E3LV formulations with 30% w/w (of dried tablet weight) mannitol, produced thermograms which displayed several thermal transitions which correspond to the thermal profile of mannitol (Figures not shown). Glass transitions were observed at around -30 and -24ºC, respectively, and crystallisation peaks were observed at around -19ºC. The glass transitions observed at around -30ºC were attributable to the mannitol phase plasticised by unfrozen water, whilst the glass transitions observed at around -24ºC correspond to the amorphous freeze-concentrate of mannitol (Cavatur et al. 2002). The crystallisation peaks observed at around -19ºC, were attributable to both mannitol hydrate and unfrozen water (Cavatur et al. 2002).

The thermal transitions recorded with the 10 and 11% w/w Methocel™ E3LV with 30% w/w (of dried tablet weight) mannitol formulations, were also observed with the Methocel™ K100LV formulations, which included 70% w/w (of dried tablet weight) mannitol, as shown in Figure 3.4I. Interestingly, no thermal transitions were observed with the Methocel™ K100LV formulations which included 30% w/w (of dried tablet weight) mannitol as shown in Figure 3.4H.

Results from the above investigations also provide conclusive evidence on the freeze drying regime followed in the formulation of various concentrations of the different binders. All the studies were carried out at -40ºC which was below the transition temperatures recorded for the different compositions investigated. The study also provides further evidence of concentration dependence for thermal transitions with smaller concentration of the total solid material providing multiple thermal events when compared to formulations comprising of higher water soluble content.

3.4.1.3 Viscosity and Mechanical Properties Analyses of Methocel™ Low Viscosity Grades Formulations and Tablets

Concentration profiling and macroscopic evaluation of Methocel™ low viscosity grades formulations revealed that the preparation of Methocel™ A15LV and K100LV formulations were limited due to the high viscosity of the formulations at low Methocel™

concentrations. With the A15LV and K100LV grades, formulations could only be prepared up to a concentration of 5 and 3% w/w, respectively.

Due to formulation viscosity being the limiting factor in preventing formulations being prepared at higher concentrations, viscosity analysis of the preparations were carried out to identify the "workable" viscosity range for the different grades of Methocel™. Additionally, analyses of the mechanical properties of the prepared tablets were carried out in parallel to formulation viscosity analysis to investigate the relationship between formulation viscosities (which is related to Methocel™ concentration) and the formation of intact tablets.

The viscosity analysis results for Methocel™ A15LV formulations are shown in Figure 3.3. For the formulations containing 30 and 70% w/w (of dried tablet weight) mannitol, an increase in methylcellulose concentration did not show any significant change in formulation viscosity ($p > 0.05$). Comparing the viscosities of the formulations consisting of 30% w/w (of dried tablet weight) mannitol, with their corresponding formulations which consisted of 70% w/w (of dried tablet weight) mannitol, revealed no significant difference in formulation viscosity ($p > 0.05$).

Viscosity analysis results of Methocel™ E3LV and K3LV formulations, shown in Figures 3.5A and 3.5B respectively, revealed comparable results. The results indicated that increasing Methocel™ concentration resulted in an increase in formulation viscosity ($p < 0.05$). This can be attributed to an increase in the density of the polymer network and an increase in the interaction between polymer chains. This particular trend was observed with both the mannitol-free formulations and the formulations which included mannitol.

Comparing the viscosities of the mannitol-free formulations with the formulations containing mannitol, the results showed that there was no significant difference observed with the 1% and 5% w/w formulations, for both E3LV and K3LV formulations ($p > 0.05$).

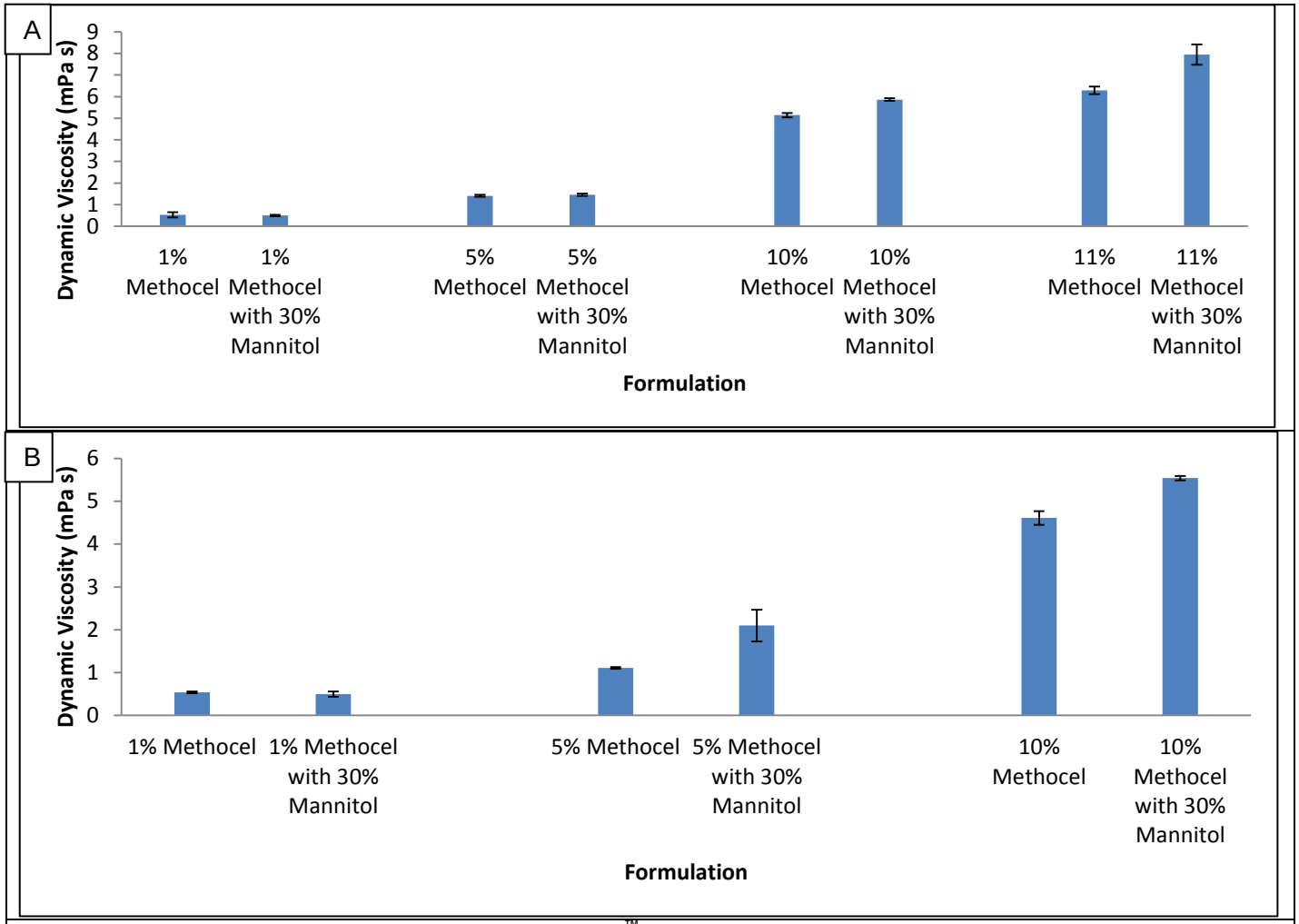


Figure 3.5 Viscosity analysis results of Methocel™ low viscosity grade formulations; (A) E3LV and (B) K3LV formulations. Viscosity analyses of the formulations were carried out in triplicate and the mean values ± standard deviation are reported.

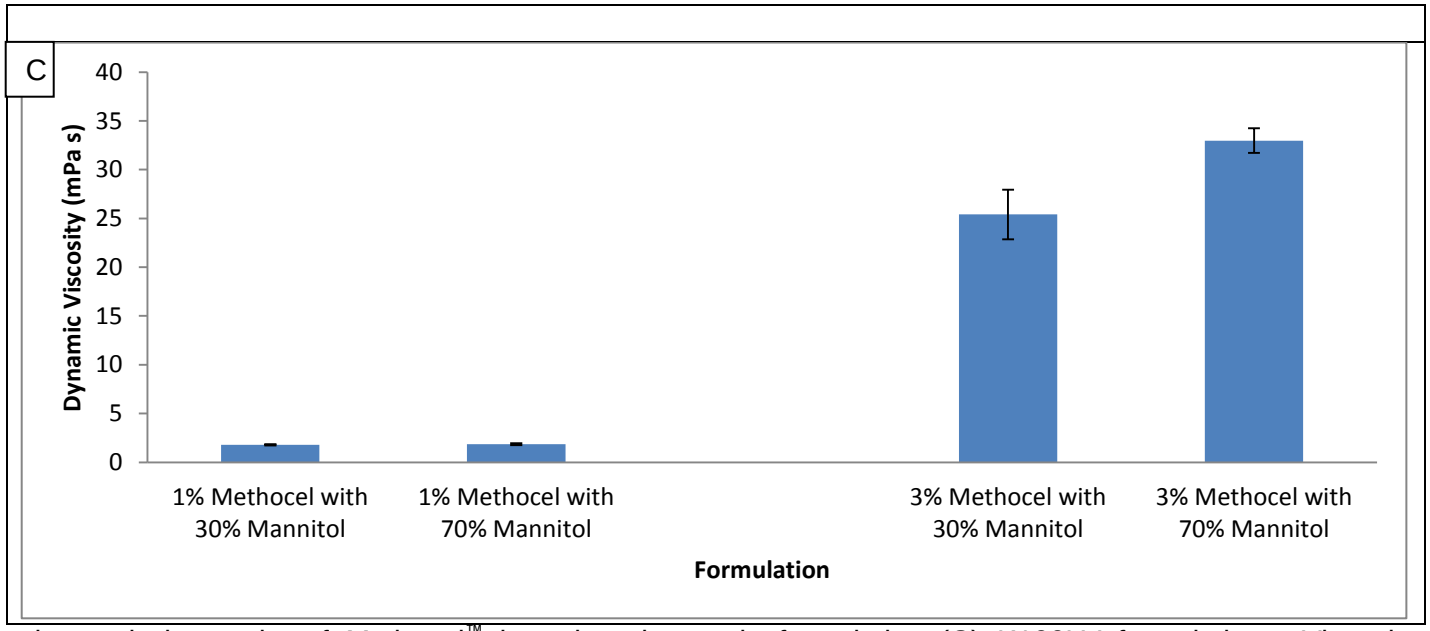


Figure 3.5 Viscosity analysis results of Methocel™ low viscosity grade formulation (C) K100LV formulations. Viscosity analyses of the formulations were carried out in triplicate and the mean values ± standard deviation are reported.

5 and 10% w/w Methocel™ K3LV formulations together with the 10 and 11% w/w Methocel™ E3LV formulations, revealed that the viscosities of the formulations containing mannitol exhibited greater viscosities than their mannitol-free formulation counterparts ($p < 0.05$). This could be due to the increased amount of solute in the formulations, and therefore increased intermolecular interactions in the formulations (Miyawaki et al. 2003), which resulted in these formulations exhibiting greater viscosities.

In terms of Methocel™ K100LV formulations shown in Figure 3.5C, the results revealed that increasing Methocel™ concentration resulted in an increase in formulation viscosity ($p < 0.05$). This trend of results was observed with both formulations which consisted of 30 and 70% w/w (of dried tablet weight) mannitol. Comparing the viscosities of the formulations consisting of 30% w/w (of dried tablet weight) mannitol with the viscosities of the formulations consisting of 70% w/w (of dried tablet weight) mannitol, revealed that comparable results were observed with the 1% w/w Methocel™ formulations ($p > 0.05$). However, with the 3% w/w Methocel™ formulations, increasing mannitol concentration from 30 to 70% w/w (of dried tablet weight) resulted in an increase in formulation viscosity ($p < 0.05$). The working viscosity range for Methocel™ K100LV formulations varied from 1.79 ± 0.06 to 32.97 ± 1.27 mPa s.

Following the analyses of the viscosities of the formulations, the mechanical properties of the prepared tablets were evaluated. Tablet hardness and fracturability analysis of the formulations were carried out in order to provide an assessment of the mechanical properties of the tablets. Tablet hardness and fracturability testing differ in that hardness testing involves an assessment of the compressibility of the tablet with a 5 mm diameter probe, whilst fracturability testing involves the penetration of a 1 mm diameter probe into the tablet binder matrix of the tablet. Tablet hardness testing therefore assesses the mechanical properties of the tablet as a whole, whilst fracturability testing focuses on the mechanical properties of the internal tablet binder matrix structure of the tablets.

Tablet hardness analysis of Methocel™ A15LV formulations showed that only two tablet formulations were suitable for testing; formulations which consisted of 3 and 5% w/w Methocel™ A15LV with 70% w/w (of dried tablet weight) mannitol. The other tablet formulations were not suitable for testing as some of the tablets were simply too soft to provide a result while some of the tablets could not be removed from the moulds due to their fragility.

Tablet fracturability measurements of Methocel™ A15LV formulations did not take place, as a number of formulations from this grade of Methocel™ did not produce tablets suitable for characterisation/analysis. As a result, extracting trends from the results would not have been possible.

Looking at the viscosity analysis in parallel with the mechanical properties analysis data, the results indicated that the high viscosity (low workable range) of some of the Methocel™ grades was responsible for weak mechanical properties of the resultant tablets. Even then, from the 8 formulations prepared in total, only two polymer grades were suitable for hardness testing. The high viscosity of these formulations prevented greater concentrations of Methocel™ being used to prepare more robust tablets, which would have been suitable for tablet hardness testing.

Tablet formulations not being suitable for testing was also an issue for the 1% w/w Methocel™ K3LV and E3LV formulations, respectively, together with the 1% w/w Methocel™ K100LV formulation consisting of 70% w/w (of dried tablet weight) mannitol.

Of the Methocel™ A15LV formulations which did provide results, the 3% w/w Methocel™ formulation with 70% w/w (of dried tablet weight) mannitol produced tablets which exhibited a hardness of 7.26 ± 2.07 N, whilst the corresponding 5% w/w Methocel™ formulation tablets displayed a hardness of 13.23 ± 7.02 N. There was no significant difference in tablet hardness between these two formulations ($p > 0.05$).

Tablet hardness results of Methocel™ E3LV formulations, shown in Figure 3.6, indicated that for the tablets produced from mannitol-free formulations, increasing Methocel™ concentration from 5 to 10 and 11% w/w, resulted in an increase in tablet hardness ($p < 0.05$). This trend of increasing polymer concentration resulting in an increase in tablet hardness can be attributed to the formation of a more extensive and robust tablet binder matrix being formed as polymer concentration was increased. This particular trend was also observed with the tablets of formulations which included mannitol ($p < 0.05$).

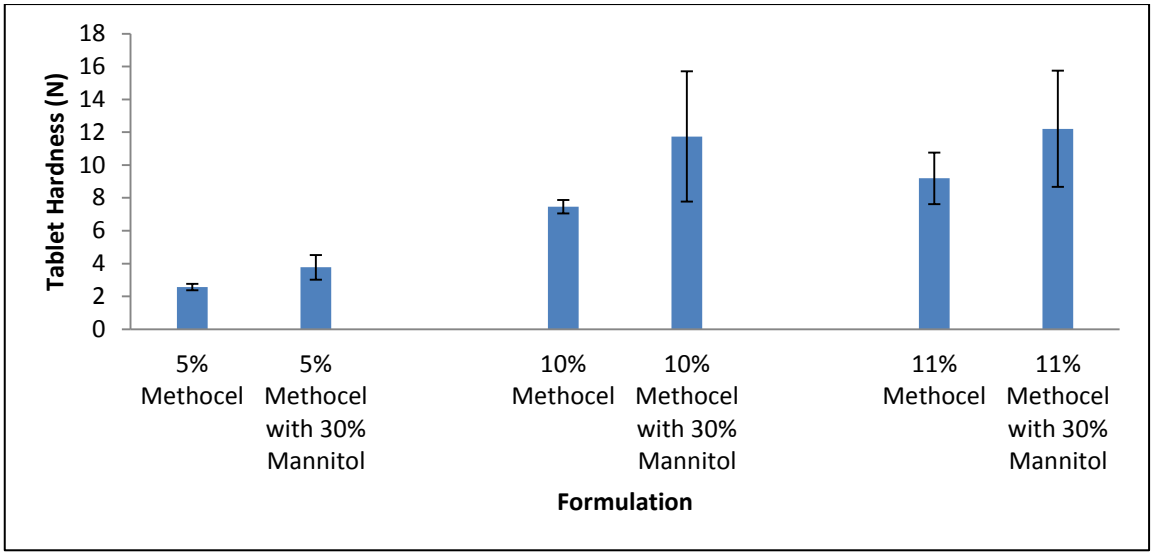


Figure 3.6 Tablet hardness analysis results for Methocel™ E3LV formulations tablets. Tablet hardness analyses of the formulations were performed in triplicate and the mean values ± standard deviation are reported.

Ahmed et al. (2011) reported in a study which evaluated the use of maltodextrin as a matrix former along with several cellulosic binders in the preparation of freeze-dried ODTs, that the concentration of cellulosic binders (Methocel™ E5LV, E15LV and A15LV) had a significant effect on the friability of the tablets. The authors attributed this to the higher binding capacity of the binders at higher concentrations. This can be related to the tablet hardness results, where it was observed that increasing Methocel™ E3LV concentration resulted in an increase in tablet hardness.

Comparing the tablet hardness of the mannitol-free formulations, with the tablets produced from formulations which included mannitol, indicated no significant difference in the results ($p > 0.05$). This was similarly observed with the 10% w/w Methocel™ K3LV formulations, of which the results are shown in Table 3.13. However, in the case of the 5% w/w formulations, the inclusion of mannitol in the formulation resulted in a reduction in tablet hardness.

Table 3.13 Tablet hardness analysis results for Methocel™ K3LV formulations tablets. Tablet hardness analyses of the formulations were performed in triplicate and the mean values ± standard deviation are reported.

Formulation	Tablet Hardness (N) ± S.D.
5% w/w Methocel K3LV	5.510 ± 0.57
5% w/w Methocel K3LV with 30% w/w (of dried tablet weight) Mannitol	3.077 ± 0.99
10% w/w Methocel K3LV	14.616 ± 5.68
10% w/w Methocel K3LV with 30% w/w (of dried tablet weight) Mannitol	13.913 ± 5.32

The results for Methocel™ K3LV formulations also revealed that increasing Methocel™ concentration from 5 to 10% w/w, did not result in an increase in tablet hardness ($p > 0.05$). This trend of results was observed with both the mannitol-free and containing formulations.

Tablet fracturability analysis results of Methocel™ E3LV and K3LV formulations, shown in Tables 3.14 and 3.15, displayed comparable results. With the mannitol-free formulations, increasing Methocel™ concentration from 5 to 10% w/w, resulted in an increase in the robustness of the tablet binder matrices, as greater force was required to penetrate the tablets. This observation can be attributed to the formation of a more extensive tablet binder matrix as Methocel™ concentration is increased, as a consequence the tablets are harder and more robust.

Table 3.14 Tablet fracturability analysis results for Methocel™ E3LV formulations tablets. Tablet fracturability analyses of the formulations were performed in triplicate, and the mean values ± standard deviation are reported.

Formulation	Tablet Fracturability (N) ± S.D.
5% w/w Methocel E3LV	1.956 ± 1.00
5% w/w Methocel E3LV with 30% w/w (of dried tablet weight) Mannitol	2.197 ± 0.19
10% w/w Methocel E3LV	8.577 ± 1.07
10% w/w Methocel E3LV with 30% w/w (of dried tablet weight) Mannitol	2.848 ± 0.30
11% w/w Methocel E3LV	4.491 ± 0.65
11% w/w Methocel E3LV with 30% w/w (of dried tablet weight) Mannitol	2.289 ± 0.68

Table 3.15 Tablet fracturability analysis results for Methocel™ K3LV formulations tablets. Tablet fracturability analyses of the formulations were performed in triplicate, and the mean values ± standard deviation are reported.

Formulation	Tablet Fracturability (N) ± S.D.
5% w/w Methocel K3LV	2.930 ± 0.34
5% w/w Methocel K3LV with 30% w/w (of dried tablet weight) Mannitol	2.573 ± 0.85
10% w/w Methocel K3LV	4.300 ± 0.64
10% w/w Methocel K3LV with 30% w/w (of dried tablet weight) Mannitol	2.760 ± 0.34

With the Methocel™ E3LV formulations, an unexpected/anomalous result was observed in which the mannitol-free formulation of the 11% w/w Methocel™ formulation exhibited a lower tablet fracturability value than the 10% w/w formulation. It was expected that the 11% w/w formulation would display a greater value due to the formation of more robust tablets at a higher concentration of Methocel™.

The results for the mannitol-included formulations revealed that increasing Methocel™ concentration did not affect tablet fracturability, as comparable results were observed ($p > 0.05$). This of course was not the case with the mannitol-free formulations, which suggests that the inclusion of mannitol in the formulations, influences tablet fracturability. To investigate this further, the tablet fracturability of the mannitol-free formulations were compared with the tablet fracturability of the mannitol-included formulations.

Comparing the tablet fracturability of the mannitol-free formulations with that of the mannitol-included formulations, the results revealed that in the case of the 5% w/w Methocel™ formulations, comparable results were observed ($p > 0.05$). However, with the 10% w/w Methocel™ formulations and the 11% w/w Methocel™ E3LV formulations, interesting results were observed in which the mannitol-included formulations displayed lower tablet fracturability values than the mannitol-free formulations.

These results suggested that the inclusion of mannitol in the formulations had a detrimental effect on tablet fracturability. It appeared that mannitol disrupted and subsequently weakened the tablet binder matrix, as a result the robustness of the tablets deteriorated. It therefore can be concluded that mannitol had a plasticisation effect, which subsequently modified the tablet binder matrix structure.

These results could possibly be related to the DSC results reported earlier. *E.g.* the 10 and 11% w/w Methocel™ E3LV formulations with 30% w/w (of dried tablet weight) mannitol, exhibited the crystallisation of mannitol. It may be possible that the crystallisation of mannitol disrupted the tablet binder matrix of Methocel™, which resulted in the deterioration of the robustness of the tablets.

Tablet hardness results of Methocel™ K100LV formulations, shown in Table 3.16, revealed that increasing Methocel™ concentration from 1 to 3% w/w resulted in an increase in tablet hardness ($p < 0.05$). There was no significant difference in tablet hardness between the two 3% w/w Methocel™ formulations ($p > 0.05$).

Table 3.16 Tablet hardness analysis results for Methocel™ K100LV formulations tablets. Tablet hardness analyses of the formulations were carried out in triplicate and the mean values ± standard deviation are reported.

Formulation	Tablet Hardness (N) ± S.D.
1% w/w Methocel K100LV with 30% w/w (of dried tablet weight) Mannitol	0.111 ± 0.09
3% w/w Methocel K100LV with 30% w/w (of dried tablet weight) Mannitol	4.179 ± 1.40
3% w/w Methocel K100LV with 70% w/w (of dried tablet weight) Mannitol	4.829 ± 3.25

Tablet fracturability measurements of Methocel™ K100LV formulations did not take place, as a number of formulations from this grade of Methocel™ did not produce tablets suitable for characterisation/analysis.

3.4.1.4 Tablet Disintegration Time Analysis and Porosity Measurements of Methocel™ Low Viscosity Grades Formulations

Freeze-Dried ODTs are extremely porous in nature, as a result of undergoing sublimation during the freeze-drying process. It has been identified that the highly porous structure of the tablets is responsible for this type of dosage form demonstrating instantaneous disintegration in the mouth following administration (Seager, 1998). In order to investigate the relationship between tablet porosity and disintegration time further, the effect of Methocel™ concentration and the inclusion of mannitol in the formulation on tablet porosity and subsequently disintegration time was assessed.

Tablet disintegration time analysis results of Methocel™ A15LV formulations, showed that only two tablet formulations were suitable for testing; formulations which consisted of 3 and 5% w/w Methocel™ A15LV with 70% w/w (of dried tablet weight) mannitol, as discussed with the tablet hardness results.

Both of these formulations exhibited disintegration times of greater than 180 s. This was also observed with the 10 and 11% w/w mannitol-free formulations of Methocel™ E3LV, along with the mannitol-free formulation of 10% w/w Methocel™ K3LV. These high tablet disintegration times can be attributed to the formation of highly extensive and robust tablet binder matrices which require a significant amount of time to disintegrate.

Porosity measurement of Methocel™ A15LV formulations did not take place, as a number of formulations from this grade of Methocel™ did not produce tablets suitable

for characterisation/analysis. As a result, extracting trends from the results would not have been possible.

The tablet disintegration time results of Methocel™ K3LV and E3LV formulations, as shown in Figures 3.7A and 3.7B, showed comparable results.

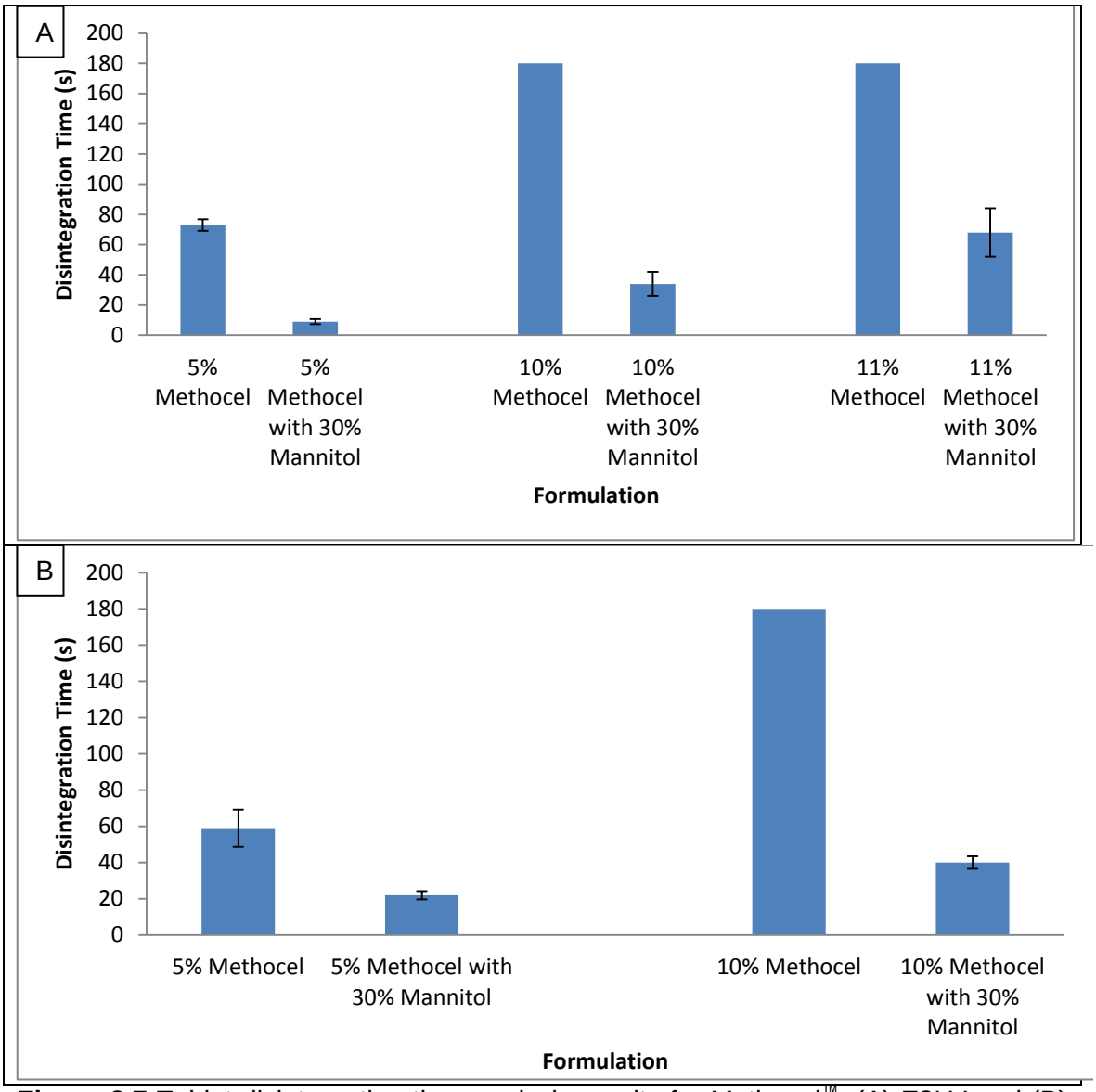


Figure 3.7 Tablet disintegration time analysis results for Methocel™; (A) E3LV and (B) K3LV formulations. Tablet disintegration time analyses of the formulations were carried out in triplicate, and the mean values ± standard deviation are reported.

The results indicated that polymer concentration had a significant effect on tablet disintegration time, as increasing polymer concentration resulted in an increase in tablet disintegration time (p <0.05). This trend of results can be attributed to the formation of a more extensive tablet binder matrix as polymer concentration is

increased (Abdelbary et al. 2004). This tendency was observed with the mannitol-free and included formulations.

Ahmed et al. (2011) similarly reported that increasing Methocel™ concentration resulted in an increase in *in vitro* and *in vivo* disintegration time of freeze-dried ODTs. The authors attributed this to the higher binding capacity of Methocel™ at higher concentrations.

These results correlated with the tablet porosity results, shown in Tables 3.17 and 3.18, which showed that increasing Methocel™ concentration from 5-10% w/w, resulted in a reduction in tablet porosity ($p < 0.05$). This observation was found with the mannitol-free and containing formulations, respectively, which can be attributed to the formation of a more extensive binder matrix, as a result of increasing Methocel™ concentration. Consequently, the porosity of the binder matrix is reduced. This relates to the disintegration time results as it is hypothesised that a reduction in tablet porosity results in an increase in tablet disintegration time, as the ability of disintegrating medium to ingress the porous structure of freeze-dried ODTs is restricted.

Table 3.17 Tablet porosity analysis results for Methocel™ E3LV formulations tablets. Tablet porosity analyses of the formulations were carried out in triplicate, and the mean values ± standard deviation are reported.

Formulation	Tablet Porosity (%) ± S.D.
5% w/w Methocel E3LV	100.21 ± 0.18*
5% w/w Methocel E3LV with 30% w/w (of dried tablet weight) Mannitol	98.36 ± 0.16
10% w/w Methocel E3LV	96.18 ± 0.33
10% w/w Methocel E3LV with 30% w/w (of dried tablet weight) Mannitol	93.11 ± 0.14
11% w/w Methocel E3LV	95.39 ± 0.33
11% w/w Methocel E3LV with 30% w/w (of dried tablet weight) Mannitol	92.58 ± 0.62

*Tablet porosity values of 100 % have been reported. The results suggest that this method has reached the limit of its operation, possibly due to the high porosity of the samples. An alternative method of measuring tablet porosity might be more suitable for these samples.

Table 3.18 Tablet porosity analysis results for Methocel™ K3LV formulations tablets. Tablet porosity analyses of the formulations were carried out in triplicate, and the mean values ± standard deviation are reported.

Formulation	Tablet Porosity (%) ± S.D.
5% w/w Methocel K3LV	100.25 ± 0.23*
5% w/w Methocel K3LV with 30% w/w (of dried tablet weight) Mannitol	98.66 ± 0.25
10% w/w Methocel K3LV	94.40 ± 0.57
10% w/w Methocel K3LV with 30% w/w (of dried tablet weight) Mannitol	93.57 ± 0.27

*Tablet porosity values of 100 % have been reported. The results suggest that this method has reached the limit of its operation, possibly due to the high porosity of the samples. An alternative method of measuring tablet porosity might be more suitable for these samples.

Comparing the tablet disintegration times of tablets from mannitol-free formulations, with the tablets from formulations which included mannitol has shown that the inclusion of mannitol in the formulations had a significant effect (Figure 3.7 A and B). Tablets which included mannitol in their formulation exhibited significantly shorter disintegration times, than tablets produced from mannitol-free formulations (p <0.05). The inclusion of mannitol in the formulations, has therefore shown to play a significant role is assisting tablet disintegration, and has shown to significantly shorten tablet disintegration time, in comparison to tablets produced from mannitol-free formulations.

Porosity measurement of 10 and 11% w/w Methocel™ E3LV mannitol-included formulations revealed that these formulations exhibited comparable results (p > 0.05), however, the mannitol-free formulations revealed that the 11% w/w Methocel™ formulation displayed a lower porosity than the corresponding 10% w/w Methocel™ formulation (Table 3.17). This result indicated that the presence of mannitol in the formulation influences tablet porosity. For this reason, the porosity of mannitol-free formulations were compared to their mannitol-included formulations counterparts, to investigate the influence of mannitol further.

Comparing the tablet porosities of the mannitol-free formulations with the formulations which included mannitol, the results revealed a clear trend. The inclusion of mannitol in the formulations resulted in a reduction in tablet porosity (Table 3.17) (p < 0.05). A possible explanation for this observation is that the presence of mannitol in the tablets reduces the porous space in the tablet binder matrix due to an increase in total solid content which therefore has a detrimental impact on the total porosity of the resultant tablet. This observation was similarly reported by Corveleyn and Remon (1997 and 1998), in which increasing maltodextrin (a matrix forming agent) concentration resulted

in a decrease in tablet porosity. The authors reported that the ability of ice crystals to grow is reduced when a higher solute concentration is used (Corveleyn and Remon, 1997 and 1998), and as the pores in freeze-dried products are formed from the ice crystals after sublimation (Dawson and Hockley, 1991), a reduction in pore size and subsequently porosity is observed.

The tablet porosity results (Table 3.17) contradicted the tablet disintegration time results (Figure 3.7 A). The porosity results revealed a decrease in tablet porosity following the inclusion of mannitol in the formulations, whilst the tablet disintegration time results revealed a reduction in disintegration time following the addition of mannitol to the formulations. Theoretically speaking, a reduction in tablet porosity would be expected to have resulted in an increase in tablet disintegration time, as hypothesised and discussed above.

These results suggested that with mannitol-included formulations, disintegration of the tablets is driven by the presence of mannitol in the formulations and not by tablet porosity, which subsequently reaffirms the role of mannitol as a disintegration enhancing agent (AlHusban et al. 2010b). The role of mannitol functioning as a disintegration enhancing agent is proposed to be attributed to its highly soluble nature. As when a tablet is introduced into disintegration medium, mannitol dissolves instantly which exposes the tablet binder matrix to the disintegrating medium to a greater extent than would be expected with a mannitol-free formulation tablet.

Disintegration time analysis results of Methocel™ K100LV formulations tablets are shown in Table 3.19. The results revealed that increasing Methocel™ concentration resulted in an increase in tablet disintegration time ($p < 0.05$), which as discussed above can be attributed to the formation of a more extensive tablet binder matrix. There was no significant difference in tablet disintegration time, between the two 3% w/w formulations ($p > 0.05$), which showed that increasing mannitol concentration from 30 to 70% w/w (of dried tablet weight), did not have an influence.

Table 3.19 Tablet disintegration time analysis results for Methocel™ K100LV formulations tablets. Tablet disintegration time analyses of the formulations were carried out in triplicate and the mean values ± standard deviation are reported.

Formulation	Tablet Disintegration Time (s) ± S.D.
1% w/w Methocel K100LV with 30% w/w (of dried tablet weight) Mannitol	24.00 ± 11.36
3% w/w Methocel K100LV with 30% w/w (of dried tablet weight) Mannitol	89.00 ± 13.05
3% w/w Methocel K100LV with 70% w/w (of dried tablet weight) Mannitol	70.00 ± 13.89

Porosity measurement of Methocel™ K100LV formulations did not take place, as a number of formulations from this grade of Methocel™ did not produce tablets suitable for characterisation/analysis. As a result, extracting trends from the results would not have been possible.

3.4.2 High Viscosity Grades of Methocel™

3.4.2.1 Concentration Profiling and Macroscopic Evaluation of the Formulations of High Viscosity Grades of Methocel™

Tables 3.20-3.22 represent the macroscopic evaluation of the tablets prepared from the various formulations of the high viscosity grades of Methocel™ respectively.

Table 3.20 Concentration profiling and macroscopic evaluation of the various Methocel™ A4M formulations prepared.

Formulation	Comments
1% w/w Methocel A4M with 30% w/w (of dried tablet weight) mannitol	Intact tablets were formed. The tablets were soft with a "spongy/foamy" consistency. The tablets were easy to remove from the bijou tubes, and were difficult to deform during handling (i.e. when compressed, the tablets return to their original shape).
1% w/w Methocel A4M with 70% w/w (of dried tablet weight) mannitol	Intact tablets formed. However the tablets were very soft/fragile, and broke/disintegrated when handled. The tablets could not be removed from the moulds.
2% w/w Methocel A4M with 30% w/w (of dried tablet weight) mannitol	Intact tablets were formed. The tablets were tough, with a "spongy/foamy" consistency. The tablets were easy to remove from the bijou tubes, and were difficult to deform during handling (i.e. when compressed, the tablets return to their original shape).
2% w/w Methocel A4M with 70% w/w (of dried tablet weight) mannitol	Intact tablets were formed. The tablets were hard/solid, with a noticeable elastic consistency. The tablets did not break when handled. The tablets were easily removed from the moulds.

Table 3.21 Concentration profiling and macroscopic evaluation of the various Methocel™ K4M formulations prepared.

Formulation	Comments
1% w/w Methocel K4M with 30% w/w (of dried tablet weight) mannitol	Intact tablets formed. Tablets were soft, with a "spongy/foamy" consistency. Tablets retained shape when handled, and did not deform permanently.
1% w/w Methocel K4M with 70% w/w (of dried tablet weight) mannitol	Intact tablets formed. However the tablets were very soft/fragile, and the tablets broke/disintegrated easily when handled. The tablets could not be removed from the moulds intact.
2% w/w Methocel K4M with 30% w/w (of dried tablet weight) mannitol	Intact tablets formed. Tablets were soft, although harder than the lower concentration formulations. The tablets had a "spongy/foamy" consistency. The tablets retained shape when handled, and did not deform permanently.
2% w/w Methocel K4M with 70% w/w (of dried tablet weight) mannitol	Intact tablets formed. The tablets were hard and physically stable. The tablets were relatively easily removed from the moulds.

Table 3.22 Concentration profiling and macroscopic evaluation of the various Methocel™ K100M formulations prepared.

Formulation	Comments
1% w/w Methocel K100M with 30% w/w (of dried tablet weight) mannitol	Intact tablets formed. Tablets were very soft, with a "spongy/foamy" consistency. Tablets retained shape when handled, and did not deform permanently.
1% w/w Methocel K100M with 70% w/w (of dried tablet weight) mannitol	Intact tablets formed, however the tablets were very soft/fragile, and the tablets broke/disintegrated easily when handled. The tablets could not be removed from the moulds intact.
2% w/w Methocel K100M with 30% w/w (of dried tablet weight) mannitol	Intact tablets formed. Tablets were soft, and noticeably harder than the lower concentration formulation. The tablets had a "spongy" consistency, and retained shape when handled. The tablets did not deform permanently when handled.
2% w/w Methocel K100M with 70% w/w (of dried tablet weight) mannitol	Intact tablets formed, however the tablets were soft/fragile, and the tablets broke/disintegrated relatively easy when handled. The tablets could not be removed from the moulds intact.

Observations made during the preparation of the formulations of Methocel™ A4M, K4M and K100M, revealed that these grades of Methocel™ could be used up to 2% w/w and above this concentration the formulations proved too difficult to dose, due to their high viscosities. The viscosity results (Figures 3.8A and 3.8B) revealed that the workable viscosity ranges for these grades of Methocel™, in order to prepare tablets were; 0.46 ± 0.36 to 1.60 ± 0.89, 19.63 ± 3.23 to 137.00 ± 4.82 and 78.63 ± 6.04 to 81.83 ± 5.11 mPa s, respectively.

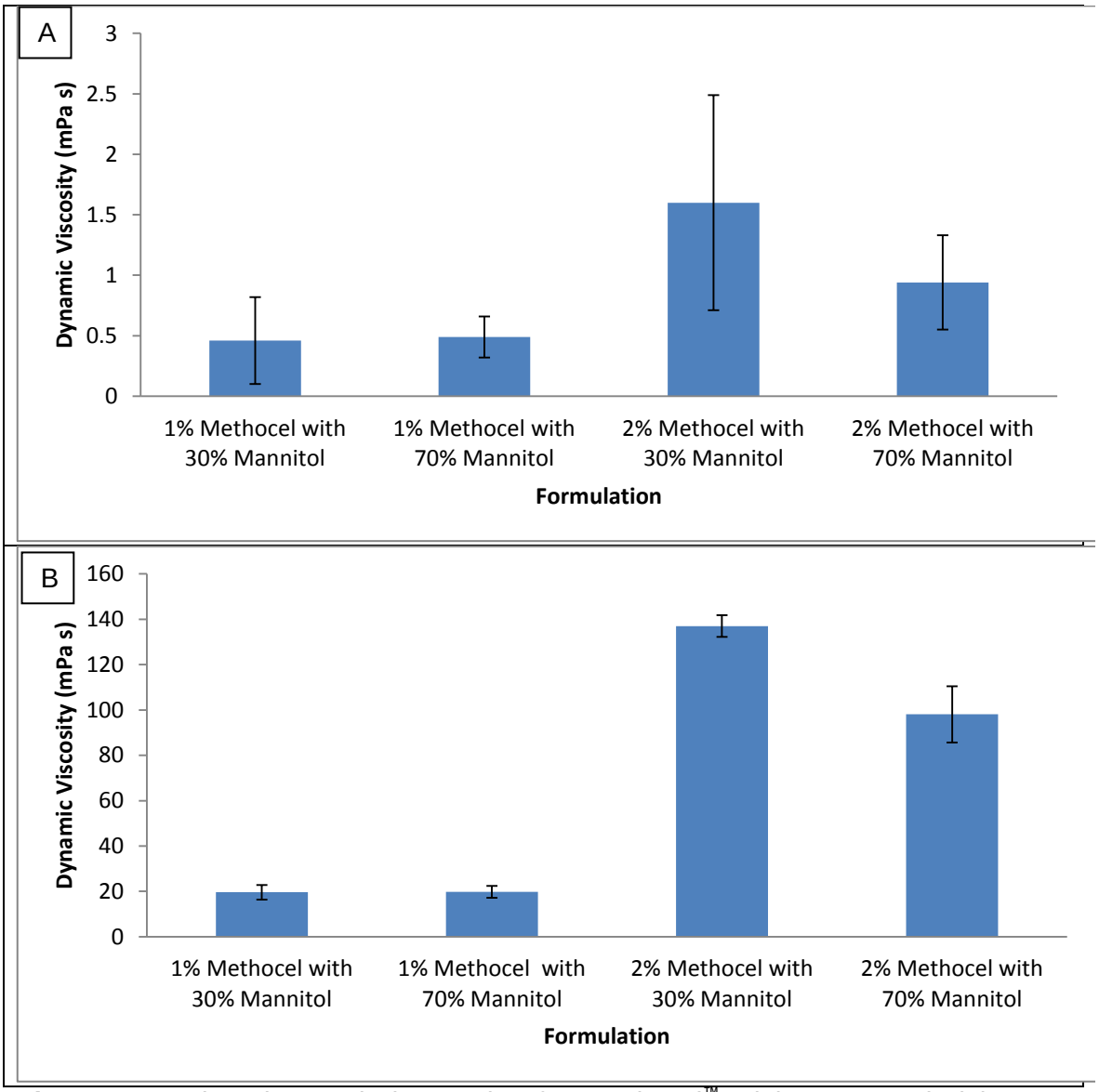


Figure 3.8 Viscosity analysis results for Methocel™; (A) A4M and (B) K4M formulations. Viscosity analyses of the formulations were performed in triplicate and the mean values ± standard deviation are reported.

Macroscopic evaluation of the tablets made from Methocel™ A4M formulations, revealed that intact tablets were produced from all of the formulations. The formulation containing 1% w/w methylcellulose and 70% w/w (of dried tablet weight) mannitol,

produced tablets which were very soft/fragile and broke/disintegrated easily when handled. These tablets could not be removed from the moulds. This is in contrast to the formulation consisting of 1% w/w Methocel™ A4M and 30% w/w (of dried tablet weight) mannitol, that produced soft tablets with a "spongy/foamy" consistency, i.e. when compressed, returned to their original shape.

The formulation containing 2% w/w Methocel™ A4M and 30% w/w (of dried tablet weight) mannitol, produced tough tablets with "spongy/foamy" consistency. When they were handled and compressed, the tablets returned to their original state. Increasing mannitol concentration from 30 to 70% w/w (of dried tablet weight) resulted in harder tablets with a noticeable elastic consistency. The tablets did not break/disintegrate when handled, which is an indication of satisfactory physical stability. The improvement in the physical appearance of the tablets as a result of adjusting mannitol concentration can possibly be related to two factors. Firstly, an increase in total solid content of the formulation produced tablets with an increase in density. Secondly, crystallisation behaviour of mannitol possibly played a part, as a result of increased concentration of the excipient. Kim et al. (1998) reported that the physical state of mannitol after freeze-drying is concentration dependent, and therefore this could be a factor in explaining why the physical appearance of the tablets improved following the adjustment of mannitol concentration.

Tablet formulations made from the K4M grade produced intact tablets. In terms of the 1% w/w formulations, increasing mannitol concentration from 30 to 70% w/w (of dried tablet weight), resulted in tablets which were very soft and fragile, which broke/disintegrated easily when handled. Increasing Methocel™ concentration to 2% w/w resulted in an improvement in the mechanical properties of the tablets. Increasing mannitol concentration also appeared to produce tablets which were harder and more physically stable.

Macroscopic evaluation of the tablets prepared from Methocel™ K100M formulations, revealed that in terms of the 1% w/w formulations, it was observed that increasing mannitol concentration from 30 to 70% w/w (of dried tablet weight), resulted in the deterioration in the mechanical properties of the tablets as the tablets appeared softer and more fragile, which broke/disintegrated easily when handled. Increasing Methocel™ concentration to 2% w/w, resulted in an improvement in the mechanical properties of the tablets. In line with the 1% w/w Methocel™ formulations, increasing

mannitol concentration from 30 to 70% w/w (of dried tablet weight), resulted in the deterioration of the mechanical properties of the tablets.

3.4.2.2 Evaluation of the Thermal Properties of the Formulations of High Viscosity Grades of Methocel™

The evaluation of the thermal properties of Methocel™ A4M, K4M and K100M formulations yielded comparable results. No thermal transitions attributable to Methocel™ or mannitol were observed with the 1 and 2% w/w Methocel™ with 30% w/w (of dried tablet weight) mannitol formulations, as shown in Figures 3.9A, 3.9C and 3.9E.

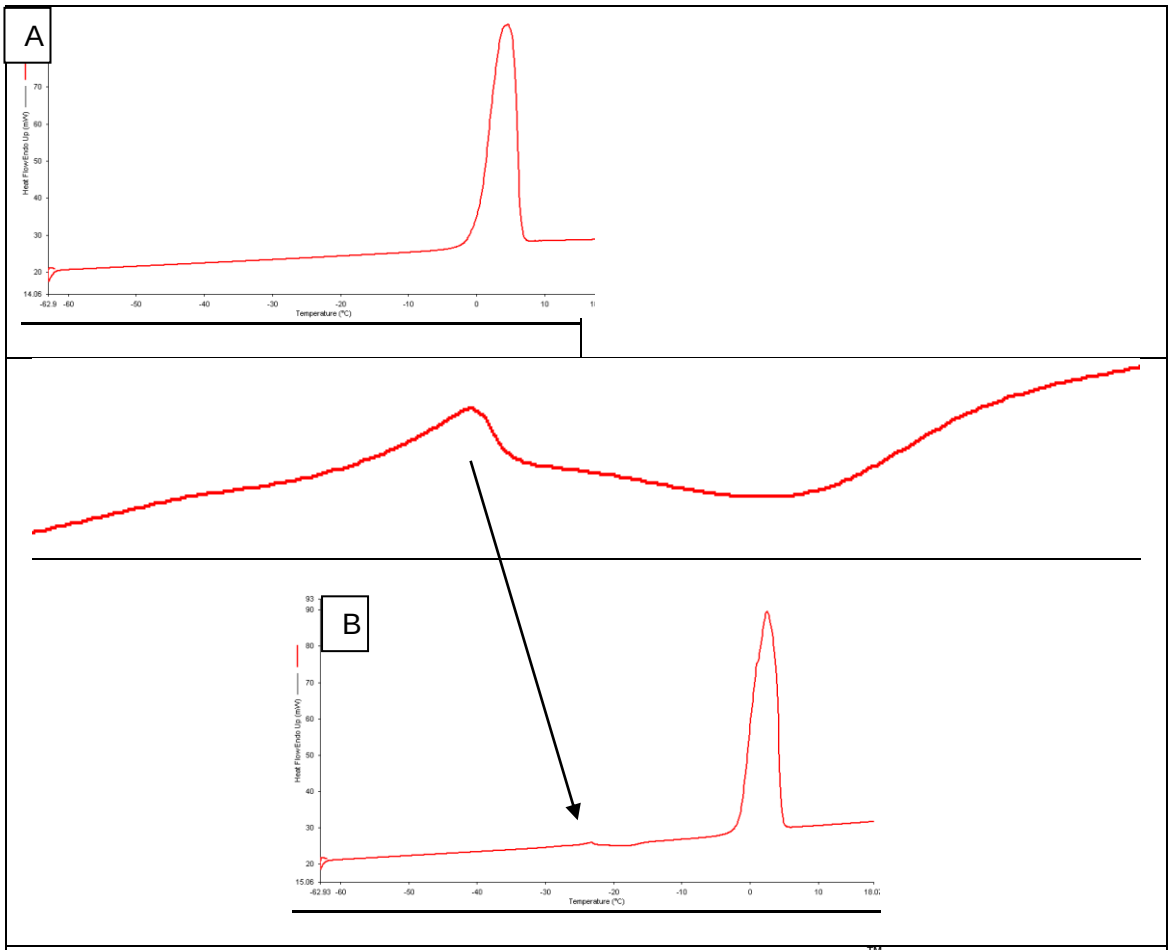


Figure 3.9 DSC analysis thermogram of; (A) 1% w/w Methocel™ A4M with 30% w/w mannitol and (B) 1% w/w Methocel™ A4M with 70% w/w mannitol, all during the heating phase of the DSC method. The concentration of mannitol is expressed as % w/w of dried tablet weight.

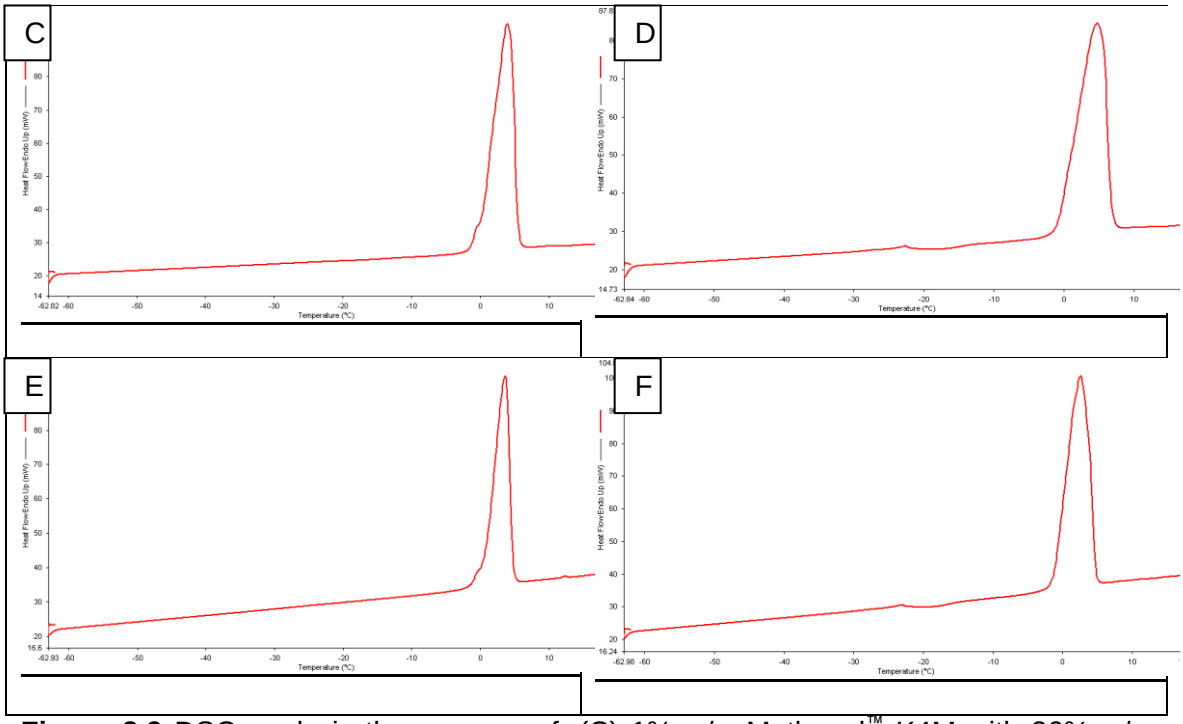


Figure 3.9 DSC analysis thermogram of; (C) 1% w/w Methocel™ K4M with 30% w/w mannitol, (D) 1% w/w Methocel™ K4M with 70% w/w mannitol, (E) 1% w/w Methocel™ K100M with 30% w/w mannitol and (F) 1% w/w Methocel™ K100M with 70% w/w mannitol, all during the heating phase of the DSC method. The concentration of mannitol is expressed as % w/w of dried tablet weight.

Thermal evaluation of the formulations consisting of 70% w/w (of dried tablet weight) mannitol revealed several thermal transitions, which were attributed to mannitol, as shown in Figures 3.9B, 3.9D and 3.9F. Glass transitions were observed at around -30 and -23ºC, respectively, whilst exothermic crystallisation peaks were observed at around -18ºC.

The glass transitions observed at around -30ºC were attributed to the glass transition of the mannitol phase plasticised by unfrozen water (Cavatur et al. 2002). Similarly, the glass transitions observed at around -23ºC were associated with the amorphous freeze-concentrate of mannitol (Cavatur et al. 2002). Exothermic crystallisation peaks observed at around -18ºC could be due to the crystallisation of both mannitol hydrate and the unfrozen water, associated with the amorphous phase of mannitol (Cavatur et al. 2002).

3.4.2.3 Viscosity, Tablet Hardness and Disintegration Time Analysis of Methocel™ High Viscosity Grades Formulations and Tablets

Similar to the low viscosity grade formulations, concentration profiling and macroscopic evaluation of Methocel™ high viscosity grades formulations, indicated that the

preparation of Methocel™ A4M, K4M and K100M formulations were restricted due to the high viscosity of the formulations. With all three high viscosity grades of Methocel™, formulations could only be prepared up to concentrations of 2% w/w.

As these grades of Methocel™ could only be used up to a concentration of 2% w/w, and their use being limited in this regard, viscosity analysis of the formulations were performed in order to establish the "workable" viscosity range for these grades of Methocel™. Additionally, the impact of this on the formation of intact and robust tablets was investigated, by undertaking tablet hardness analysis. These tablets were also examined for their disintegration times.

The viscosity analysis results for Methocel™ A4M formulations, are shown in Figure 3.8A. The results revealed that increasing methylcellulose concentration from 1 to 2% w/w did not result in any significant change in formulation viscosity ($p > 0.05$). Increasing mannitol concentration from 30 to 70% w/w (of dried tablet weight) also showed not to influence formulation viscosity ($p > 0.05$). This was observed with both the 1 and 2% w/w Methocel™ formulations. The "workable" viscosity range for this grade of Methocel™ that allowed satisfactory dosing was 0.46 ± 0.36 to 1.60 ± 0.89 mPa s.

Methocel™ K100M formulations also displayed comparable results. The 1% w/w Methocel™ formulation with 30% w/w (of dried tablet weight) mannitol, exhibited a viscosity of 81.83 ± 5.11 mPa s, whilst the formulation consisting of 70% w/w (of dried tablet weight) mannitol displayed a viscosity of 78.63 ± 6.04 mPa s ($p > 0.05$). The 2% w/w Methocel™ formulations, proved too viscous to analyse, as a result the "workable" viscosity range for this grade of Methocel™ was found as being 78.63 ± 6.04 to 81.83 ± 5.11 mPa s.

Analysis of the viscosities of Methocel™ K4M formulations, shown in Figure 3.8B, revealed that increasing Methocel™ concentration resulted in an increase in formulation viscosity ($p < 0.05$). This can be attributed to an increase in the density of the polymer network which ultimately results in the increase in the interaction between polymer chains.

Increasing mannitol concentration from 30 to 70% w/w, revealed comparable results with the 1% w/w Methocel™ formulations ($p > 0.05$). However, with the 2% w/w Methocel™ formulations, increasing mannitol concentration resulted in a decrease in

formulation viscosity ($p < 0.05$). The formulation consisting of 30% w/w mannitol displayed a viscosity of 137.00 ± 4.82 mPa s, whilst the formulation comprising of 70% w/w mannitol exhibited a viscosity 98.07 ± 12.45 mPa s ($p < 0.05$). This could possibly be attributed to mannitol acting as a plasticiser, at higher concentrations. Increasing mannitol concentration from 30 to 70% w/w appeared to weaken and decrease the molecular chain interactions of Methocel™ K4M polymer chains, and therefore resulted in a reduction in viscosity. A similar observation was reported in a study which investigated the effect of polyols on plasticising corn starch (Qiao et al. 2011).

Qiao et al. (2011) studied the effect of using mixtures of conventional plasticiser; glycerol, and higher molecular weight polyols, *e.g.* xylitol, on the plasticisation of corn starch. Qiao et al. (2011) reported that high molecular weight polyols reduced the strong molecular chain interactions (hydrogen-bonds) in starch. Moreover, the molecular chain mobility of starch increases following the addition of small plasticiser molecules, which results in decreasing melt viscosity (Qiao et al. 2011).

The "workable" viscosity range for Methocel™ K4M formulations was found as being 19.63 ± 3.23 to 137.00 ± 4.82 mPa s.

Due to the high viscosity of the formulations at low Methocel™ concentrations, and the fact that the Methocel™ grades could only be used up to concentrations of 2% w/w, tablet hardness analysis of the formulations, revealed that the majority of the formulations did not provide results when tested, which suggested that the tablets were too soft/fragile. It is apparent that greater concentrations of Methocel™ are required in order to form robust tablets, suitable for hardness testing. The formulation viscosity of these high viscosity grades of Methocel™ has shown to be the limiting factor, for the successful formation of intact and robust tablets, which exhibit satisfactory mechanical properties.

In terms of formulations that were too soft/fragile to provide results, both the 1% w/w Methocel™ A4M formulations were not suitable. This was similarly observed with the Methocel™ K4M and K100M formulations. Additionally, the 2% w/w Methocel™ K4M and K100M formulations with 70% w/w (of dried tablet weight) mannitol, were not suitable for testing.

Focussing on the formulations which did provide results, the 2% w/w Methocel™ A4M formulation with 30% w/w (of dried tablet weight) mannitol displayed a tablet hardness

of 2.61 ± 0.04 N. Increasing mannitol concentration to 70% w/w, yielded comparable tablet hardness as a value of 5.05 ± 1.38 N was observed ($p > 0.05$). The results therefore suggested that increasing mannitol concentration did not result in improving the mechanical properties of the tablets.

This observation could possibly be attributed to the molecular weight of Methocel™ A4M relative to that of mannitol. Due to the vast difference in molecular weight between these two excipients, it may be possible that increasing mannitol concentration did not exert a significant effect on supporting the tablet binder matrix of Methocel™ A4M, consequently its influence was minimal.

The K4M and K100M formulations (2% w/w Methocel™ with 30% w/w (of dried tablet weight) mannitol) yielded similar tablet hardness results. The K4M formulation displayed a value of 2.62 ± 0.10 N, whilst the K100M formulation exhibited a tablet hardness of 2.66 ± 0.15 N ($p > 0.05$).

In terms of the tablet disintegration time analysis of Methocel™ A4M formulations, increasing mannitol concentration from 30 to 70% w/w (with the 2% w/w Methocel™ formulations), resulted in an increase in tablet disintegration time ($p < 0.05$). The formulation consisting of 30% w/w (of dried tablet weight) mannitol displayed a disintegration time of 59.00 ± 6.51 s, whilst the formulation comprising of 70% w/w (of dried tablet weight) mannitol exhibited a disintegration time of 99.00 ± 12.12 s ($p < 0.05$).

Tablet disintegration time analysis of Methocel™ high viscosity grades formulations, therefore revealed that there was similarity in trend as with tablet hardness analysis results as the majority of the formulations were not suitable for testing. The formulations which were suitable for analysis were the same as for tablet hardness analysis.

Corveleyn and Remon (1997 and 1998) reported that increasing maltodextrin (matrix forming agent) concentration resulted in an increase in tablet disintegration time, due to a reduction in tablet porosity. It is therefore possible that increasing mannitol concentration in the Methocel™ A4M formulations, reduced tablet porosity and as a result the ability of water to penetrate the tablet binder matrix and disintegrate the tablet was restricted, which resulted in an increase in tablet disintegration time.

The Methocel™ K4M and K100M formulations (both 2% w/w Methocel™ with 30% w/w (of dried tablet weight) mannitol) produced tablets which displayed comparable disintegration times ($p > 0.05$). The Methocel™ K4M formulation exhibited a disintegration time of 93.00 ± 20.03 s, whilst the K100M formulation demonstrated a disintegration time of 116.00 ± 16.56 s ($p > 0.05$).

3.5 Conclusions

Evaluating the several grades of Methocel™, which comprised of both low and high viscosity grades, revealed that the low viscosity grades, in particular; Methocel™ E3LV and K3LV, were suitable as binders in freeze-dried ODTs.

With the higher viscosity grades, due to the highly viscous nature of these polymers, only low concentration formulations were prepared, as with higher concentrations dosing was not possible. As only low concentration formulations were prepared, the resulting tablets did not exhibit satisfactory mechanical properties, as extensive tablet binder matrices were not formed.

Using the lower viscosity grades of Methocel™ produced more robust tablets, as formulations consisting of greater concentrations of polymer were produced, and as a result tablets exhibited more extensive binder matrices.

Methocel™ E3LV appeared the most suitable grade for an application as a binder in freeze-dried ODTs. This grade of Methocel™ showed to produce tablets exhibiting a hardness of 11.74 ± 3.97 N and a disintegration time of 34.00 ± 7.94 s. This grade of Methocel™ was taken forward to future studies, in order to prepare HPMC-based formulations.

Investigating the Application of Polyox™ (Synthetic Polyethylene Oxide) in Freeze-Dried ODTs

4.1 Introduction

As discussed previously in Chapter One, one of the advantages/benefits of ODTs is that they can enhance the clinical effects of APIs through pre-gastric absorption, which can lead to an increase in bioavailability and reduction in adverse drug reactions (Seager, 1998).

It has been reported that following the administration of freeze-dried ODTs, the contents of the formulations disperse over the buccal mucosa and are retained for a considerable length of time, following the rapid disintegration of the dosage form in the oral cavity (Wilson et al. 1987). Additionally, Seager (1998) reported that particles from Zydis® (freeze-dried fast-dissolving dosage form) formulations coat the buccal, pharyngeal and gastric mucosa, following administration of the dosage form.

It can be concluded that as freeze-dried ODTs disperse in the oral cavity and are retained for a considerable length of time in the pre-gastric regions, absorption of suitable APIs takes place resulting in rapid onset of action.

In relation to the work carried out by Wilson et al. (1987) and Seager (1998), Chandrasekhar et al. (2009) carried out a study which investigated the role of formulation excipients in the development of lyophilised fast-disintegrating tablets. In this study the authors investigated the addition of viscosity-modifying polymers (Carbopol and Pluronic) to improve and aid pre-gastric retention by increasing formulation viscosity with the ultimate objective of promoting pre gastric absorption and rapid bioavailability.

Polyethylene oxide (PEO), which is also referred to as Polyox™, is a non-ionic homopolymer of ethylene oxide and is represented by the formula:

$$(OCH_2CH_2)n$$

in which *n* represents the average number of oxyethylene groups (United States Pharmacopeial Convention, 2003).

PEO appears as a white to off-white, free-flowing powder, which has a slight ammonical odour (Maximilien, 2011). It is soluble in water and a range of organic solvents such as chloroform, acetonitrile and methylene chloride, whilst it is insoluble in most alcohols, ethylene glycol and aliphatic hydrocarbons (Bailey and Kolesky, 1976).

PEO is available in a wide range of molecular weight grades, from 100,000 – 8,000,000 and is widely applicable in pharmaceutical formulations including; tablet binding, controlled release solid dose matrix systems, mucosal bioadhesives and transdermal drug delivery systems (DOW Chemical Company, 2002). PEO also has an application as a viscosity-increasing agent (Maximilien, 2011). In terms of its tablet binding capability, PEO can be used at concentrations of 5-85% (Maximilien, 2011).

PEO has been reported to be very effective as a mucoadhesive polymer (Bottenberg et al. 1991). Its application as a mucoadhesive polymer is attributed to its water solubility, hydrophilicity, high molecular weight, hydrogen bonding functionality and its biocompatibility (DOW Chemical Company, 2002). Additionally, as PEO exists as a long linear chain structure, this allows strong interpenetrating networks to form with mucus (DOW Chemical Company, 2002). It is therefore evident that PEO has a number of properties/attributes which makes it extremely applicable and effective as a mucoadhesive polymer.

As a continuation of the study by Chandrasekhar et al. (2009), Polyox™, which as discussed has applications as a; viscosity-increasing agent, mucoadhesive agent and tablet binding agent, is proposed to be investigated for its applications in freeze-dried ODTs. It is hypothesised that the viscosity-increasing, mucoadhesive and binding properties of Polyox™, could potentially be utilised to increase the retention of active ingredients in the pre-gastric regions, such as the buccal and pharyngeal mucosa, and subsequently encourage pre-gastric absorption.

4.2 Materials

Polyethylene oxide (Polyox™; N10 (Lot No. DT353617), 1105 (Lot No. DT314288), N-60K (Lot No. DT305827) and Coagulant (Lot No. DT307830) were supplied by Colorcon Ltd. (Dartford, UK). Mannitol was supplied by Sigma-Aldrich Chemicals (Poole, UK).

4.3 Methods

4.3.1 Preparation of Freeze-Dried Tablets

For the preparation of polyethylene oxide-based tablets, the required amount of polymer was added to double-distilled water at ambient temperature, followed by the addition of mannitol to form a solution. 1.5 g of the resulting solutions were dosed into a tablet mould, frozen at -70°C for a minimum of sixty minutes and freeze-dried (ADVANTAGE, Freeze-Dryer, VIRTIS), according to the following regime; primary drying for forty eight hours at a shelf temperature of -40°C, secondary drying for ten hours at a shelf temperature of 20°C, and vacuum pressure of 6.67 Pa (50 mTorr). A minimum of ten tablets were prepared for each formulation.

4.3.2 Formulation Viscosity Analysis

For all formulations, the viscosities of 100 mL samples were measured using a DV-I+ Brookfield digital viscometer (Harlow, UK). The viscosities of the formulations were measured at 37 ± 2°C, in order to replicate the viscosity of the formulations in the oral cavity, at physiological temperature. The viscometer spindles were selected based on the viscosity of the individual formulations, as each spindle is suitable for analysing a certain viscosity range. The rotational speed of the spindle was set at 100 rpm. Each formulation was analysed in triplicate, and the mean values ± standard deviation is reported.

4.3.3 Concentration Profiling and Macroscopic Evaluation of the Formulations

Table 4.1 shows the grades of Polyox™ which were evaluated in this study, and as can be seen the grades differ greatly in terms of approximate molecular weight and subsequently viscosity, which allowed a broad evaluation of Polyox™ as an excipient.

Table 4.1 Information detailing the different grades of Polyox™ evaluated in this study, which highlights the differences between the grades.

Polyox™ Grade	Approximate Number of Repeating Units	Approximate Molecular Weight	Viscosity at 25°C (mPa s)
N10	2,275	100,000	30-50 (5% solution)
1105	20,000	900,000	8,800 – 17,600 (5% solution)
N-60K	45,000	2,000,000	2,000 – 4,000 (2% solution)
Coagulant	114,000	5,000,000	5,500 – 7,500 (1% solution)

During the preparation of the formulations, several visual assessments were carried out in order to evaluate the polymers as binders. The solubility of the material was assessed by observing the ease at which the polymer went into solution. The ease of dosing of the formulations was also assessed, in terms of the viscosity of the prepared solution/suspension and also the consistency of the formulation, i.e. if it had a cohesive or non-cohesive appearance.

In terms of the visual and physical assessment of the tablets, several assessments were carried out in order to evaluate the ability of the polymers to form robust tablets with satisfactory mechanical strength. The first assessment involved the evaluation of the ability of the formulations to form intact tablet shaped dosage forms, following freeze-drying. The physical appearance of the tablets was assessed, classifying them as either soft or hard, and having either elastic or a more robust/solid consistency. The ability of the tablets to be removed from the moulds was also assessed. Finally, the performance of the tablets when handled was assessed, to observe if they deformed permanently or retained their shape.

4.3.4 Differential Scanning Calorimetry (DSC)

DSC (Pyris Diamond DSC and Intercooler 2P: Perkin Elmer, Wellessey, USA) was used to determine the glass transition temperature (T_g) and other thermal properties of the formulations in their frozen state (before freeze-drying). 10-15 mg of liquid samples of the formulations were loaded into aluminium pans, cooled from 25ºC to -65ºC at a rate of 5ºC/min, and then heated to 20ºC at a rate of 5ºC/min, with a nitrogen purge of 20 mL/min. An empty aluminium pan was used as a reference for all measurements.

The resulting thermograms were analysed by Pyris manager software. T_g values were determined from the intersection of relative tangents to the baseline, and other thermal

properties of the formulations were also assessed. Three measurements were taken for each formulation, and the mean values ± standard deviation were reported.

4.3.5 Tablet Hardness and Fracturability

The hardness and fracturability of the tablets was investigated using a texture analyzer (QTS 25: Brookfield, Essex, UK) equipped with a 25 kg load cell. The instrument was calibrated by standard weights of 500 g and 5 kg. The tablets were placed in a holder with a cylindrical hole. The hardness was taken as the peak force after 1 mm penetration of a 5 mm diameter probe at a rate of 6 mm/min. The fracturability was taken as the peak force after 3 mm penetration of a 1 mm diameter probe at a rate of 6 mm/min. Three measurements were taken for each formulation, and the mean ± standard deviation is reported.

4.3.6 Disintegration Time of the Tablets

The disintegration time of the tablets was determined using a USP disintegration tester (Erweka, ZT3). 800 mL of double distilled water, which was kept at 37 ± 2ºC, was used as the medium and the basket was raised and lowered at a fixed rate of 30 rpm. Three tablets were evaluated from each formulation, and the mean values ± standard deviation is reported.

4.3.7 Investigation of Tablet Porosity

The porosity of the tablets was measured using helium pycnometry (MULTIPYCNOMETER, Quantachrome Instruments, Hampshire, UK). Two freeze-dried tablets were placed in a suitably sized sample cup and subjected to helium pycnometry, to determine the true density of the tablets. The true density value was then inserted into the equation, as shown below, to determine the porosity of the tablets.

$$\text{Porosity} = 1 - \left[\frac{\text{bulk density}}{\text{true density}}\right] \times 100\%$$

(Equation 4.1)

Bulk density was determined by considering; the mass of the tablets, the diameter of the tablets, and the height/depth of the tablets. The diameter and height/depth of the tablets were determined using a screw gauge (LINEAR Farnell). Three porosity measurements were taken for each formulation, and the mean values ± standard deviation is reported. The porosity of the tablets is expressed as a percentage.

4.3.8 Statistical Analysis

Statistical analyses of the formulation and tablet characterisation results were performed using GraphPad Prism 5 statistical analysis software. One-way analysis of variance (ANOVA) was the statistical analysis test performed, using the Tukey post test. A significance level of $p < 0.05$ (95% confidence interval) was judged as being statistically significant.

4.4 Results and Discussion

4.4.1 Concentration Profiling and Macroscopic Evaluation of the Formulations

Due to the versatility of Polyox™, the aim of this chapter was to carry out an evaluation of its applications in lyophilised ODTs, in terms of both low and high viscosity grades. Several formulations were prepared based on each of the four grades of Polyox™ evaluated in this study. Each formulation prepared was assessed for its ability to form intact tablets, which were subsequently characterised for hardness and disintegration time. The viscosity and thermal properties (determined by DSC) of the formulations were also assessed.

The first phase of the investigation was focussed on concentration profiling of the four grades of Polyox™, in order to understand its function as an excipient in freeze-dried ODTs. Tables 4.2-4.5 represent the various formulations that were prepared with the four grades of Polyox™. Tables 4.6-4.9 represent the macroscopic evaluation of the resultant tablets respectively. Both mannitol inclusive and free formulations were tested to determine the effect of inclusion of matrix supporting agents in the formulation and to identify the specific role of Polyox™ within the tablets.

Table 4.2 Formulation details of Polyox™ N10 formulations, detailing the concentrations of Polyox™ and mannitol used in each formulation.

Polyox™ Concentration (% w/w)	Mannitol Concentration (% w/w of dried tablet weight)
1	0
1	30
5	0
5	30
10	0
10	30

Table 4.3 Formulation details of Polyox™ 1105 formulations, detailing the concentrations of Polyox™ and mannitol used in each formulation.

Polyox™ Concentration (% w/w)	Mannitol Concentration (% w/w of dried tablet weight)
1	0
1	30
1	70
3	0
3	30
3	70

Table 4.4 Formulation details of Polyox™ N-60K formulations, detailing the concentrations of Polyox™ and mannitol used in each formulation.

Polyox™ Concentration (% w/w)	Mannitol Concentration (% w/w of dried tablet weight)
1	0
1	30
1	70
2	0
2	30
2	70

Table 4.5 Formulation details of Polyox™ Coagulant formulations, detailing the concentrations of Polyox™ and mannitol used in each formulation.

Polyox™ Concentration (% w/w)	Mannitol Concentration (% w/w of dried tablet weight)
1	0
1	30
1	70
2	0
2	30
2	70

Tables 4.6-4.9 detail the macroscopic evaluation of the prepared tablets, from the various grades and subsequent formulations.

Table 4.6 Concentration profiling and macroscopic evaluation of the various formulations prepared with the Polyox™ N10 grade.

Formulation	Comments
1% w/w Polyox™ N10	Intact tablets formed. Tablets were very soft and deformed permanently (plastic deformation) when handled. Tablets did not have elastic properties, i.e. did not retain shape when handled. Low viscosity grade Polyox™ N10 was not suitable as a binder in ODTs, at a concentration of 1% w/w.
1% w/w Polyox™ N10 with 30% w/w (of dried tablet weight) Mannitol	Intact tablets formed. Tablets were very soft and deformed permanently (plastic deformation) when handled. Tablets did not have elastic properties, i.e. did not retain shape when handled. Low viscosity grade Polyox™ N10 at a concentration of 1% w/w with 30% w/w (of dried tablet weight) mannitol, was not suitable as a formulation for ODTs.
5% w/w Polyox™ N10	Intact tablets formed. Tablets were harder than the 1% w/w tablets but still deformed permanently (plastic deformation) when handled. Tablets did not retain shape when handled. Low viscosity grade Polyox™ N10 was not suitable as a binder in ODTs, at a concentration of 5% w/w.
5% w/w Polyox™ N10 with 30% w/w (of dried tablet weight) Mannitol	Intact tablets formed. Tablets were soft and deformed permanently when handled. Tablets did not retain shape when handled. Polyox™ N10 at a concentration of 5% w/w with 30% w/w (of dried tablet weight) mannitol, was not suitable as a formulation for ODTs.
10% w/w Polyox™ N10	Intact tablets formed. Tablets were very hard and robust, and retained shape when handled. However, the tablets did deform permanently when excessive force was applied, when handled. Tablets were relatively difficult to remove from the moulds. Low viscosity grade Polyox™ N10 at a concentration of 10% w/w, was suitable as a binder in ODTs.
10% w/w Polyox™ N10 with 30% w/w (of dried tablet weight) Mannitol	Intact tablets formed. Tablets were hard and robust, and retained shape when handled. However, tablets did deform when excessive force was applied, when handled. Tablets were relatively difficult to remove from the moulds. Low viscosity grade Polyox™ N10 at a concentration of 10% w/w with 30% w/w (of dried tablet weight) mannitol, was a suitable formulation for ODTs.

Table 4.7 Concentration profiling and macroscopic evaluation of the various formulations prepared with the Polyox™ 1105 grade.

Formulation	Comments
1% w/w Polyox™ 1105	Intact tablets were formed. The tablets were very soft, with a "spongy/foamy" consistency. The tablets were relatively easy to remove from the bijou tubes. The tablets deformed-permanently (i.e. lost shape) very easily when handled.
1% w/w Polyox™ 1105 with 30% w/w (of dried tablet weight) mannitol	
1% w/w Polyox™ 1105 with 70% w/w (of dried tablet weight) mannitol	
3% w/w Polyox™ 1105	Intact tablets were formed. The tablets were soft, but harder than the lower polymer concentration tablets. The tablets had a "spongy/foamy" consistency. The tablets were difficult to remove from the bijou tubes. The tablets deformed-permanently (i.e. lost shape) very easily when handled.
3% w/w Polyox™ 1105 with 30% w/w (of dried tablet weight) mannitol	
3% w/w Polyox™ 1105 with 70% w/w (of dried tablet weight) mannitol	Intact tablets were formed. The tablets were very tough, with a noticeable "spongy/foamy" consistency. The tablets were very difficult to remove from the bijou tubes. The tablets deformed-permanently (i.e. lost shape) when handled.

Table 4.8 Concentration profiling and macroscopic evaluation of the various formulations prepared with the Polyox™ N-60K grade.

Formulation	Comments
1% w/w Polyox™ N-60K	Intact tablets were formed. The tablets were soft with an "elastic" consistency. The tablets were relatively easy to remove from the bijou tubes. The tablets deformed-permanently (i.e. lost shape) easily when handled.
1% w/w Polyox™ N-60K with 30% w/w (of dried tablet weight) mannitol	
1% w/w Polyox™ N-60K with 70% w/w (of dried tablet weight) mannitol	
2% w/w Polyox™ N-60K	Intact tablets were formed. The tablets were soft but harder than the lower polymer concentration tablets. The tablets had an "elastic" consistency. The tablets were difficult to remove from the bijou tubes, and deformed-permanently (i.e. lost shape) easily when handled.
2% w/w Polyox™ N-60K with 30% w/w (of dried tablet weight) mannitol	
2% w/w Polyox™ N-60K with 70% w/w (of dried tablet weight) mannitol	Intact tablets were formed. The tablets were tough, with an "elastic" consistency. The tablets were difficult to remove from the bijou tubes, and deformed-permanently (i.e. lost shape) easily when handled.

Table 4.9 Concentration profiling and macroscopic evaluation of the various formulations prepared with the Polyox™ Coagulant grade.

Formulation	Comments
1% w/w Polyox™ Coagulant	Intact tablets were formed. The tablets were soft with an "elastic" consistency. The tablets were relatively easy to remove from the bijou tubes, and deformed-permanently (i.e. lost shape) easily when handled.
1% w/w Polyox™ Coagulant with 30% w/w (of dried tablet weight) mannitol	
1% w/w Polyox™ Coagulant with 70% w/w (of dried tablet weight) mannitol	
2% w/w Polyox™ Coagulant	Intact tablets were formed. The tablets were soft with an "elastic" consistency. The tablets were difficult to remove from the bijou tubes, and deformed-permanently (i.e. lost shape) easily when handled.
2% w/w Polyox™ Coagulant with 30% w/w (of dried tablet weight) mannitol	
2% w/w Polyox™ Coagulant with 70% w/w (of dried tablet weight) mannitol	

The macroscopic evaluation of Polyox™ N10 formulations and tablets are shown in Table 4.6. Formulations comprising of 1, 5 and 10% w/w Polyox™ N10 were prepared in order to observe and evaluate the effects of Polyox™ N10 concentration on the properties of the formulations and tablets, over a broad concentration range. This low viscosity grade Polyox™ polymer has shown to produce intact tablets. With a polymer concentration of 1% w/w, the tablets were very soft and deformed permanently (i.e. lost their shape) when handled. Therefore, Polyox™ N10 at a concentration of 1% w/w was not suitable as a binder in ODTs. The inclusion of mannitol in the formulation did not appear to have a significant effect on the physical appearance of the tablets. Similar to the mannitol-free formulation, the tablets were very soft and lost their shape when handled. This composition which includes mannitol, was not therefore suitable as a formulation for ODTs.

Increasing Polyox™ N10 concentration to 5% w/w, produced tablets which were harder than the 1% w/w tablets, however the 5% w/w formulation tablets still lost their shape when handled. Polyox™ N10 at a concentration of 5% w/w was not suitable as a binder in ODTs. Including mannitol at a concentration of 30% w/w (of dried tablet weight), in the formulation, did not appear to significantly improve or deteriorate the physical appearance of the tablets. The tablets were soft, although noticeably harder than the 1% w/w Polyox™ N10 tablets, and lost their shape when handled. As a result, this concentration was not suitable as a formulation for ODTs, as this formulation did not form hard and robust tablets which can withstand manual handling.

The formulation which consisted of 10% w/w Polyox™ N10 produced tablets which were hard and robust, which also retained their shape when handled. However, when excessive force was applied during handling the tablets, they did deform. The tablets were also more difficult to remove from the moulds, than the tablets which consisted of 1 and 5% w/w Polyox™ N10, respectively. The inclusion of mannitol in the formulation did not significantly improve or deteriorate the physical appearance of the tablets. The tablets were hard and robust, which could retain their shape when handled. This particular composition is a suitable formulation for ODTs.

A possible reason why tablets which consisted of 1 and 5% w/w Polyox™ N10 underwent permanent deformation (i.e. lost their shape/could not retain their shape), could be due to the fact that Polyox™ is a linear chain polymer and is thermoplastic in nature (DOW, 2002). Polyox™ is based on the polyethylene structure which is

considered as a plastic material, which exhibits limited elasticity (i.e. the tablets do not retain their shape when handled/the tablets do not return to their original shape when compressed), and is formable (i.e. can undergo plastic deformation) (Houwink and De Decker, 1971).

Tablets which consisted of 10% w/w Polyox™ N10 were harder and more robust than the lower concentration Polyox™ N10 based formulation tablets, and were more resistant to deformation possibly because of the formation of a denser and extensive polymer binder network which produces harder and more robust tablets.

Tablets which were produced from formulations which consisted of 1 and 5% w/w Polyox™ N10, respectively, were considered not suitable for tablet characterisation analysis. These tablets deformed permanently when handled (i.e. lost their shape). It was therefore decided not to analyse these tablets for; mechanical properties, porosity and disintegration time, as it would appear that the tablets would have provided inaccurate results and inaccurate representation of the tablets.

The macroscopic evaluation of Polyox™ 1105 formulations and tablets are shown in Table 4.7. In terms of the observations made for Polyox™ 1105 formulations, it was possible to use this polymer up to 3% w/w in the formulations. Above this concentration, the formulations were too viscous to dose. The viscosity analysis results (Figure 4.1B) revealed that the workable viscosity range for this grade of Polyox™, was 2.98 ± 0.11 to 71.33 ± 1.21 mPa s.

Macroscopic evaluation of Polyox™ N-60K formulations and tablets are shown in Table 4.8. In terms of the observations made for Polyox™ N-60K formulations, it was possible to use this polymer up to 2% w/w in the formulations. Above this concentration, the formulations were too viscous to dose. The viscosity analysis results (Figure 4.1C) revealed that the workable viscosity range for this grade of Polyox™, was 8.56 ± 0.93 to 33.17 ± 2.45 mPa s.

The macroscopic evaluation of Polyox™ Coagulant formulations and tablets are shown in Table 4.9. In terms of the observations made for Polyox™ Coagulant formulations, it was possible to use this polymer up to 2% w/w in the formulations. Above this concentration, the formulations were too viscous to dose. The viscosity analysis results

(Figure 4.1D) revealed that the workable viscosity range for this grade of Polyox™, was 7.02 ± 0.91 to 58.67 ± 7.53 mPa s.

The formulations prepared form Polyox™ grades; 1105, N-60K and Coagulant, were also very cohesive in nature, which made dosing very difficult in terms of accurate uniform dosing of the formulations. This is another reason why these grades could only be used at low concentrations.

The tablets prepared from Polyox™; 1105, N-60K and Coagulant formulations underwent permanent deformation when handled, i.e. the tablets would not return to their original shape following manual compression and when handled.

These three grades of Polyox™ have shown not to be suitable as binders in freeze-dried ODTs, as the prepared tablets deformed permanently when handled, and therefore the prepared tablets were deemed not suitable for characterisation. However, it is hypothesised that the highly viscous and cohesive nature of these grades of Polyox™ could potentially be utilised in lower concentrations (below 0.5%w/w) to increase the retention of the formulations in the pre-gastric regions and subsequently encourage pre-gastric absorption.

4.4.2 Viscosity Analysis of Polyox™ Formulations

Viscosity analysis of freeze-dried ODT formulations is particularly important, as it can provide an indication of the ease of dosing of formulations, which can ultimately affect content uniformity of the resulting tablets. During concentration profiling of the formulations it was discovered that the Polyox™ 1105 grade could only be used up to 3% w/w, whilst Polyox™ N-60K and Coagulant grades could only be used up to 2% w/w, respectively, due to the highly viscous and cohesive nature of the formulations. Viscosity analysis of the formulations will provide greater information regarding the “workable” viscosity range, which permits satisfactory dosing.

Analysis of the viscosities of Polyox™ formulations, as shown in Figures 4.1A-D, showed that for the mannitol-free formulations, increasing Polyox™ concentration resulted in an increase in formulation viscosity ($p < 0.05$). This was also observed with the formulations which included mannitol ($p < 0.05$).

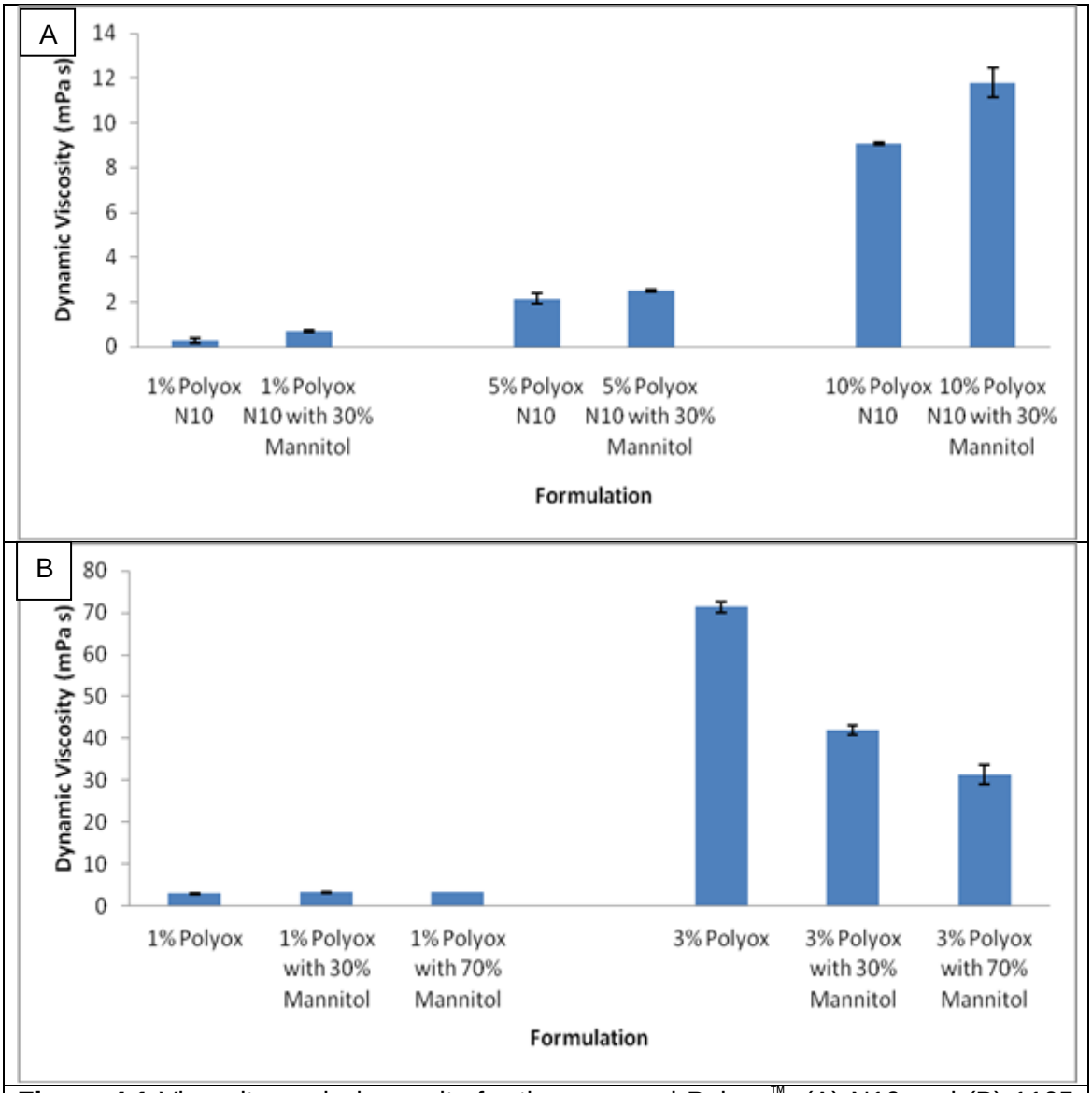


Figure 4.1 Viscosity analysis results for the prepared Polyox™; (A) N10 and (B) 1105 formulations. Viscosity analyses of the formulations were performed in triplicate, and mean values ± standard deviation are reported.

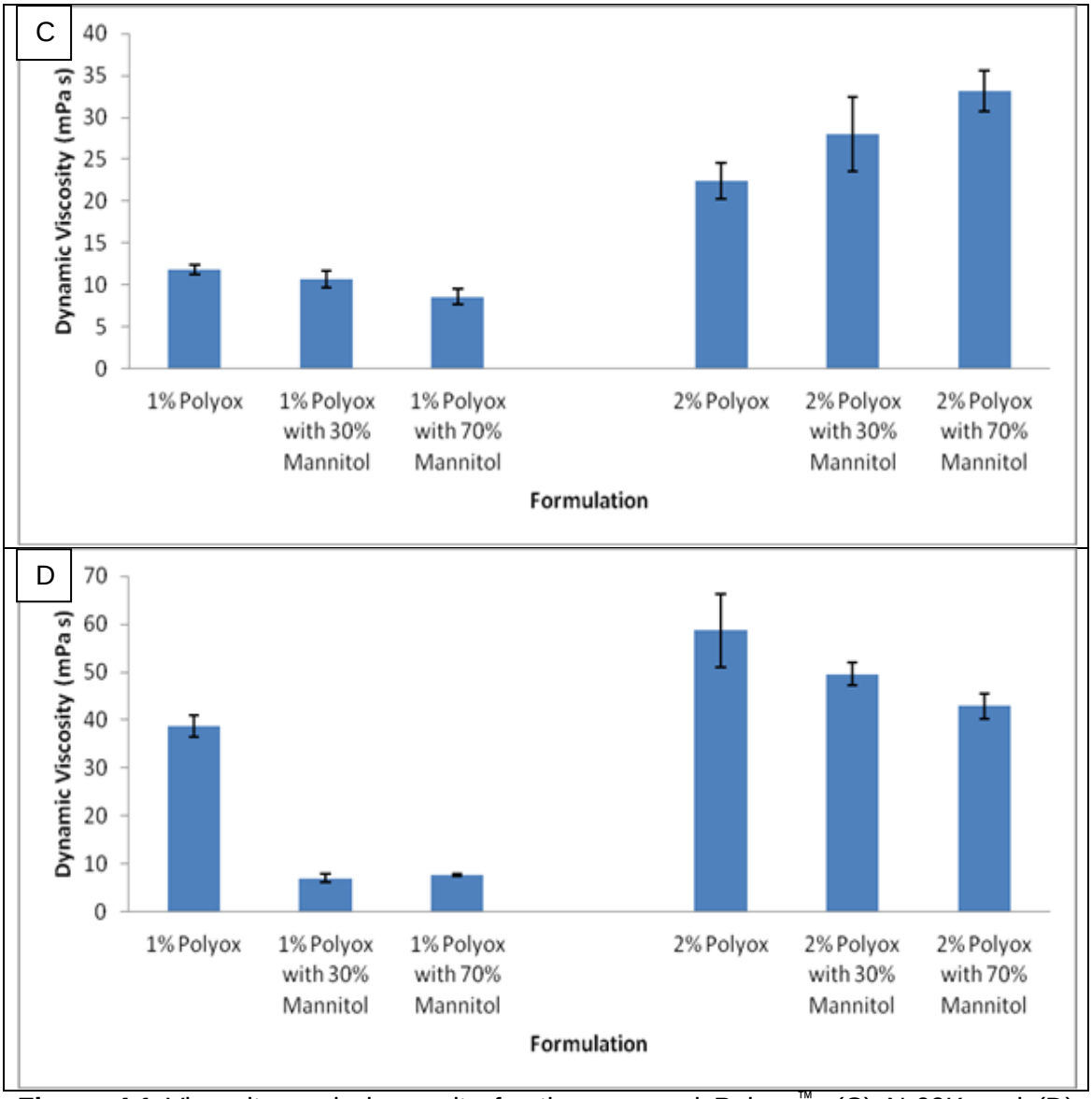


Figure 4.1 Viscosity analysis results for the prepared Polyox™; (C) N-60K and (D) Coagulant formulations. Viscosity analyses of the formulations were performed in triplicate, and mean values ± standard deviation are reported.

An increase in Polyox™ concentration resulted in an increase in formulation viscosity possibly due to an increase in the density of the polymer network which resulted in amplification in the interaction between polymer chains.

With the Polyox™ N10 formulations, comparing the viscosities of the mannitol-free formulations with the formulations which included 30% w/w (of dried tablet weight) mannitol, indicated that the inclusion of mannitol resulted in an increase in formulation viscosity, in the case of the 1 and 10% w/w Polyox™ N10 formulations ($p < 0.05$).

The inclusion of mannitol in the formulations resulting in an increase in viscosity could be attributed to the increased amount of solute in the formulations which results in

increased intermolecular interactions exhibiting greater viscosities (Miyawaki et al. 2003).

Both formulations based on 5% w/w Polyox™ N10, exhibited comparable viscosity ($p > 0.05$). The mannitol-free formulation exhibited a viscosity of 2.16 ± 0.23 mPa s, whilst the formulation which included 30% w/w (of dried tablet weight) mannitol displayed a viscosity of 2.51 ± 0.06 mPa s.

In terms of the 1% w/w formulations of the Polyox™ 1105 grade, the addition of mannitol at a concentration of 30% w/w (of dried tablet weight) showed not to affect formulation viscosity ($p > 0.05$). However, increasing mannitol concentration to 70% w/w (of dried tablet weight), resulted in an increase in formulation viscosity ($p < 0.05$).

Interestingly, in the case of the 3% w/w Polyox™ 1105 formulations, the addition of mannitol at concentrations of 30 and 70% w/w (of dried tablet weight), respectively, resulted in a reduction in formulation viscosity ($p < 0.05$). This observation can possibly be attributed to mannitol acting as a plasticiser. The addition of mannitol appears to weaken and decrease the molecular chain interactions of Polyox™ 1105 polymer chains, and therefore results in a reduction in viscosity. A similar observation was reported in a study which investigated the effect of polyols on plasticising corn starch (Qiao et al. 2011).

Qiao et al. (2011) investigated the plasticisation of corn starch by using mixtures of conventional plasticiser glycerol and higher molecular weight polyols, *e.g.* xylitol. Qiao et al. (2011) reported that the introduction of high molecular weight polyols significantly reduced the strong molecular chain interactions (hydrogen-bonds) in starch. Furthermore, the molecular chain mobility of starch increases following the addition of small plasticiser molecules, which results in decreasing melt viscosity (Qiao et al. 2011).

Comparing the viscosities of mannitol-free formulations of Polyox™ N-60K, with the formulations which included mannitol, revealed that in the case of the 1% w/w formulations, the addition of mannitol at a concentration of 30% w/w (of dried tablet weight) showed no effect on viscosity ($p > 0.05$). However, increasing mannitol concentration to 70% w/w (of dried tablet weight), resulted in an increase in viscosity ($p < 0.05$). This particular trend was also observed with the 2% w/w formulations.

In the case of the 1% w/w Polyox™ Coagulant formulations, the addition of mannitol at concentrations of 30 and 70% w/w (of dried tablet weight), respectively, resulted in a reduction in formulation viscosity ($p < 0.05$). No significant difference was observed in formulation viscosity, between the two mannitol-containing formulations ($p > 0.05$). Similar trends were observed for formulations containing 2%w/w Polyox™ Coagulant.

In terms of the workable viscosity ranges of Polyox™; 1105, N-60K and Coagulant formulations, Polyox™ 1105 formulations could be successfully dosed which composed of viscosities between 2.98 ± 0.11 and 71.33 ± 1.21 mPa s. On the other hand, Polyox™ N-60K and Coagulant formulations could be successfully dosed which comprised of viscosities between 8.56 ± 0.93 and 33.17 ± 2.45, and 7.02 ± 0.91 and 58.67 ± 7.53 mPa s, respectively.

4.4.3 Tablet Hardness, Fracturability and Thermal Analysis

Tablet hardness and fracturability analysis of freeze-dried ODTs provides an assessment of the mechanical properties of the tablets. It is well acknowledged that freeze-dried ODTs generally exhibit poor mechanical properties than conventional tablets due to the porous framework of the formulation. Assessment of their mechanical properties is a vital parameter to determine the mechanical integrity of the formulation during handling, transportation and packaging.

Tablet hardness analysis results for Polyox™ N10 formulations tablets, as shown in Table 4.10, indicate that both formulations produced tablets which exhibited comparable tablet hardness.

Table 4.10 Tablet hardness analysis results for the prepared Polyox™ N10 formulations tablets. Tablet hardness analyses of the formulations were performed in triplicate, and the mean values ± standard deviation are reported.

Formulation	Tablet Hardness (N) ± S.D.
10% w/w Polyox N10	2.364 ± 1.268
10% w/w Polyox N10 with 30% w/w (of dried tablet weight) Mannitol	4.192 ± 0.875

The 10% w/w Polyox™ N10 mannitol-free formulation produced tablets which exhibited tablet hardness of 2.36 ± 1.27 N, whilst the formulation which included mannitol, produced tablets with hardness of 4.19 ± 0.88 N ($p > 0.05$).

The inclusion of mannitol in the formulation has therefore shown not to improve or deteriorate tablet hardness.

Table 4.11 shows the results from the analysis of tablet fracturability of Polyox™ N10 formulations. The results indicated that both formulations produced tablets which displayed similar tablet fracturability.

Table 4.11 Tablet fracturability analysis results for the prepared Polyox™ N10 formulations tablets. Tablet fracturability analyses of the formulations were performed in triplicate, and the mean values ± standard deviation are reported.

Formulation	Tablet Fracturability (N) ± S.D.
10% w/w Polyox N10	1.259 ± 0.461
10% w/w Polyox N10 with 30% w/w (of dried tablet weight) Mannitol	1.824 ± 0.789

Tablets produced from the 10% w/w Polyox™ N10 mannitol-free formulation, displayed tablet fracturability of 1.26 ± 0.46 N, whilst the formulation which included mannitol exhibited tablet fracturability of 1.82 ± 0.79 N ($p > 0.05$).

Including mannitol in the formulation has therefore shown not to affect the mechanical properties of the tablets. The resistance of the tablets to the penetrating fracturability probe is comparable for both the tablets formed from mannitol-free and mannitol included formulations. These results are in agreement with the tablet hardness results (Table 4.10), where it was also observed that the incorporation of mannitol in the formulation did not influence the mechanical properties of the tablets.

It has been previously reported that mannitol provides crystallinity and subsequently hardness to freeze-dried ODTs (Seager, 1998). In order to investigate further the influence of mannitol on the mechanical properties of the tablets, the thermal properties of the formulations were evaluated using DSC.

DSC analysis of mannitol-free formulations of Polyox™ N10, 1105, N-60K and Coagulant, produced thermograms which exhibited two endothermic melting peaks, as shown in Figures; 4.2A, 4.2C, 4.2E and 4.2G, respectively. The two melting peaks were observed at around -8ºC and at around 0ºC. The melting peaks observed at around -8°C, were attributed to the melting of strongly bound water (PEO-water hydrate) (Graham et al. 1990), in which water molecules bind strongly to polyethylene oxide molecules, resulting in strongly bound water referred to as non-freezeable water (Takei et al. 2002). This “strongly bound water” involves hydrogen bonding between the hydrogen of water molecules and the ether oxygen of polyethylene oxide molecules (Graham et al. 1990). The second melting peaks observed at around 0°C,

were attributed to the melting of free (bulk) water, which is also referred to as freezeable water (Takei et al. 2002).

DSC analysis of mannitol-included formulations of Polyox™ N10, 1105, N-60K and Coagulant, produced thermograms which exhibited several thermal transitions, as shown in Figures; 4.2B, 4.2D and 4.2F, respectively. Glass transitions were observed with an onset of around -32ºC, which were attributed to the glass transition of the mannitol phase plasticised by unfrozen water (Cavatur et al. 2002). Second glass transitions were observed with an onset of around -25ºC, which were attributed to the glass transition of the amorphous freeze-concentrate of mannitol (Cavatur et al. 2002).

Exotherms were also observed at around -22ºC, which were attributed to the crystallisation of both mannitol hydrate and the unfrozen water, which were associated with the amorphous phase of mannitol (Cavatur et al. 2002). The reason that crystallisation of both mannitol hydrate and the unfrozen water occurred so suddenly after the second glass transition, was due to the high molecular mobility of the amorphous freeze-concentrate above the glass transition temperatures.

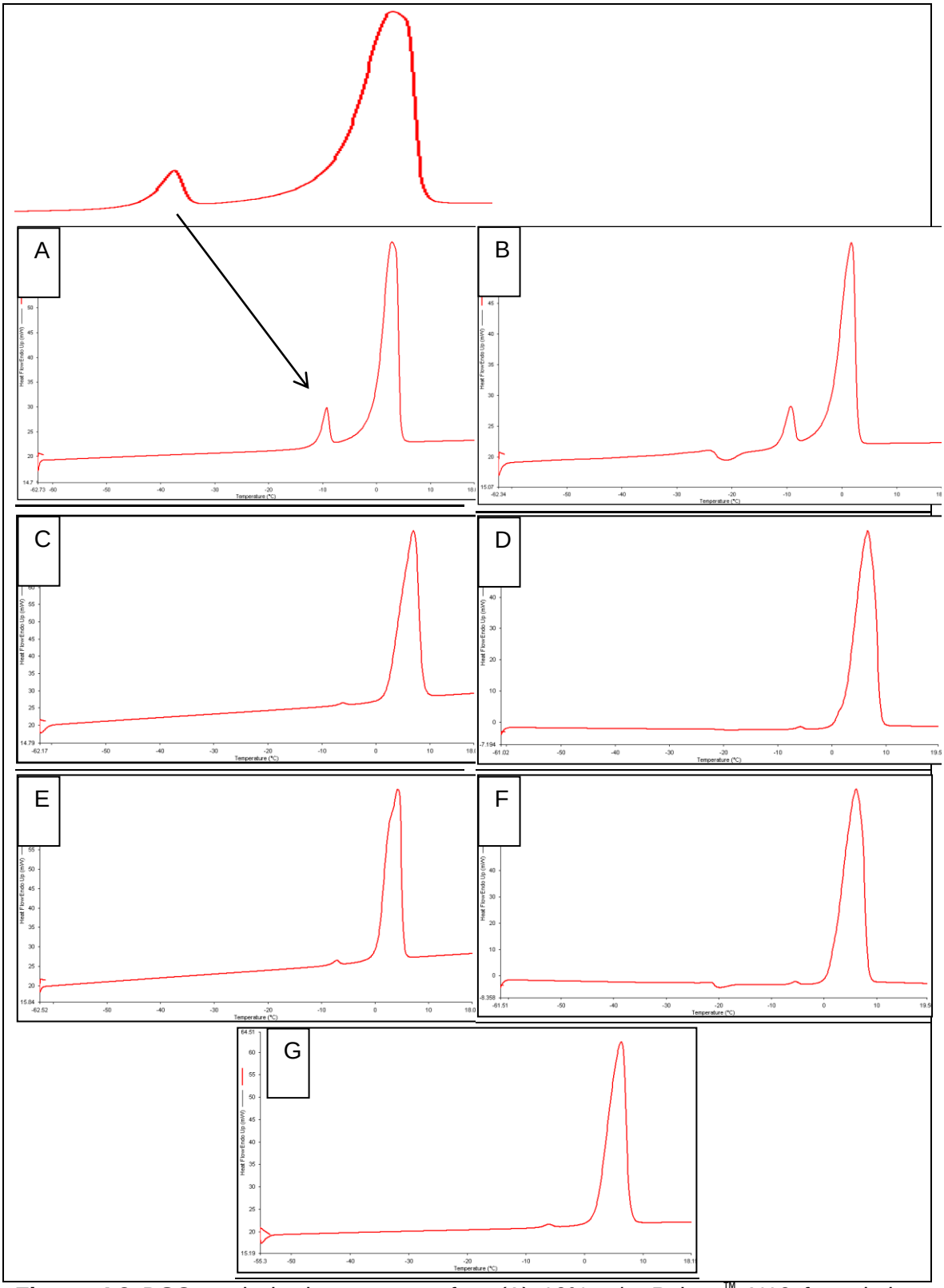


Figure 4.2 DSC analysis thermogram of a; (A) 10% w/w Polyox™ N10 formulation sample, (B) 10% w/w Polyox™ N10 with 30% w/w (of dried tablet weight) mannitol formulation sample, (C) 1% w/w Polyox™ 1105 formulation sample, (D) 1% w/w Polyox™ 1105 with 30% w/w (of dried tablet weight) mannitol formulation sample, (E) 2% w/w Polyox™ N-60K formulation sample, (F) 1% w/w Polyox™ N-60K with 70% w/w (of dried tablet weight) mannitol formulation sample and (G) 2% w/w Polyox™ Coagulant formulation sample, all during the heating phase of the DSC method.

The thermograms of the mannitol-included formulations also revealed two endothermic melting peaks at around -8 and 0ºC, respectively. As discussed previously, the melting peaks observed at around -8ºC, were attributed to the melting of "strongly bound water". The second melting peaks observed at around 0ºC, were attributed to the melting of free (bulk) water.

Evaluation of the thermal properties of both the mannitol-free and mannitol-included formulations of 10% w/w Polyox™ N10, revealed that with the mannitol-included formulation, mannitol hydrate underwent crystallisation during the heating phase of the DSC method. Despite this thermal transition, the inclusion of mannitol in the formulation did not improve the mechanical properties of the tablets, as comparable tablet hardness and fracturability results were observed between the two formulations.

Mannitol as a matrix supporting agent/structure former, has been reported to influence tablet hardness through a number of mechanisms. It has been reported that mannitol, due to its crystalline nature, imparts hardness to freeze-dried ODTs, when used in combination with a water-soluble polymer, which is generally amorphous in nature (Sastry et al. 2000, Kearney, 2003 and Seager, 1998).

Another reported mechanism by which mannitol influences tablet hardness, is by functioning as a matrix supporting agent which acts by cementing the porous framework provided by the water soluble polymer (AlHusban et al. 2010b). Highlighting the influence of mannitol on the mechanical properties (tablet hardness and fracturability) of freeze-dried ODTs, Chandrasekhar et al. (2009) previously reported on the improvement/increase of the mechanical properties as a consequence of increasing mannitol concentration.

DSC analysis of 2% w/w Polyox™ N-60K formulations with 30 and 70% w/w (of dried tablet weight) mannitol was not performed due to the highly viscous and cohesive nature of the formulations where sample preparation for DSC analysis could not be performed. This was also the case with 1 and 2% w/w Polyox™ Coagulant formulations consisting of 30 and 70% w/w (of dried tablet weight) mannitol.

4.4.4 Tablet Disintegration Time Analysis and Porosity Measurement of Polyox™ N10 Formulations

The rapid disintegration time of freeze-dried ODTs, is the principal feature which distinguishes this type of dosage form from conventional tablets. Consequently, assessing tablet disintegration time is a prerequisite. The highly porous nature of freeze-dried ODTs, as a result of the formulations undergoing freeze-drying, is one factor which promotes the rapid disintegration of this type of dosage form. Assessing tablet porosity therefore provides useful information which allows further understanding of how the tablets disintegrate when exposed to the disintegrating medium.

Analysis of the tablet disintegration time of Polyox™ N10 formulations, as shown in Table 4.12, has shown that the inclusion of mannitol in the formulation had a significant effect on tablet disintegration time.

Table 4.12 Tablet disintegration time analysis results for the prepared Polyox™ N10 formulations tablets. Tablet disintegration time analyses of the formulations were performed in triplicate, and the mean values ± standard deviation are reported.

Formulation	Tablet Disintegration Time (s) ± S.D.
10% w/w Polyox N10	69.67 ± 5.03
10% w/w Polyox N10 with 30% w/w (of dried tablet weight) Mannitol	32.00 ± 7.94

The tablets produced from 10% w/w Polyox™ N10 mannitol-free formulation, exhibited a tablet disintegration time of 69.67 ± 5.03s, whilst the formulation which included mannitol displayed a tablet disintegration time of 32.00 ± 7.94s ($p < 0.01$). The inclusion of mannitol in the formulation has therefore shown to significantly reduce tablet disintegration time.

A possible explanation of why the incorporation of mannitol in the formulations produced tablets which exhibited a reduced disintegration time, compared to tablets produced from mannitol-free formulations, could be due to the solubility of mannitol. As mannitol is soluble in water, when the tablet is exposed to the disintegration medium, mannitol instantly dissolves, which exposes the tablet binder matrix to the disintegration medium to a greater extent than would be expected with tablets produced from mannitol-free formulations. As a result, the tablets disintegrate in a shorter time.

The presence of mannitol in the formulation of freeze-dried ODTs has been reported to function not only as a matrix supporting agent/structure former, but also as a disintegration enhancing agent (AlHusban et al. 2010a). Mannitol therefore has dual functionality as an excipient, not only to influence the mechanical properties of the tablets by imparting hardness, but also to enhance the rapid disintegration of the tablets, due to its highly hydrophilic nature (AlHusban et al. 2010a).

Analysis of the tablet porosity of Polyox™ N10 formulations, as shown in Table 4.13, has shown that the inclusion of mannitol in the formulation had a significant effect on tablet porosity.

Table 4.13 Tablet porosity analysis results for the prepared Polyox™ N10 formulations tablets. Tablet porosity analyses of the formulations were performed in triplicate, and the mean values ± standard deviation are reported.

Formulation	Tablet Porosity (%) ± S.D.
10% w/w Polyox N10	95.38 ± 0.54
10% w/w Polyox N10 with 30% w/w (of dried tablet weight) Mannitol	92.27 ± 0.24

Tablets formulated from the 10% w/w Polyox™ N10 mannitol-free formulation exhibited a tablet porosity of 95.38 ± 0.54%, whilst tablets formulated from the formulation which included mannitol, displayed a tablet porosity of 92.27 ± 0.24% ($p < 0.05$). The inclusion of mannitol in the formulation has therefore shown to significantly reduce tablet porosity.

A possible explanation of this observation is that the inclusion of mannitol in the formulation, resulted in an increase in the amount of solid material in the tablets, therefore the porous space in the tablets was reduced, which resulted in a reduction in tablet porosity.

Evaluating the disintegration time and porosity results together, interestingly revealed that the reduction in tablet disintegration time as a result of the addition of mannitol in the formulation was not related to tablet porosity, as a reduction in tablet porosity was observed, which would usually suggest an increase in disintegration time. It can therefore be concluded that the reduction in tablet disintegration time can be attributed to mannitol functioning as a disintegration enhancing agent (AlHusban et al. 2010b).

A number of formulation parameters have been identified which are reported to be responsible for the mechanism of disintegration of freeze-dried ODTs. Seager (1998)

and Kearney (2003) reported that the highly porous nature of freeze-dried ODTs, which allows the rapid ingress of saliva into the tablet matrix structure, is responsible for the instantaneous disintegration of these tablets in the mouth. Meanwhile, AlHusban et al. (2010a) identified the high hydrophilic/soluble nature of matrix supporting/disintegration enhancing agents, as being responsible. These materials dissolve upon hydration with saliva, which results in the disruption and ultimately disintegration of the tablets (AlHusban et al. 2010a).

AlHusban and Mohammed (2011) and AlHusban et al. (2010b) identified that high tablet porosity and the high wettability of excipients, results in the swift disintegration of the tablets, as the short wetting time of excipients translates to quick disintegration of the tablets. AlHusban et al. (2010b) and Bi et al. (1996) reported linear positive correlations between the wetting time of excipients/tablets and tablet disintegration time.

4.5 Conclusions

Following the evaluation of the several grades of Polyox™ for potential applications with freeze-dried ODTs, the lowest viscosity grade (Polyox™ N10) produced robust tablets at a concentration of 10% w/w which were suitable for tablet hardness and disintegration testing. Tablets were produced which exhibited a hardness of 4.19 ± 0.88 N and a disintegration time of 32.00 ± 7.94 s. This grade of Polyox™ therefore appeared most suitable as a binder in freeze-dried ODTs, from the four grades investigated.

Formulations of Polyox™ 1105, N-60K and Coagulant were very viscous and cohesive at low concentrations. The viscosity analysis results highlighted the potential of these grades of Polyox™ to increase the retention of ODT formulations in the pre-gastric regions, and subsequently encourage pre-gastric absorption of APIs.

With the higher viscosity grades of Polyox™ (1105, N-60K and Coagulant), due to the highly viscous and cohesive nature of the formulations, concentrations up to 2% w/w could only be used with the N-60K and Coagulant grades, whilst concentrations up to 3% w/w could only be used with the 1105 grade, which could be successfully dosed. As a consequence, robust tablets which were suitable for tablet hardness and disintegration testing were not produced, as extensive binder matrices appeared not being formed at such low concentrations.

Additionally, the tablets produced with these three grades of Polyox™, underwent permanent deformation when handled, which could possibly be attributed to the linear chain nature of this polymer, which exhibits limited elasticity and can undergo plastic deformation.

Chapter Five: Investigating the Application of Kollicoat® IR (Polyvinyl Alcohol-Polyethylene Glycol Co-Polymer) as a Binder in Freeze-Dried ODTs

5.1 Introduction

Kollicoat® IR is a polyvinyl alcohol-polyethylene glycol graft co-polymer which has a principal application as an instant-release coating for tablets (BASF SE, 2010). The copolymer is composed of 75% polyvinyl alcohol units and 25% polyethylene glycol units, and is reported to be freely-soluble in water (BASF SE, 2010). In fact, due to the chemical structure of this co-polymer, it dissolves readily in acidic, neutral and alkaline aqueous media (BASF SE, 2010). It has been reported that Kollicoat® IR is effective as a coating material, as the polyvinyl alcohol component of the polymer provides good film-forming properties whilst the polyethylene glycol component functions as an internal plasticiser which provides flexibility to the material (Fouad et al. 2011). Kollicoat® IR is also favourable as a coating material as it displays lower viscosity than cellulose derivatives which allows it to be used at higher concentrations (Fouad et al. 2011).

Kollicoat® IR has also been shown to have binding properties in both mixer and fluid bed wet granulation methods which can be attributed to its film-forming ability (Fouad et al. 2011). Furthermore, a reported application of Kollicoat® IR is as a binder for rapidly dispersible/soluble granules or tablets (BASF SE, 2010). In terms of the preparation of solid dispersions (using a hot stage extruder, spray drying or freeze-drying), it has been reported that Kollicoat® IR can enhance the dissolution rate of bio-pharmaceutics classification system class II drugs due to its high aqueous solubility and the resulting low viscosity of the aqueous medium. In addition, it also offers a wide range of advantages including its pH-independent solubility, surface activity and low viscosity (when it is dissolved in water) (Fouad et al. 2011).

Kollicoat® IR has also been reported to be used in controlled/sustained-release tablet coatings owing to its pore forming ability (BASF SE, 2010). Siepmann et al. (2007) reported the effect of Kollicoat® IR in an ethylcellulose film. Siepmann et al. (2007) reported that the addition of Kollicoat® IR significantly increased the rate and extent of water uptake and the permeability of the films increased as Kollicoat® IR content increased. Adjusting the content of Kollicoat® IR in ethylcellulose-coated dosage forms can therefore control/modify drug release rates (Siepmann et al. 2007). The use of Kollicoat® IR in freeze-dried preparations (solid dispersions) has been reported previously (El-Badry et al. 2010). El-Badry et al. (2010) reported that freeze-dried solid dispersions of omeprazole-Kollicoat® IR mixtures exhibited dissolution at a rate seven times that of the physical mixture.

The current work aims to exploit the dual property (high aqueous solubility and its ability to function as a binder) offered by Kollicoat® IR in the formulation of freeze-dried orally disintegrating tablets (ODTs). This would have distinct advantages including low viscosity of the binder solution ensuring uniform dosing of samples into the moulds, faster disintegration rate of the tablets, solubility enhancement of BCS class II drugs and higher hardness of the resultant tablets.

5.2 Materials

Kollicoat® IR was supplied by BASF SE (Ludwigshafen, Germany). Mannitol was supplied by Sigma-Aldrich Chemicals (Poole, UK).

5.3 Methods

5.3.1 Preparation of Freeze-Dried Tablets

For the preparation of Kollicoat® IR formulations, the required amount of polymer was added to the desired amount of double-distilled water under stirring at ambient temperature, followed by the addition of mannitol (for formulations which included mannitol). The formulations were then stirred, until all formulation ingredients dissolved.1.5 g of the resulting solutions were dosed into a tablet mould, frozen at -70°C for a minimum of sixty minutes and freeze-dried (ADVANTAGE, Freeze-Dryer, VIRTIS), according to the following regime; primary drying for forty eight hours at a shelf temperature of -40°C, secondary drying for ten hours at a shelf temperature of 20°C, and vacuum pressure of 6.67 Pa (50 mTorr). A minimum of ten tablets were prepared for each formulation.

5.3.2 Formulation Viscosity Analysis

For all formulations, the viscosities of 100 mL samples were measured using a DV-I+ Brookfield digital viscometer (Harlow, UK). The viscosities of the formulations were measured at 37 ± 2°C, in order to replicate the viscosity of the formulations in the oral cavity at physiological temperature. The viscometer spindles were selected based on the viscosity of the individual formulations, as each spindle is suitable for analysing a certain viscosity range. The rotational speed of the spindle was set at 100 rpm. Each formulation was analysed in triplicate and the mean values ± standard deviation is reported.

5.3.3 Concentration Profiling and Macroscopic Evaluation of the Formulations

During the preparation of the formulations, several visual assessments were carried out in order to evaluate the polymers as binders. The solubility of the material was assessed by observing the ease at which the polymer went into solution. The ease of dosing of the formulations was also assessed, in terms of the viscosity of the prepared solution/suspension and also the consistency of the formulation, i.e. if it had a cohesive or non-cohesive appearance.

In terms of the visual and physical assessment of the tablets, several assessments were carried out in order to evaluate the ability of the polymers to form robust tablets with satisfactory mechanical strength. The first assessment involved the evaluation of the ability of the formulations to form intact tablet shaped dosage forms following freeze-drying. The physical appearance of the tablets was assessed, classifying them as either soft or hard, and having either elastic or a more robust/solid consistency. The ability of the tablets to be removed from the moulds was also assessed. Finally, the performance of the tablets when handled was assessed to observe if they deformed permanently or retained their shape.

The first phase of the investigation was focussed on concentration profiling of Kollicoat® IR to determine the optimum binder concentration and understand its function as a binder or binder/disintegrant. Table 5.1 represents the various formulations that were prepared with Kollicoat® IR. Both mannitol inclusive and free formulations were tested to determine the effect of inclusion of matrix supporting agents in the formulation and to identify the specific role of Kollicoat® IR within the tablets.

Table 5.1 Formulation details of Kollicoat® IR formulations detailing the concentrations of Kollicoat® IR and mannitol used in each formulation.

Kollicoat® IR Concentration (% w/w)	Mannitol Concentration (% w/w of dried tablet weight)
1	0
1	30
5	0
5	30
10	0
10	30
10	50
15	0
15	30
20	0
20	30

5.3.4 Differential Scanning Calorimetry (DSC)

DSC (Pyris Diamond DSC and Intercooler 2P: Perkin Elmer, Wellessey, USA) was used to determine the glass transition temperature (T_g) and thermal properties of the formulations in their frozen state (before freeze-drying). 10-15 mg of liquid samples of the formulations were loaded into aluminium pans, cooled from 25ºC to -65ºC at a rate

of 5ºC/min and then heated to 20ºC at a rate of 5ºC/min with a nitrogen purge of 20 mL/min. An empty aluminium pan was used as a reference for all measurements.

The resulting thermograms were analysed by Pyris manager software. T_g values were determined from the intersection of relative tangents to the baseline, and other thermal properties of the formulations were also assessed. Three measurements were taken for each formulation and the mean values ± standard deviation were reported.

5.3.5 Tablet Hardness Analysis

The hardness of the tablets were investigated using a texture analyzer (QTS 25: Brookfield, Essex, UK) equipped with a 25 kg load cell. The instrument was calibrated by standard weights of 500 g and 5 kg. The tablets were placed in a holder with a cylindrical hole. The hardness was taken as the peak force after 1 mm penetration of a 5 mm diameter probe at a rate of 6 mm/min. Three measurements were taken for each formulation and the mean ± standard deviation is reported.

5.3.6 Disintegration Time of the Tablets

The disintegration time of the tablets was determined using a USP disintegration tester (Erweka, ZT3). 800 mL of double distilled water, which was kept at 37 ± 2ºC, was used as the medium and the basket was raised and lowered at a fixed rate of 30 rpm. Three tablets were evaluated from each formulation and the mean values ± standard deviation is reported.

5.3.7 Investigation of Tablet Porosity

The porosity of the tablets was measured using helium pycnometry (MULTIPYCNOMETER, Quantachrome Instruments, Hampshire, UK). Two freeze-dried tablets were placed in a suitably sized sample cup and subjected to helium pycnometry, to determine the true density of the tablets. The true density value was then inserted into the equation as shown below to determine the porosity of the tablets.

$$\text{Porosity} = 1 - \left[\frac{\text{bulk density}}{\text{true density}}\right] \times 100\%$$

(Equation 5.1)

Bulk density was determined by considering the mass of the tablets, the diameter of the tablets, and the height/depth of the tablets. The diameter and height/depth of the tablets were determined using a screw gauge (LINEAR Farnell). Three porosity

measurements were taken for each formulation, and the mean values ± standard deviation is reported. The porosity of the tablets is expressed as a percentage.

5.3.8 Statistical Analysis

Statistical analyses of the formulation and tablet characterisation results were performed using GraphPad Prism 5 statistical analysis software. One-way analysis of variance (ANOVA) was the statistical analysis test performed, using the Tukey post test. A significance level of $p < 0.05$ (95% confidence interval) was judged as being statistically significant.

5.4 Results and Discussion

5.4.1 Concentration Profiling and Macroscopic Evaluation of the Formulations

The visual and physical assessment of Kollicoat® IR formulations and tablets are shown in Table 5.2. Kollicoat® IR formulations were formulated using concentrations of 1, 5, 10, 15 and 20% w/w. These concentration values were selected in order to analyse the effects of Kollicoat® IR concentration on the properties of the formulations and tablets over a broad concentration range.

Tablets which consisted of 1% w/w Kollicoat® IR appeared intact, however once any contact was made with the tablets, they disintegrated. These tablets were extremely soft, and did not withstand manual handling. Kollicoat® IR at a concentration of 1% w/w did not appear suitable as a binder for ODTs. Including mannitol in the formulation did not have an effect on the physical properties of the tablets. Similar to the mannitol-free formulation tablets, the tablets were soft and could not withstand manual handling. Once any contact was made with the tablets, they disintegrated.

Table 5.2 Concentration profiling and macroscopic evaluation of the various Kollicoat® IR-based formulations prepared.

Formulation	Visual and Physical Assessment
1% w/w Kollicoat® IR	Intact tablets were formed, however once any contact was made with the tablets, they simply disintegrated. The tablets were extremely soft and did not have physical integrity, i.e. the tablets could not withstand manual handling. Kollicoat® IR at a concentration of 1% w/w does not appear suitable as a binder in ODTs.
1% w/w Kollicoat® IR with 30% w/w (of dried tablet weight) Mannitol	Intact tablets formed, however when any contact was made with the tablets they disintegrated. The tablets appeared extremely soft and did not retain their shape when handled. The tablets did not have any physical integrity and therefore could not withstand any physical contact. Kollicoat® IR at a concentration of 1% w/w with 30% w/w (of dried tablet weight) mannitol, does not appear suitable as a formulation for ODTs.
5% w/w Kollicoat® IR	Intact tablets formed. However, the tablets were very difficult to remove from the moulds. The tablets appeared very soft and did not have significant physical integrity. Once it was attempted to remove the tablets from the moulds, the tablets completely disintegrated. Kollicoat® IR at a concentration of 5% w/w was not suitable as a binder in ODTs.
5% w/w Kollicoat® IR with 30% w/w (of dried tablet weight) Mannitol	Intact tablets formed, however when the tablets were handled or attempted to remove from the moulds, they disintegrated. The tablets were soft and did not retain their shape when handled. Kollicoat® IR at a concentration of 5% w/w with 30% w/w (of dried tablet weight) mannitol, does not result in a suitable formulation of ODT.
10% w/w Kollicoat® IR With and Without 30 and 50% w/w (of dried tablet weight) Mannitol	Intact tablets formed. The tablets were very hard and robust, with a noticeable slightly elastic/“spongy” nature. They retained their shape when handled, and were easily removed from the moulds. Kollicoat® IR at a concentration of 10% w/w is suitable as a binder in ODTs.
15% w/w Kollicoat® IR With and Without 30% w/w (of dried tablet weight) Mannitol	Intact tablets formed. The tablets were very hard and robust, which were noticeably harder than the 10% w/w Kollicoat® IR formulations tablets. The tablets retained their shape when handled and were easily removed from the moulds.
20% w/w Kollicoat® IR With and Without 30% w/w (of dried tablet weight) Mannitol	Intact tablets formed. The tablets appeared very hard and robust, which were noticeably harder than the 15% w/w Kollicoat® IR formulations tablets. The tablets retained their shape when handled and were easily removed from the moulds.

Similar results as that for 1% w/w were obtained upon increasing the concentration of Kollicoat® IR to 5% w/w which resulted in fragile and easily deformable tablets. The tablets were soft and did not retain their shape when handled. Furthermore, addition of mannitol as a matrix supporting agent did not have any influence on the outcome for the formulation. As a result, tablets produced from formulations which comprised of either 1 or 5% w/w Kollicoat® IR, were not suitable for characterisation analysis/studies. The tablets of these formulations could not be removed from their moulds intact; therefore these tablets could not undergo characterisation analysis/studies. A possible explanation for the weak tablet properties could be the inability of Kollicoat® IR to produce an extensive binder matrix which would provide a robust and strong network within the formulation upon sublimation of water. In addition, it is also possible that the extremely low viscosity of Kollicoat® IR formulations (refer to the Viscosity Analysis of Kollicoat® IR Formulations), results in limited/insufficient interaction between the polymer chains and hence when the formulations were freeze-dried, the resulting tablets were soft and could not withstand manual handling.

Evaluation of 10% w/w Kollicoat® IR formulation produced tablets which were hard and robust, with a noticeable mild elastic nature. The tablets retained their shape when handled and were easily removed from their moulds. The inclusion of mannitol in the formulation produced tablets which displayed similar physical properties to the tablets formulated from the mannitol-free formulation. Similarly, tablets produced from the formulation which consisted of 10% w/w Kollicoat® IR with 50% w/w (of dried tablet weight) mannitol, were intact and appeared very hard and robust which retained their shape and were easily removed from the moulds.

Increasing the concentration of Kollicoat® IR to 15 and 20%w/w resulted in intact tablets with considerable integrity upon handling. Similar macroscopic properties (as that of 10%w/w formulations) were recorded upon the inclusion of mannitol for the both the concentrations. Findings from these investigations demonstrated that a threshold concentration above 5% w/w of the polymer is needed for the formation of intact tablets and that extensive network formation within the tablet upon sublimation in the freeze drier occurs at higher concentrations.

5.4.2 Evaluation of thermal properties

Thermal characterisation of the formulation or its excipients provides vital information on optimisation of freeze drying protocol, excipient compatibility and stability considerations for the resultant formulation. Evaluation of excipient compatibility is

essential as previous research has shown that interaction between excipients could result in depression of crystallisation temperature or could detrimentally affect the quality of the final product after freeze-drying (Chandrasekhar et al., 2009).

DSC analysis of mannitol-free formulations of Kollicoat® IR comprising of 1, 5, 10, 15 and 20% w/w respectively produced thermograms which exhibited only a single thermal transition/event, as shown in Figure 5.1A. This thermal transition/event was attributed to the melting of ice/water at around 0ºC (Harris, 1992). No thermal transitions/events were observed which were attributed to Kollicoat® IR within the temperature range investigated.

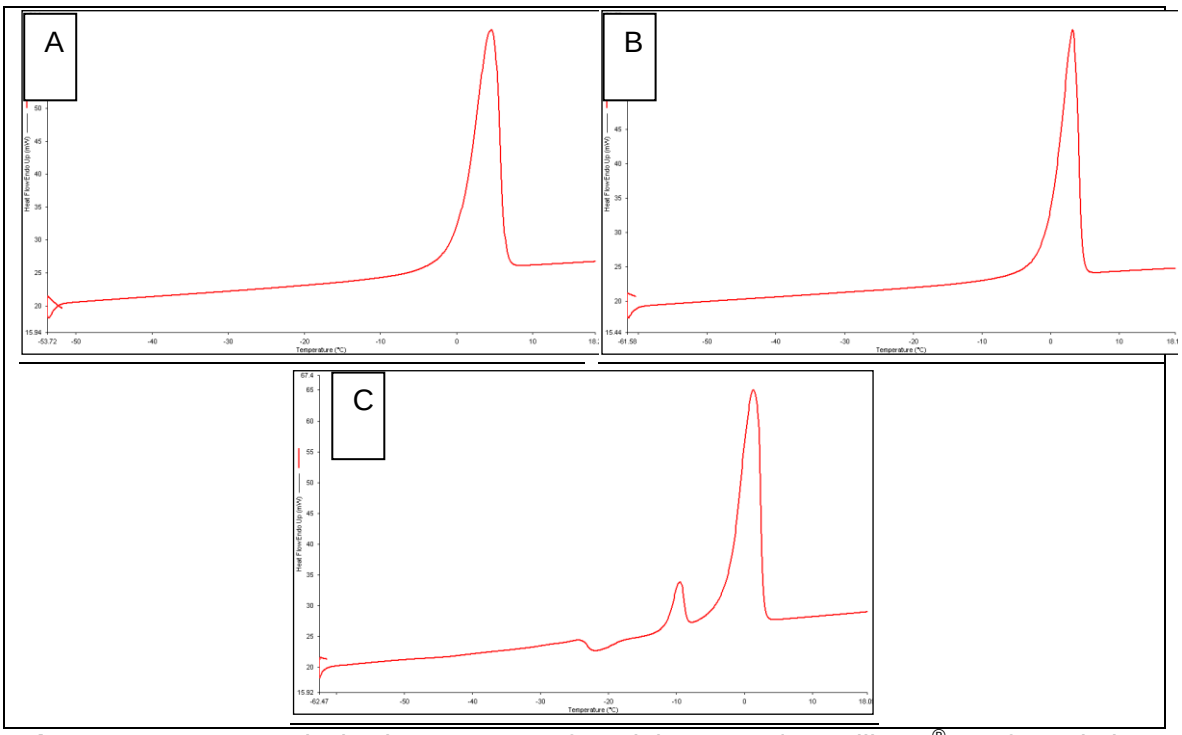


Figure 5.1 DSC analysis thermogram of a; (A) 10% w/w Kollicoat® IR formulation sample, (B) 5% w/w Kollicoat® IR with 30% w/w (of dried tablet weight) mannitol formulation sample and (C) 10% w/w Kollicoat® IR with 30% w/w (of dried tablet weight) mannitol formulation sample, all during the heating phase of the DSC method.

DSC analysis of the formulations which included 30% w/w (of dried tablet weight) mannitol, revealed that 1, 5, 15 and 20% w/w Kollicoat® IR formulations exhibited different thermograms compared to the thermograms of the 10% w/w Kollicoat® IR formulations. Formulations with 1, 5, 15 and 20% w/w Kollicoat® IR formulations showed thermal signal which revealed no thermal transitions/events which were attributed to Kollicoat® IR or mannitol, as shown in Figure 5.1B. The only thermal transition/event which was present was the endothermic peak of the melting of ice/water at around 0ºC (Harris, 1992).

DSC analysis of 10% w/w Kollicoat® IR with 30 and 50% w/w (of dried tablet weight) mannitol formulations produced thermograms which exhibited several thermal transitions/events as shown in Figure 5.1C. Two glass transitions were observed at -32 and -25ºC, respectively, followed by immediate crystallisation at around -22ºC. The thermograms also exhibited two endothermic melting peaks at around -8 and 0ºC, respectively. The thermal transitions/events observed at -32, -25 and -22ºC, respectively, were attributed to mannitol. The transition at -32ºC was attributed to the glass transition of the mannitol phase plasticised by unfrozen water (Cavatur et al. 2002). Similarly, the glass transitions observed at -25ºC were associated with the transition of amorphous freeze-concentrate of mannitol (Cavatur et al. 2002). Exothermic crystallisation peaks at around -22ºC could be due to the crystallisation of both mannitol hydrate and the unfrozen water associated with the amorphous phase of mannitol (Cavatur et al. 2002).

In terms of the endothermic melting peaks observed at around -8 and 0ºC, respectively, the peak at around -8ºC were attributed to an association between water and Kollicoat® IR. Kollicoat® IR is a graft co-polymer of polyvinyl alcohol and polyethylene glycol (PEG) (BASF, 2010) and it has been reported that water binds strongly to PEG (Tirosh et al. 1998). It has also been reported in literature that DSC analysis of PEG solutions produces two endothermic melting peaks (Harris, 1992). The first at around -8ºC were attributed to the melting of a PEG-water hydrate in which water is strongly bound to Kollicoat® IR (Harris, 1992), which is also referred to as non-freezable water (Takei et al. 2002). The second endothermic melting peak at around 0ºC were attributed to the melting of ice, i.e. unbound “free” water (Harris, 1992).

The thermal events which were attributed to mannitol and Kollicoat® IR with 10% w/w Kollicoat® IR and 30 and 50% w/w (of dried tablet weight) mannitol formulations, were concentration dependent as these events were not observed with 1, 5, 15 and 20% w/w Kollicoat® IR (with 30% w/w of dried tablet weight) and mannitol formulations.

Interestingly, the endothermic melting peak at around -8ºC which was observed in 10% w/w Kollicoat® IR with 30 and 50% w/w (of dried tablet weight) mannitol formulations (Figure 5.1C), were not seen with the corresponding mannitol-free formulations, as shown in Figure 5.1A.

A likely explanation is that the inclusion of mannitol in the formulation increases the fraction of unfrozen/non-freezable water in the formulations (Ghosh and Coupland, 2008 and McClements, 2004), resulting in the formation of PEG-water hydrate with melting temperature at around -8ºC.

5.4.3 Viscosity Analysis of Kollicoat® IR Formulations

Viscosity analysis was carried out for all the concentration ranges investigated irrespective of the inability of lower concentrations to form intact and robust tablets. The results showed that increase in concentration of the polymer resulted in the increase in viscosity (Figure 5.2) ($p < 0.05$). Similar trend of results was also observed with the formulations which included mannitol ($p < 0.05$). The increase in viscosity with increase in concentration can be attributed to an increase in the density of the polymer network which ultimately results in an increase in the interaction between polymer chains.

Upon comparing formulation viscosities of the mannitol-free formulations with the formulations which included mannitol (for each concentration studied), the results revealed that the formulation viscosity increased with the inclusion of mannitol ($p < 0.05$). A possible explanation of this observation could be the increased amount of solute in the formulations, which increases the intermolecular interactions within the liquid resulting in greater viscosity than the corresponding mannitol-free formulation (Miyawaki et al. 2003). However, the only exception to this trend was observed with 10% w/w Kollicoat® IR and the corresponding formulation which included 30% w/w (of dried tablet weight) mannitol which were comparable ($p > 0.05$).

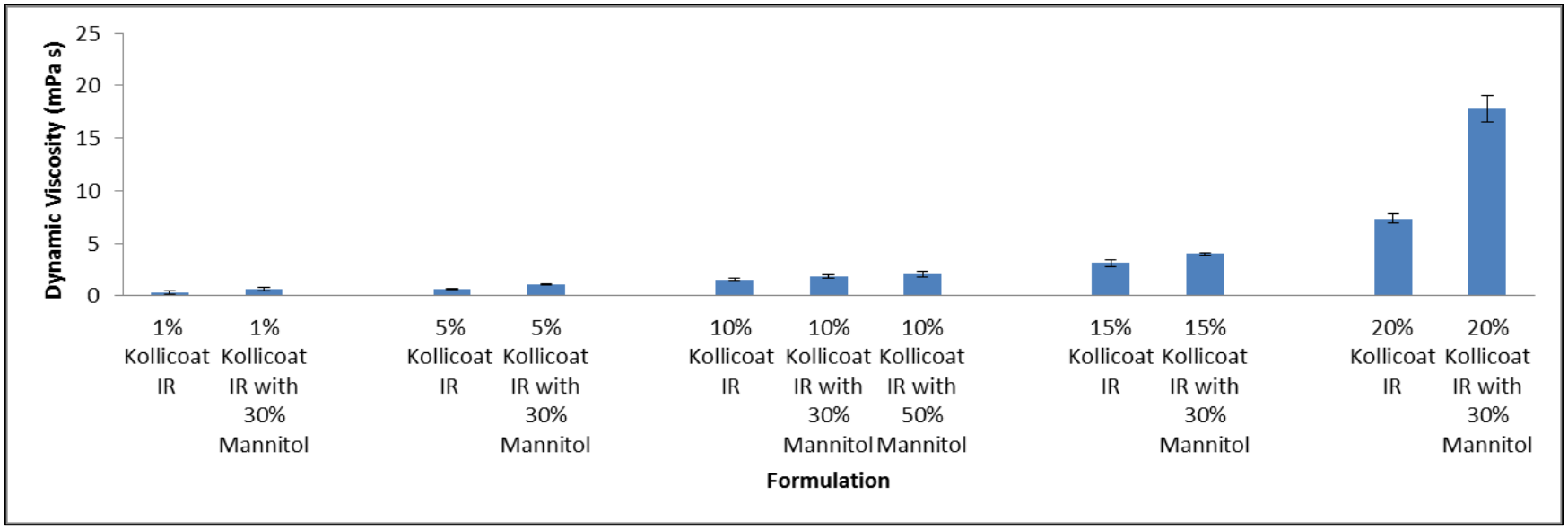


Figure 5.2 Viscosity analysis results for the Kollicoat® IR formulations prepared. Viscosity analyses of the formulations were performed in triplicate and the mean values ± standard deviation are reported.

5.4.4 Tablet Hardness Analysis of Kollicoat® IR Based Formulations

One of the significant properties of freeze-dried ODTs that are assessed in addition to the disintegration time of the tablets is mechanical properties, including hardness. Hardness assessment in formulation development is key as it can have significant implication on the cost of the finished product due to the need for specialist packaging material. Freeze dried ODTs are made of a porous frame work which is strengthened by the inclusion of matrix supporting agents and as a consequence hardness measurement is vital for both packaging requirements as well as product performance.

Tablet hardness analysis results for Kollicoat® IR formulations tablets are presented in Table 5.3. The results indicated that for the mannitol-free formulations, increasing Kollicoat® IR concentration from 10% through to 20% w/w resulted in an increase in tablet hardness ($p < 0.05$). Similar trend of results was also observed with the formulations which included mannitol ($p < 0.05$).

Table 5.3 Tablet hardness analysis results for the prepared Kollicoat® IR formulations tablets. Tablet hardness analyses of the formulations were performed in triplicate, and the mean values ± standard deviation are reported.

Formulation	Tablet Hardness (N) ± S.D.
10% w/w Kollicoat IR	5.944 ± 1.305
10% w/w Kollicoat IR with 30% w/w (of dried tablet weight) Mannitol	11.274 ± 1.947
10% w/w Kollicoat IR with 50% w/w (of dried tablet weight) Mannitol	17.31 ± 0.83
15% w/w Kollicoat IR	20.14 ± 2.10
15% w/w Kollicoat IR with 30% w/w (of dried tablet weight) Mannitol	38.84 ± 2.57
20% w/w Kollicoat IR	41.16 ± 3.92
20% w/w Kollicoat IR with 30% w/w (of dried tablet weight) Mannitol	> 50

The trend of increasing Kollicoat® IR concentration resulting in an increase in tablet hardness, can be attributed to an increase in the total solid content of the tablets and subsequent increase in the density of the tablet matrices which results in hard and robust tablets. The results can also possibly be related to the viscosity assessments in which it was observed that increasing Kollicoat® IR concentration resulted in an increase in viscosity due to an increase in the density of the polymer network and subsequently increase in polymer chain interaction.

The incorporation of mannitol into the formulations resulted in a significant increase in tablet hardness when compared to their mannitol free counter parts ($p < 0.05$). This

increase in hardness can be attributed to a number of reasons. Firstly, the incorporation of mannitol in the formulations increases the total solid content of the tablets, and subsequently increases the density of the tablet matrices. Secondly, the results can be possibly related to the viscosity results where it was observed that the incorporation of mannitol increased the amount of solute in the formulations, therefore an increase in intermolecular interactions occurred which resulted in an increase in formulation viscosity. An increase in intermolecular interactions, as a result of the incorporation of mannitol in the formulations reaffirms the role of mannitol as a matrix supporting agent (Chandrasekhar et al. 2009) by strengthening the binder matrix of the tablets, which results in an increase in tablet hardness. A third explanation for the increase in hardness could be related to the DSC results. Mannitol has previously been reported to provide crystallinity and subsequently hardness to freeze-dried ODTs (Seager, 1998). Results from DSC scans (Figure 5.1C) demonstrate that mannitol undergoes crystallisation which could explain the increase in tablet hardness relative to the mannitol-free formulations.

5.4.5 Tablet Disintegration Time Analysis and Porosity Measurements

Tablet disintegration time of Kollicoat® IR formulations, as shown in Table 5.4, revealed that increasing Kollicoat® IR concentration from 10 to 15% w/w resulted in an increase in tablet disintegration time ($p < 0.05$). Whilst increasing Kollicoat® IR concentration to 20% w/w, produced tablets which exhibited disintegration times greater than three minutes.

Table 5.4 Tablet disintegration time analysis results for the prepared Kollicoat® IR formulations tablets. Tablet disintegration time analyses of the formulations were performed in triplicate and mean values ± standard deviation are reported.

Formulation	Tablet Disintegration Time (s) ± S.D.
10% w/w Kollicoat IR	37.00 ± 2.65
10% w/w Kollicoat IR with 30% w/w (of dried tablet weight) Mannitol	29.00 ± 1.73
10% w/w Kollicoat IR with 50% w/w (of dried tablet weight) Mannitol	19.33 ± 2.08
15% w/w Kollicoat IR	112.33 ± 29.37
15% w/w Kollicoat IR with 30% w/w (of dried tablet weight) Mannitol	112.00 ± 11.36
20% w/w Kollicoat IR	> 180
20% w/w Kollicoat IR with 30% w/w (of dried tablet weight) Mannitol	> 180

In terms of the formulations which included mannitol, a similar trend was observed in which increasing Kollicoat® IR from 10 to 15% w/w resulted in an increase in tablet

disintegration time ($p < 0.05$). As with the mannitol-free formulation, increasing Kollicoat® IR concentration to 20% w/w produced tablets which exhibited disintegration times greater than three minutes. An increase in Kollicoat® IR concentration resulting in an increase in tablet disintegration time, can be attributed to a decrease in tablet porosity and an increase in total solid content of the tablets, which produces a more extensive tablet binder matrix, which requires greater time to disintegrate.

To further evaluate the role of porosity on disintegration time of the tablets, helium pycnometry was used to assess total porosity of the finished dosage forms. Analysis of the tablet porosity results of Kollicoat® IR formulations, as shown in Table 5.5, revealed that increasing Kollicoat® IR concentration (for both mannitol included and mannitol free) resulted in a reduction in tablet porosity ($p < 0.05$). However, the 10% w/w Kollicoat® IR with 50% w/w (of dried tablet weight) mannitol formulation and 15% w/w Kollicoat® IR formulation exhibited comparable tablet porosities ($p > 0.05$), as did the 15 and 20% w/w Kollicoat® IR formulations ($p > 0.05$). The trend of increasing Kollicoat® IR concentration resulting in a decrease in tablet porosity could possibly be attributed to the increase in tablet density upon increase of polymer concentration.

Table 5.5 Tablet porosity analysis results for the prepared Kollicoat® IR formulations tablets. Tablet porosity analyses of the formulations were performed in triplicate and the mean values ± standard deviation are reported.

Formulation	Tablet Porosity (%) ± S.D.
10% w/w Kollicoat IR	92.35 ± 0.22
10% w/w Kollicoat IR with 30% w/w (of dried tablet weight) Mannitol	91.55 ± 0.48
10% w/w Kollicoat IR with 50% w/w (of dried tablet weight) Mannitol	86.33 ± 0.17
15% w/w Kollicoat IR	88.47 ± 0.85
15% w/w Kollicoat IR with 30% w/w (of dried tablet weight) Mannitol	85.34 ± 0.18
20% w/w Kollicoat IR	84.14 ± 0.21
20% w/w Kollicoat IR with 30% w/w (of dried tablet weight) Mannitol	81.91 ± 2.74

Upon comparing the disintegration time of mannitol-free formulations with the formulations which included mannitol, the results indicated that the 10% w/w formulations exhibited a shorter disintegration time than the mannitol-free formulation ($p < 0.05$). It was also observed that the formulation consisting of 50% w/w (of dried tablet weight) mannitol showed a shorter disintegration time than the formulation consisting of 30% w/w (of dried tablet weight) mannitol ($p < 0.05$). The results therefore suggest that mannitol plays a significant role in reducing disintegration time, and

subsequently confirms its function as a disintegration enhancing agent (AlHusban et al. 2010b).

5.5 Conclusions

Evaluation of Kollicoat® IR as an excipient in freeze-dried ODTs has shown that the polymer functions as a binder and its functionality is concentration dependent. This study has shown that concentrations above 10% w/w result in intact, hard and robust tablets. *E.g.* a formulation consisting of 10% w/w Kollicoat® IR and 50% w/w (of dried tablet weight) mannitol, produced tablets which exhibited a hardness of 17.31 ± 0.83 N and a disintegration time of under twenty seconds (19.33 ± 2.08 s). Additionally, Kollicoat® IR possesses a number of advantages which makes it suitable as a binder in freeze-dried ODTs. Due to its low viscous nature, Kollicoat® IR can be used at high concentrations which allow the formation of very hard and robust tablets. Furthermore, as it's freely-soluble in water, Kollicoat® IR formulations can be prepared at room/ambient temperature, which makes formulation preparation convenient. Moreover, Kollicoat® IR tablets exhibit a short disintegration time, especially when formulated in combination with mannitol.

Chapter Six: A Design of Experiments (DOE) Study Evaluating the Role of Excipients on the Properties of Freeze-Dried ODTs

6.1 Introduction

Design of experiments (DOE) is defined as a structured, organised method for determining the relationship between factors affecting a process and the output of that process (Food and Drug Administration, 2009). In order to carry out a DOE investigation, factorial design is commonly used which involves the variation of two or more experimental variables or factors in a planned manner (in which the factors are being studied at two or more levels) (Armstrong and James, 1996). Factorial design establishes the relative order of importance of the factors and can indicate if factors interact (Armstrong and James, 1996).

The principles of factorial design can be applied to pharmaceutical formulation development in which factors such as formulation excipients (concentration, function) can be investigated for their effect on the properties of pharmaceutical products. This ultimately allows formulations to be optimised in order for the finished products to exhibit the required properties/performance.

The use of DOE and factorial design in the development and optimisation of freeze-dried ODT formulations has been reported previously. Ahmed et al. (2011) reported the use of factorial design to investigate the use of maltodextrin as a sugar-matrix former along with cellulosic binders. The effects of formulation parameters on ODT properties were evaluated in order to identify an optimum formulation for the delivery of Nimesulide. AlHusban et al. (2011) meanwhile employed the use of DOE and factorial design to optimise an ODT formulation suitable for delivering enteric coated multiparticulates of Omeprazole. The impacts of several formulation variables on the crucial properties of the formulations were evaluated.

The aim of this chapter was to carry out a DOE study in order to evaluate the role of excipients on the properties of freeze-dried ODTs and subsequently optimise a formulation comprising of Methocel™ E3LV, Polyox™ N10 and mannitol, with the objective of investigating the performance of the tablets in terms of disintegration time, hardness and *in vitro* oral retention time.

Methocel™ E3LV was selected as the binder in this DOE study based on the observations made in Chapter Three (Investigating the Application of Methocel™ (Hydrophilic Cellulose Ethers) as Binders in Freeze-Dried ODTs). Methocel™ E3LV was identified as the most suitable grade of Methocel™, from the several grades investigated, for an application as a binder in freeze-dried ODTs. Mannitol was chosen

to utilise its matrix supporting/structure forming and disintegration enhancing functionality. Polyox™ N10 was selected as an excipient to be investigated in this DOE study, based on the results from Chapter Four (Investigating the Application of Polyox™ (Synthetic Polyethylene Oxide) in Freeze-Dried ODTs) in order to exploit its mucoadhesive characteristics (Bottenberg et al. 1992) and its subsequent influence on *in vitro* oral retention of the formulations.

As discussed above, one of the properties of the formulations investigated in this study was *in vitro* oral retention in order to give an assessment of the mucoadhesiveness of the formulations. Young and Smart (1998) reported a novel apparatus to measure the mucoadhesiveness of liquid or semi-solid formulations in the mouth and oesophagus. The Porcine Oesophageal Mucoadhesion Test System comprised of a test cell constructed from Perspex™ into which a previously isolated mucosa approximately 150mm long was clamped to yield a test plane 120mm in length and 15mm wide, inclined at 30º to the horizontal. The apparatus was maintained at 37ºC and artificial saliva was applied at the top of the test plane to mimic salivary flow and prevent surface desiccation. Testing of formulations involved placing a sample at the top of the test plane and assessment of the resultant eluent fractions.

The Porcine Oesophageal Mucoadhesion Test System has similarly been employed to study the elution behaviour of microparticles placed on a mucosal surface, in which the polymeric microspheres were investigated for drug delivery to the oral mucosa (Kockisch et al. 2003). A similar *in vitro* model was reported by Batchelor et al. (2002) in which the model assessed the adhesion of alginate solutions to porcine oesophageal tissue. The methodology involved a retention apparatus (which was relatively similar to The Porcine Oesophageal Mucoadhesion Test System apparatus) onto which porcine oesophageal tissue was mounted. Fluorescently labelled alginate solutions were dispersed onto the tissue and a washing solution was applied at a fixed rate to mimic saliva flow and the eluted material collected. Fluorimetric analysis allowed dose retention to be assessed as a function of time.

Zhang (1998) employed the *in vitro* retention model, which was reported by Batchelor et al. (2002), to measure the oral retention of mucoadhesive buccal films with the exception that sabouraud agar was used as an alternative to porcine oesophageal tissue as the retention substrate. Agar was used as an alternative to porcine oesophageal tissue as it has been shown to have an *in vitro-in vivo* correlation when

comparing the adhesion of water soluble polymers to agar (*in vitro*) and nasal mucoadhesion (*in vivo*) (Nakamura et al. 1996).

Bigucci et al. (2008) and Bertram and Bodmeier (2006) also used agar as the retention substrate in their methods which were adapted from Nakamura et al. (1996). Bigucci et al. (2008) used agar/mucin plates to measure the residence time of pectin-based microspheres used for the colon-specific delivery of vancomycin. Bertram and Bodmeier (2006) similarly used agar/mucin plates to measure the adhesion potential of nasal inserts. Agar gel has also been reported as a substitute for the oral mucosa, in order to test the adhesion of mucoadhesive films to the oral mucosa *in vitro* (Kuroya and Inoue, 1992).

It was proposed to use the same apparatus/model which was reported by Batchelor et al. (2002) and Zhang (1998) in order to measure the *in vitro* oral retention time of ODT formulations from the DOE study. Agar was used as the retention substrate, which has been widely reported to be used in measuring mucoadhesion. The method involved placing a tablet on top of the test plane followed by addition of double-distilled water which mimicked saliva encountered *in vivo* and the time taken for the formulations to be completely removed from the test plane was recorded and referred to as retention time.

Following the DOE study, a model API, nystatin was used in order to assess if the *in vitro* retention time of this API could be modified based on the composition of the formulations, using the *in vitro* oral retention apparatus. Nystatin is an antifungal agent used in the treatment of oropharyngeal candidiasis, which is usually available as an oral suspension (Peppas et al. 2009). When administering nystatin oral suspension, patients are advised that one-half of the dose should be placed in each side of the mouth and retained for as long as possible before being swallowed (Fougera and Co., 2005). Formulating nystatin in the form of a freeze-dried ODT, is an innovative approach to delivering the antifungal agent to the mouth and subsequently treating oropharyngeal candidiasis. Modifying the retention of the API in the mouth by adjusting the composition of the formulation could potentially improve the treatment of oropharyngeal candidiasis.

6.2 Materials

Polyethylene oxide (Polyox™ WSR N10, Lot No.: DT353617) and hydroxypropyl methylcellulose (Methocel™ E3LV, Lot No.: DT220342) were supplied by Colorcon Ltd. (Dartford, UK). Mannitol (Lot No.: BCBF4739V) was supplied by Sigma-Aldrich (Poole, UK). Agar Technical (Agar No. 3) was supplied by Oxoid Ltd. (Basingstoke, Hampshire, UK). Nystatin (≥ 4400 USP units/mg) was supplied by Scientific Laboratory Supplies (Nottingham, UK).

6.3 Methods

6.3.1 Preparation of Freeze-Dried Tablets

For the preparation of the various formulations, firstly, the required amounts of mannitol and/or polyethylene oxide were added to half the required amounts of water (at ambient temperature) under stirring, until fully dissolved. These solutions were then heated to around 90ºC under stirring, followed by the addition of hydroxypropyl methylcellulose. Cold water was then added to solubilise the hydroxypropyl methylcellulose and form the final solutions.

For formulations which did not contain both polyethylene oxide and mannitol, half the required amounts of water were heated to around 90ºC under stirring, followed by the addition of hydroxypropyl methylcellulose. Cold water was then added to solubilise the hydroxypropyl methylcellulose and form the final solutions.

For the formulations which included nystatin, the required amounts of mannitol and/or polyethylene oxide were added to half the required amounts of water (at ambient temperature) under stirring, until fully dissolved. Nystatin was then added under stirring. These preparations were then heated to around 90ºC under stirring, followed by the addition of hydroxypropyl methylcellulose. Cold water was then added to solubilise the hydroxypropyl methylcellulose and form the final preparations.

1.5g of the resulting preparations were dosed into a tablet mould, frozen at -70°C for a minimum of sixty minutes and freeze-dried (ADVANTAGE, Freeze-Dryer, VIRTIS), according to the following regime; primary drying for forty eight hours at a shelf temperature of -40°C, secondary drying for ten hours at a shelf temperature of 20°C, and vacuum pressure of 6.67 Pa (50 mTorr). A minimum of ten tablets were prepared for each formulation.

6.3.2 Formulation Viscosity Analysis

For all formulations, the viscosities of 100 mL samples were measured using a DV-I+ Brookfield digital viscometer (Harlow, UK). The viscosities of the formulations were measured at 37 ± 2°C, in order to replicate the viscosity of the formulations in the oral cavity, at physiological temperature. The viscometer spindles were selected based on the viscosity of the individual formulations, as each spindle is suitable for analysing a certain viscosity range. The rotational speed of the spindle was set at 100 rpm. Each formulation was analysed in triplicate, and the mean values ± standard deviation is reported.

6.3.3 Tablet Hardness

The hardness of the tablets was investigated using a texture analyzer (QTS 25: Brookfield, Essex, UK) equipped with a 25 kg load cell. The instrument was calibrated by standard weights of 500 g and 5 kg. The tablets were placed in a holder with a cylindrical hole. The hardness was taken as the peak force after 1 mm penetration of a 5 mm diameter probe at a rate of 6 mm/min. Three measurements were taken for each formulation, and the mean ± standard deviation is reported.

6.3.4 Disintegration Time of the Tablets

The disintegration time of the tablets was determined using a USP disintegration tester (Erweka, ZT3). 800 mL of double distilled water, which was kept at 37 ± 2ºC, was used as the medium and the basket was raised and lowered at a fixed rate of 30 rpm. Three tablets were evaluated from each formulation, and the mean values ± standard deviation is reported.

6.3.5 *In Vitro* Oral Retention Time

In vitro oral retention time was measured (as a means of assessing *in vitro* oral mucoadhesion of the formulations) using an *in vitro* oral retention apparatus adapted from Zhang (2008) and Batchelor et al. (2002). The apparatus consisted of a Perspex® mounting block with dimensions; 100mm in length by 60mm in width and 15mm deep. A groove was cut into this block with dimensions; 100mm in length by 20mm in width and 5mm deep, into which liquid agar was dosed and allowed to set to mimic oral mucosal tissue. The mounting block was attached to a clamp (which was further attached to a stand) at an angle of 30º to the horizontal. The stand was placed within a temperature and humidity controlled environment (a Perspex® cabinet containing a water bath and a platform onto which the stand was placed).

Temperature and humidity were maintained at 37 ± 2ºC and > 90% RH, respectively, with these conditions monitored using a LogIT-Voyager temperature and humidity logging instrument (DCP Microdevelopments Ltd., Great Ellingham, Norfolk, UK). The cabinet had partially sealed glove access to enable procedures to be performed whilst carrying out measurements. Distilled water was used to mimic saliva and was delivered to the agar section of the Perspex® mounting block at a rate of 1 mL/min via a Gilson Minipuls 3 peristaltic pump (Gilson UK, Luton, Bedfordshire, UK). The flow was split into four channels to provide an even distribution of the distilled water over the agar section.

In order to determine *in vitro* oral retention time of the formulations, a tablet was placed on the uppermost part of the agar section and the time taken for the tablet to be removed was recorded with the naked eye and recorded as the retention time. Three measurements were taken for each formulation and the mean values are reported.

To determine the *in vitro* oral retention of nystatin, a tablet was placed on the uppermost part of the agar section and at 30s intervals, the material washed from the surface of the agar was collected into plastic vials. The vials were changed at 30s intervals in order to examine the *in vitro* oral retention of nystatin. Analysis of the amount of nystatin collected at 30s intervals was performed using HPLC analysis (method information detailed below), which allowed the *in vitro* oral retention of nystatin to be calculated, with reference to a previously performed calibration curve. Three measurements were taken for each formulation and the mean values are reported.

6.3.6 HPLC (High Performance Liquid Chromatography) Analysis

HPLC analysis of the collected nystatin samples were performed using reversed phase HPLC (Dionex AS50 autosampler, GP50 gradient pump and UVD170U detector: Dionex, Leeds, UK) at room temperature using a Phenomenex Gemini 5 µm C18 150 x 4.60 mm column (Macclesfield, UK). The collected nystatin samples were filtered through a 0.45 µm nylon syringe filter (to remove undissolved API) and 30 µL of the samples were analysed, with UV detection performed at 305 nm, with methanol:water (70:30) as the mobile phase and a flow rate of 1.00 mL/min.

6.3.7 Design of Experiments

The design of experiments – factorial design used in this study was performed using MODDE software (version 8) (Umetrics AB, Umea, Sweden). The response surface modelling objective was selected, as it provides detailed modelling and optimisation of

the study. A central composite face quadratic model design was recommended by the software, which is composed of a full factorial design, which comprised 17 runs in total with 3 centre points.

The factors (independent variables) investigated in this study were the concentrations of; Methocel™ E3LV, Polyox™ N10 and mannitol. Each factor was investigated at three different levels; low, medium and high, which were referred to as; -1, 0 and 1, as detailed in Table 6.1. The responses (dependent variables) measured in this study were; formulation viscosity, tablet hardness, tablet disintegration time and *in vitro* oral retention time of the formulations. Analysis of variance (ANOVA) was the statistical analysis of the data performed in this study.

Table 6.1 Information detailing the concentration values of Methocel™ E3LV, Polyox™ N10 and mannitol relative to their levels, which were investigated in this study.

	Concentration of Excipients (% w/w)		
Level of Concentration	**Methocel™ E3LV**	**Polyox™ N10**	**Mannitol***
-1 (Low Level)	5.0	0.0	0.0
0 (Medium Level)	6.5	1.5	25.0
1 (High Level)	8.0	3.0	50.0

*Mannitol concentration expressed as % w/w (of dried tablet weight), relative to 6.5% w/w Methocel™ E3LV.

6.4 Results and Discussion

As discussed earlier, the aim of this chapter was to evaluate the role of the excipients on the properties of freeze-dried ODTs and subsequently optimise a formulation comprising of Methocel™ E3LV, Polyox™ N10 and mannitol, with the objective of studying the performance of tablets in terms of disintegration time, hardness and *in vitro* oral retention time. These three factors were investigated at three different levels/concentrations in order to evaluate their effect on the responses of the tablets/formulations.

In this study, 17 formulations were prepared in total, which were subsequently characterised for viscosity, tablet hardness, tablet disintegration time and *in vitro* oral retention time. The results from the study are shown in Table 6.2, which revealed that formulation viscosity varied from 1.41 to 21.37 mPa s, between the 17 formulations prepared and analysed. Tablet hardness and disintegration time varied from 1.29 to 20.82 N and 6.67 to 396.00 s, respectively. In terms of *in vitro* oral retention time of the formulations, the values varied from 25.67 to 164.67 s.

Table 6.2 Completed DOE study worksheet, which shows the compositions of the 17 formulations prepared and characterisation results for the formulations/tablets. Each characterisation/response result is a mean value (n=3).

		Factors (Independent Variables)			**Responses (Dependent Variables)**			
Exp. Name	**Run Order**	**Methocel E3LV (% w/w)**	**POLYOX N10 (% w/w)**	**Mannitol (% w/w)**	**Formulation Viscosity (mPa s)**	**Tablet Hardness (N)**	**Disintegration Time (s)**	**In Vitro Oral Retention Time (s)**
N1	4	5.0	0.0	0.0	1.41	1.29	59.33	55.67
N2	5	8.0	0.0	0.0	3.26	6.89	363.67	66.67
N3	14	5.0	3.0	0.0	4.65	1.45	290.00	164.67
N4	10	8.0	3.0	0.0	13.33	7.02	176.67	82.67
N5	12	5.0	0.0	50.0	2.10	8.04	6.67	25.67
N6	9	8.0	0.0	50.0	4.98	14.83	16.67	50.00
N7	3	5.0	3.0	50.0	8.59	11.48	27.00	32.67
N8	17	8.0	3.0	50.0	21.37	20.82	55.33	38.33
N9	8	5.0	1.5	25.0	3.70	5.42	79.67	45.33
N10	1	8.0	1.5	25.0	8.61	13.89	75.00	34.33
N11	15	6.5	0.0	25.0	2.54	6.91	39.33	31.67
N12	6	6.5	3.0	25.0	10.33	9.70	56.67	32.00
N13	7	6.5	1.5	0.0	4.84	3.38	396.00	40.33
N14	2	6.5	1.5	50.0	5.81	11.50	40.33	31.00
N15	16	6.5	1.5	25.0	4.34	8.63	64.00	29.67
N16	13	6.5	1.5	25.0	5.21	7.09	57.67	32.67
N17	11	6.5	1.5	25.0	5.49	7.19	127.67	34.33

The results showed wide variations in the four responses measured as a result of modifying the composition of the formulations in terms of the levels/concentrations of each factor. This suggested that the three factors investigated, exerted a significant effect on the four responses measured.

The effect each factor had both individually and interactively on the four responses is shown in Table 6.3. In Table 6.3, the quantitative effect that each factor had on the responses is expressed as "Effect" and the level of significance of the quantitative effect is represented by a *p* value, where $p < 0.05$ is considered statistically significant. As can be seen in Table 6.3, in some cases factors exerted a positive effect on responses which resulted in an increase in response, whilst in other cases factors exhibited a negative effect on responses, which resulted in a decrease in response.

Table 6.3 The quantitative effect each factor(s) has on the responses and associated level of significance, represented by *p* value, where $p < 0.05$ is considered statistically significant.

	Formulation Viscosity		**Tablet Hardness**		**Disintegration Time**		***In Vitro* Oral Retention Time**	
Factor(s)	**Effect**	***p* Value**	**Effect**	***p* Value**	**Effect**	***p* Value**	**Effect**	***p* Value**
Methocel™ E3LV	6.2160	<0.0001	7.1784	<0.0001	45.0444	0.4778	-10.297	0.4803
POLYOX™ N10	8.7958	<0.0001	2.5032	0.0091	24.0052	0.7014	24.1370	0.1241
Mannitol	3.0701	0.0052	9.3396	<0.0001	-227.881	0.0068	-46.418	0.0121
Methocel™ E3LV-Methocel™ E3LV	2.0790	0.3042	2.3269	0.2170	-56.8848	0.7099	28.8339	0.4213
POLYOX™ N10-POLYOX™ N10	2.3919	0.2428	1.1248	0.5329	-108.770	0.4829	19.3021	0.5854
Mannitol-Mannitol	0.2703	0.8895	-1.202	0.5062	228.8590	0.1630	24.3909	0.4934
Methocel™ E3LV-POLYOX™ N10	4.1909	0.0062	0.5789	0.5781	-100.067	0.2774	-28.138	0.1930
Methocel™ E3LV-Mannitol	1.2777	0.2775	1.2690	0.2418	-38.0385	0.6679	25.3727	0.2352
POLYOX™ N10-Mannitol	2.3967	0.0630	2.2597	0.0569	3.7156	0.9663	-32.526	0.1399

6.4.1 Formulation Viscosity

In terms of formulation viscosity, the results (shown in Table 6.3) revealed that Methocel™ E3LV ($p < 0.0001$), Polyox™ N10 ($p < 0.0001$), mannitol ($p < 0.01$) and an interaction between Methocel™ E3LV and Polyox™ N10 ($p < 0.01$), all had a significant positive effect on formulation viscosity. Analysing the quantitative effect of these factors revealed that Polyox™ N10 had the greatest effect on formulation viscosity, followed by Methocel™ E3LV, an interaction between Methocel™ E3LV-Polyox™ N10, and finally mannitol, respectively.

The ability of these factors to increase formulation viscosity upon increasing concentration, could possibly be attributed to an increase in the interaction between the formulation ingredients as their concentration were increased. The fact that Polyox™ N10 displayed the greatest effect on formulation viscosity highlights its functionality as a viscosity-increasing agent (Maximilien, 2011).

Interestingly, the results also revealed that Methocel™ E3LV and Polyox™ N10 exhibited a synergistic-interactive effect on formulation viscosity, as illustrated in Figure 6.1, which shows that an increase in the concentrations of Methocel™ E3LV and Polyox™ N10 resulted in an increase in formulation viscosity. The synergistic-interactive effect of Methocel™ E3LV and Polyox™ N10 on formulation viscosity can be attributed to an interaction between these two factors at a molecular level. Fuller et al. (2001) reported that HPMC and polyethylene oxide blends are miscible and behave similarly to other PEO-cellulosic blends, i.e. these polymers interact via hydrogen-bonding between the hydroxyl groups of HPMC and the ether oxygen of PEO. Interactions between PEO and cellulose-based polymers have also been previously reported and have been attributed not only to intra- but also intermolecular interactions including hydrogen bonding (Kondo et al. 1994 and Kondo and Sawatari, 1994).

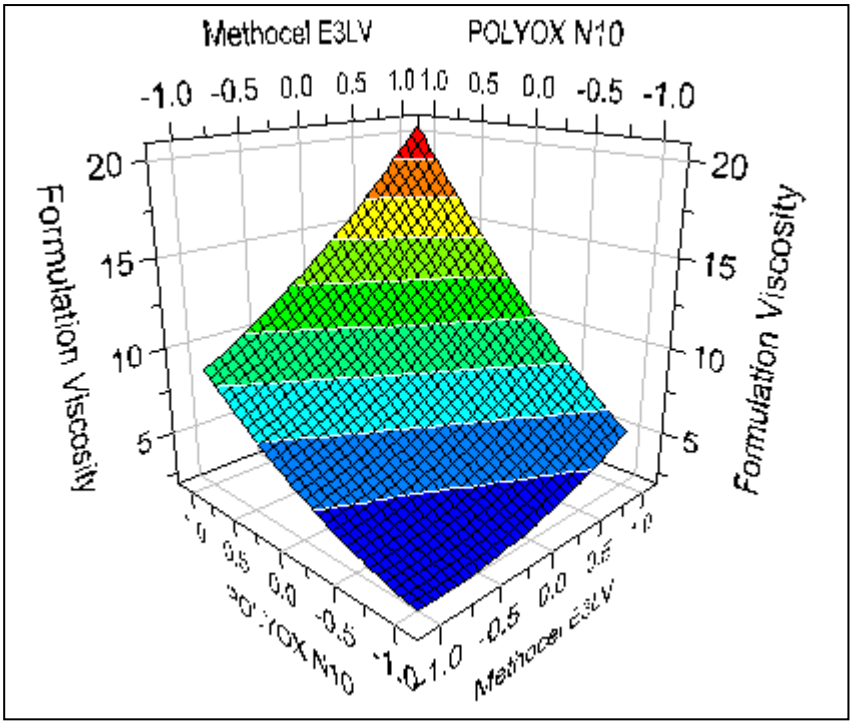


Figure 6.1 A 3D surface response plot showing the influence of varying Methocel™ E3LV and Polyox™ N10 levels on formulation viscosity. The different colours of the plot refer to contour levels, with the colour blue referring to the lowest contour level (response) and the colour red referring to the greatest contour level (response). The levels of Methocel™ E3LV and Polyox™ N10 range from -1.0 (lowest level/concentration) to 1.0 (greatest level/concentration).

In terms of the interactions between HPMC and PEO, it has been reported that using these two polymers in combination, has shown to provide an innovative matrix tablet system that permits the adjustment of the rate of API release, in comparison with pure HPMC matrix tablet systems (Fuller et al. 2001). Fuller et al. (2001) reported that the adjustment of the rate of API release could be due to a direct interaction between HPMC and PEO. The application of the interaction between HPMC and PEO with matrix tablet systems, demonstrates the potential usefulness of this polymer:polymer interaction with pharmaceutical formulations.

As discussed above, the results revealed that mannitol displayed the least effect on formulation viscosity. This could possibly be attributed to the low molecular weight and non-polymeric nature of mannitol, relative to Polyox™ N10 and Methocel™ E3LV, which possess significantly greater molecular weight and are polymeric in nature, which displayed more significant effect on formulation viscosity.

6.4.2 Tablet Hardness

The results for tablet hardness indicated that Methocel™ E3LV ($p < 0.0001$), mannitol ($p < 0.0001$) and Polyox™ N10 ($p < 0.01$), all had a significant positive effect. An evaluation of the quantitative effect of these three factors revealed that mannitol exhibited the greatest effect on tablet hardness followed by Methocel™ E3LV and Polyox™ N10. Figure 6.2 illustrates the influence of mannitol and Methocel™ E3LV concentrations on tablet hardness.

Increase in tablet hardness as a result of increasing the concentrations of these three factors, could possibly be attributed to the formation of a more extensive and robust tablet binder matrix which consequently results in an increase in tablet hardness. As discussed in Chapter Four (Investigating the Application of Polyox™ (Synthetic Polyethylene Oxide) in Freeze-Dried ODTs), mannitol has been reported to influence tablet hardness through a number of mechanisms including its crystalline nature (Sastry et al. 2000, Kearney, 2003 and Seager, 1998) and by functioning as a matrix supporting agent (AlHusban et al. 2010b).

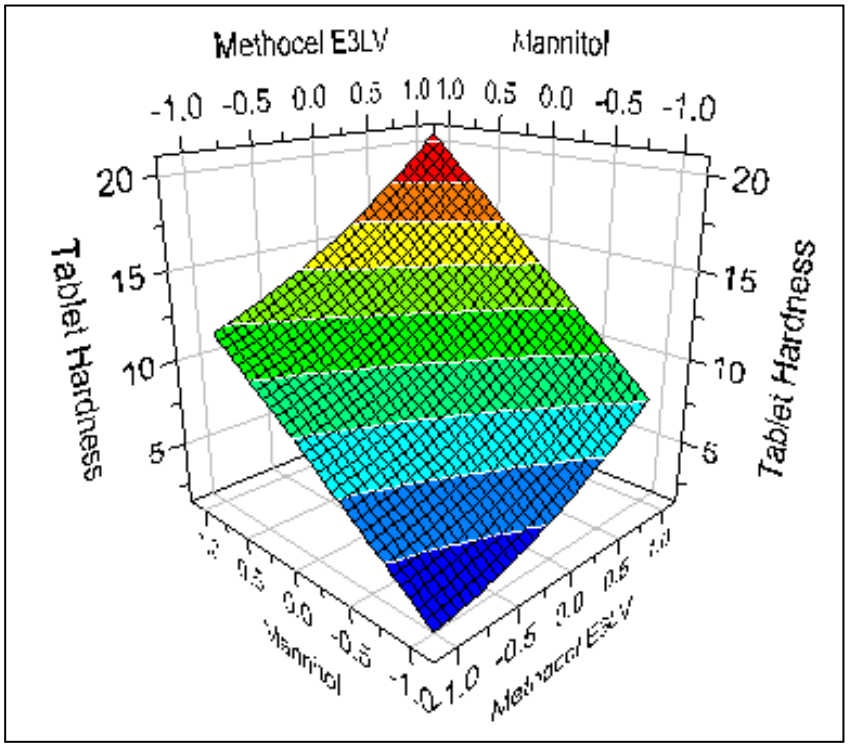


Figure 6.2 A 3D surface response plot showing the influence of varying Methocel™ E3LV and mannitol levels on tablet hardness. The different colours of the plot refer to contour levels, with the colour blue referring to the lowest contour level (response) and the colour red referring to the greatest contour level (response). The levels of Methocel™ E3LV and mannitol range from -1.0 (lowest level/concentration) to 1.0 (greatest level/concentration).

In terms of Methocel™ E3LV exerting a significant effect on tablet hardness, it has previously been reported that the binding capacity of HPMC increases as concentration is increased (Ahmed et al. 2011). Additionally, as Methocel™ E3LV functions as a binder, its function is to convey strength and robustness to the tablets (Seager, 1998). The results from this DOE investigation also provide further insight into factors influencing tablet hardness. It is generally accepted that formulations of freeze-dried ODTs requires the inclusion of both a binder as well as matrix supporting agents, with binders providing the required framework for the tablet and supporting agents cementing and strengthening binder scaffold. The study has shown that despite the formation of framework upon inclusion of Methocel™ E3LV, material properties of the supporting agents play a critical role in determining resultant tablet hardness. Mannitol in its crystalline state (which can be determined by evaluating the thermal properties of formulations, by using DSC) provides the necessary cementing support and ranks at the top in hierarchy in providing hardness to the finished dosage form which further suggests that two important criteria (hydrophilic nature and crystallinity) govern tablet properties and should be included in the development and selection of novel matrix supporting agents.

6.4.3 Tablet Disintegration Time

Tablet disintegration time was significantly influenced by a single factor; mannitol ($p < 0.01$), which demonstrated a significant negative effect, i.e. increasing the concentration of mannitol resulted in a general decrease in tablet disintegration time as illustrated in Figure 6.3. The results reaffirmed the functionality of mannitol as a disintegration enhancing agent due to its highly hydrophilic nature (AlHusban et al. 2010a), as discussed in Chapter Four (Investigating the Application of Polyox™ (Synthetic Polyethylene Oxide) in Freeze-Dried ODTs).

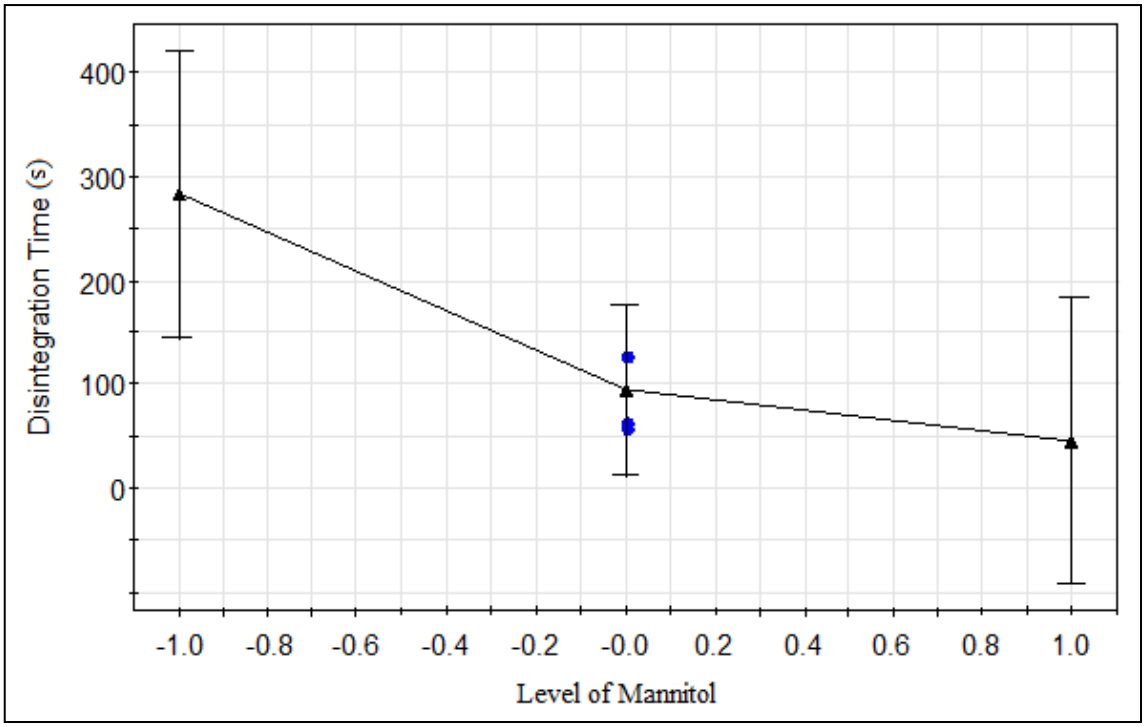


Figure 6.3 A main effects plot showing the influence of mannitol level on tablet disintegration time. The level of mannitol ranges from -1.0 (lowest level/concentration) to 1.0 (greatest level/concentration).

6.4.4 *In Vitro* Oral Retention Time

Investigation into *in vitro* oral retention time of the formulations showed similar trend as with the tablet disintegration time results, where a single factor was shown to have a significant effect; mannitol ($p < 0.05$). Mannitol exhibited a significant negative effect, i.e. increasing the concentration of mannitol resulted in decreasing *in vitro* oral retention time as illustrated in Figure 6.4.

This trend could be attributed to mannitol being a mucoactive agent (defined as an agent that has the capability of modifying the nature and composition of mucus, and/or interactions with the mucociliary epithelium (Capri et al. 1994)), as a consequence of being an osmotic agent (Daviskas et al. 2008).

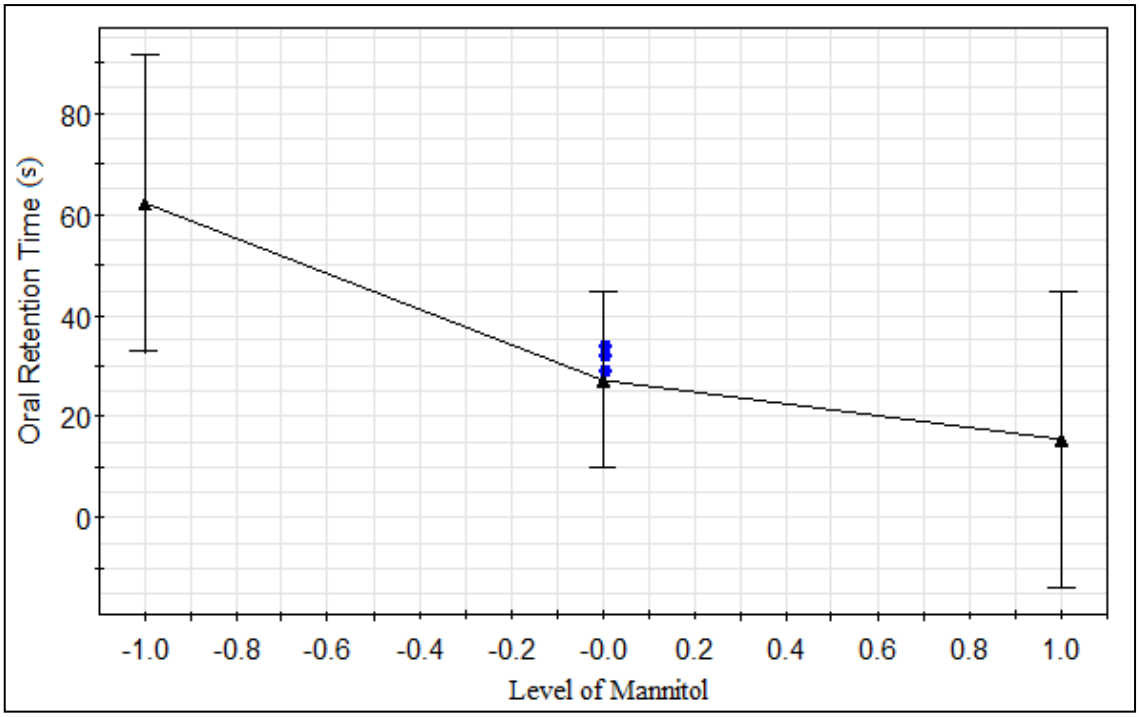


Figure 6.4 A main effects plot showing the influence of mannitol level on *in vitro* oral retention time of the formulations/tablets. The level of mannitol ranges from -1.0 (lowest level/concentration) to 1.0 (greatest level/concentration).

It can be concluded that by increasing the concentration of mannitol in the formulations/tablets, the nature and composition of the formulations/tablets is modified to such an extent that the adhesion of the formulations/tablets to the agar surface was reduced. The decrease in *in vitro* oral retention time was possibly due to modification of the interactions between the formulations/tablets and the agar surface. Interestingly a similar observation has been reported, in that the tendency for HPMC to adhere to isolated porcine oesophageal tissue was reduced by the addition of sucrose to the formulations (Marvola et al. 1983). This was attributed to the water-soluble nature of sucrose, as Marvola et al. (1983) reported that the addition of sparingly water-soluble ingredients, such as talc, increased adherence. It therefore appeared that sucrose modified the nature and composition of HPMC, which resulted in a reduction in adherence. This observation reported by Marvola et al. (1983) has similar outcome to the observed results seen with mannitol in this DOE study.

The results interestingly revealed that Polyox™ N10, although had a positive effect on *in vitro* oral retention time, statistical analysis of the data showed that effect was insignificant ($p > 0.05$) at the concentration values/range investigated, possibly due to the low viscous/low molecular weight nature of this grade of Polyox™.

6.5 Formulation Optimisation

Following the preparation and characterisation of the seventeen formulations which were proposed as part of the DOE study, the next stage of the study involved formulation optimisation, which allowed identifying a formulation that exhibited desired properties as outlined in table 6.4. The objective of the study was to maximise tablet hardness with a minimum tablet hardness value of 7 N and a maximum target of 12 N. These values for tablet hardness were selected following macroscopic evaluation of the tablets of the seventeen formulations prepared as part of the DOE study, and it was concluded that tablets exhibiting these values for tablet hardness were sufficiently robust to withstand manual handling.

Table 6.4 Proposed desired properties for the optimum formulation, detailing the acceptable and target values for; tablet hardness, tablet disintegration time and *in vitro* oral retention time.

Response	Maximise or Minimise?	Smallest or Highest Acceptable Value	Target
Tablet Hardness	Maximise	7 N	12 N
Tablet Disintegration Time	Minimise	30 s	10 s
***In Vitro* Oral Retention Time**	Maximise	50 s	100 s

For tablet disintegration time, the range chosen was between 10 and 30 seconds to comply with the criteria set by Food and Drug Administration (FDA) which stipulates that ODTs should disintegrate within 30s (FDA, 2008).

In terms of *in vitro* oral retention time, it was proposed to maximise this response with a minimum value of 50 s and a target value of 100 s. The minimum value of 50 s was selected as it has been previously reported that the mean time for 50 % buccal clearance of a freeze-dried tablet formulation was 50 s (Wilson et al., 1987).

Table 6.5 shows the composition of the optimum formulation based on the proposed desired properties.

Table 6.5 Composition of the optimum formulation, detailing the concentration of each of the three formulation ingredients.

Formulation Ingredient	Concentration (% w/w)
Methocel™ E3LV	7.95
Polyox™ N10	3.00
Mannitol	28.11*

*Mannitol concentration expressed as % w/w (of dried tablet weight), relative to 6.5% w/w Methocel™ E3LV.

The predicted and observed responses for the optimum formulation are shown in Table 6.6.

Table 6.6 Predicted and observed responses for the optimum formulation. In terms of the observed responses, three measurements were performed and the mean values ± standard deviation are reported.

Response	Predicted	Observed
Formulation Viscosity (mPa s)	16.84	11.90 ± 0.36
Tablet Hardness (N)	14.89	12.56 ± 2.92
Tablet Disintegration Time (s)	27.23	46.33 ± 1.53
***In Vitro* Oral Retention Time (s)**	41.37	41.33 ± 2.52

As Table 6.6 shows, the observed formulation viscosity was slightly less than what was predicted, with a difference of 4.94 mPa s. For tablet disintegration time, the observed value was around 20 s greater than the predicted value. Analysis of tablet hardness and in *vitro* oral retention time showed that both the tested parameters demonstrated comparable results between observed and predicted values. The results demonstrate the validity of the DOE study carried out as discussed above and provides useful information in formulation development of freeze dried ODT using the new combination of excipients. To further develop ODTs using the above strategy, the next phase of the investigation was focussed on formulating a tablet to deliver Nystatin.

6.6 Assessment of the *In Vitro* Oral Retention of Nystatin

One of the aims of this study was to assess if the *in vitro* oral retention of nystatin could be modified, based on the composition of the formulation, which was assessed using the *in vitro* oral retention apparatus. Two formulations were selected in order to assess the retention of nystatin; the optimised formulation from the DOE study and formulation N5 (refer to Table 6.2), also from the DOE study.

These formulations were selected due to their differences in composition, as the optimised formulation consisted of high concentrations of Methocel™ E3LV and

Polyox™ N10, and a medium level of mannitol. Formulation N5 meanwhile consisted of a low concentration of Methocel™ E3LV and a high concentration of mannitol (this formulation did not consist of Polyox™ N10).

The results from the assessment of the retention of nystatin (Figure 6.5) revealed that the retention of nystatin from the two formulations, differed greatly. With formulation N5, nystatin was retained up to 30s whilst with the optimised formulation, nystatin retention was observed up to 60s.

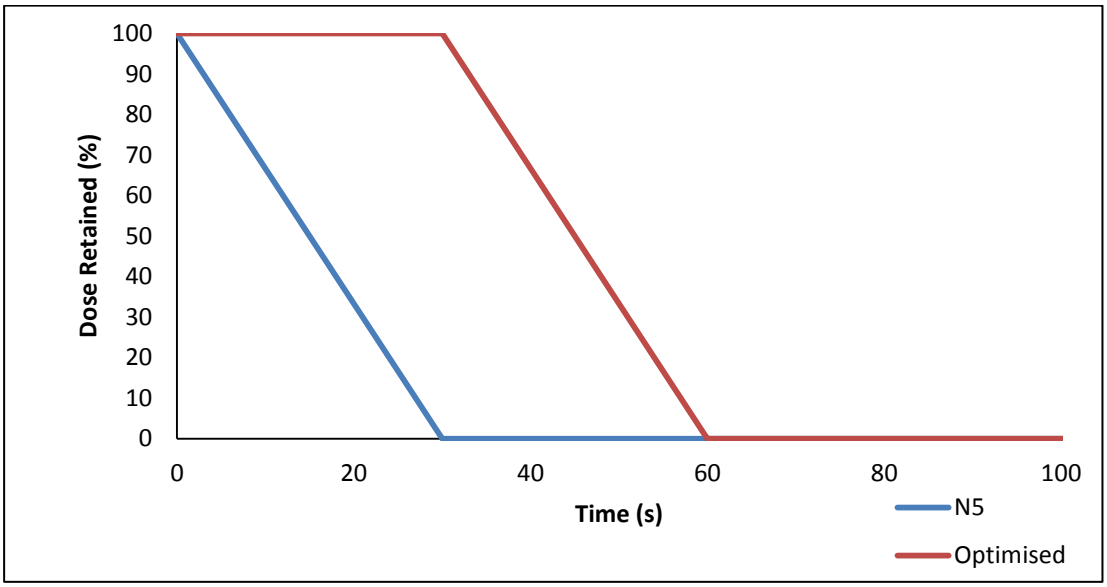


Figure 6.5 An assessment of the *in vitro* oral retention of nystatin from formulation N5 (shown in blue) and the optimised formulation (shown in red). Three measurements were taken for each formulation and the mean values ± standard deviation are shown.

As discussed previously, the results from the DOE study revealed that mannitol exhibited a significant negative effect on *in vitro* oral retention time of the formulations. Formulation N5, which consisted of a high concentration of mannitol, exhibited a nystatin retention time of 30s, whilst the optimised formulation, which comprised of a medium level/concentration of mannitol, displayed a nystatin retention time of 60s. The difference in nystatin retention between the two formulations could be attributed to the osmotic effect which is dependent on mannitol concentration within the formulations.

6.7 Conclusions

The results from the DOE study revealed that formulation viscosity was primarily influenced (significant positive effect) by both Polyox™ N10 and Methocel™ E3LV, and in fact a synergistic-interactive effect was observed with these two excipients. In terms of tablet hardness, mannitol and Methocel™ E3LV were shown to be most influential (significant positive effect). Tablet disintegration time and *in vitro* oral retention time were both significantly influenced (significant negative effect) by mannitol concentration.

An optimised formulation was identified which exhibited desired properties/responses, in terms of tablet hardness, tablet disintegration time and *in vitro* oral retention time. Assessing the *in vitro* oral retention of nystatin demonstrated that the *in vitro* oral retention of APIs can be modified based on the composition of the formulation, with particular emphasis on mannitol concentration. This opens up the possibility of potentially optimising the retention of APIs in the oral cavity and other pre-gastric regions, in order to treat localised conditions such oropharyngeal candidiasis or encourage pre-gastric absorption of APIs.

Chapter Seven: Incorporation of Nanoparticles in Freeze-Dried ODTs

7.1 Introduction

Nanoparticles are defined as solid particles ranging in size from 10 nm to 1000 nm (1 μm) (Kreuter, 1996). According to Bahl et al. (2011) there are only around four commercially available solid oral dosage formulations in the United States that contain nanoparticles. The first product to be approved for commercial production was launched in 2000; Rapamune®, which consisted of nanocrystals (the term drug nanocrystals refers to crystals with a size in the nanometre range) in a tablet form (Muller and Junghanns, 2006).

The rationale for processing an active drug into nanocrystal form and subsequently incorporating the nanocrystals into a tablet is to increase the surface area of the drug particles and consequently increase drug dissolution (Muller and Junghanns, 2006). Processing drug particles into nanocrystals is therefore highly applicable for poorly water soluble drug compounds as a means of increasing solubility.

It has been reported that around 40% of the APIs in development pipelines have solubility issues (Speiser, 1998), and that around 60% of the APIs coming from synthesis are currently poorly soluble (Merisko-Liversidge, 2002). This can be attributed to the increased use of high throughput screening methods, which results in the discovery of more drugs exhibiting poor water solubility (Muller and Junghanns, 2006). It is anticipated that due to the emergence of increasing number of poorly water soluble drugs, an increase in drug nanocrystal-based products on the market can be expected (Muller and Junghanns, 2006).

The incorporation of nanoparticles in freeze-dried ODTs has also been reported. Jain et al. (2001) investigated a rapidly disintegrating or dissolving solid oral dosage form comprising of a poorly soluble nanoparticulate active ingredient (on which a surface stabiliser is adsorbed). Jain et al. (2001) further reported that a rapidly disintegrating or dissolving solid oral dosage form has the advantage of combining rapid presentation of the poorly soluble active agent as a result of the fast disintegration and dissolution of the poorly soluble drug in the oral cavity. Other advantages of this type of formulation reported by Jain et al. (2001) included a reduction in the delay of onset of therapeutic action of the poorly soluble drug together with the enhanced opportunity for buccal absorption of the drug (Jain et al. 2001).

It is apparent that the rationale for Jain et al. (2001) to incorporate an API in nanoparticulate form in an ODT was to enhance the presentation of the poorly soluble API as a result of an improvement in dissolution.

The incorporation of nanoparticles in freeze-dried ODTs has similarly been investigated by Bahl et al. (2011). Bahl et al. (2011) reported a direct method of preparing freeze-dried ODTs containing nanoparticles. The method involved reducing the particle size (by wet milling or homogenisation) of an API dispersed in a solution containing fish gelatin to form a nanosuspension, which is subsequently freeze-dried to form ODTs (Bahl et al. 2011). Fish gelatin therefore acts as a nanoparticle stabiliser during both the nanomilling process and freeze-drying process (Bahl et al. 2011). The method avoids the need to adjust excipient composition during the process steps as fish gelatin facilitates both the particle size reduction and freeze-drying procedures (Bahl et al. 2011).

As with Jain et al. (2001) it is apparent that the rationale for Bahl et al. (2011) to incorporate nanoparticles in freeze-dried ODTs was to increase the rate and extent of dissolution of poorly soluble APIs.

Additionally, Lai et al. (2011) reported the use of nanoparticles in freeze-dried ODTs. Lai et al. (2011) reported the preparation of freeze-dried ODTs using nanocrystal formulations, with the aim of improving the dissolution properties of piroxicam, which is a poorly soluble API. Lai et al. (2011) showed that ODT formulations prepared using piroxicam nanocrystals, exhibited a higher dissolution rate than ODT formulations prepared using coarse piroxicam particles, which was due to an increase in the surface area of the nanocrystal drug particles.

Polymeric biodegradable nanoparticles have attracted attention as drug delivery systems due to their application in controlled release/delivery of APIs (Soppimath et al. 2001). Nanoparticles have particular advantages over conventional drug delivery devices in that extended drug release rates up to days, weeks or months can be achieved, and biodegradable polymeric nanoparticles can be formulated (Mundargi et al. 2008). Another significant advantage of nanoparticles is that a wide variety of APIs can be delivered including hydrophilic, hydrophobic, proteins, vaccines and biological macromolecules (Hans and Lowman, 2002). Nanoparticles based formulations can also result in increase in bioavailability, solubility and permeability of many potent APIs which are otherwise difficult to deliver via the oral route (Kumari et al. 2010). As

nanoparticles can potentially provide extended drug release rates up to days, weeks or months, administering a drug in nanoparticulate form can reduce drug dosage frequency and will consequently increase patient compliance (Kumari et al. 2010).

Oral dosage forms consisting of nanoparticles have been widely reported including a suspension in which the vehicles used for preparing the oral suspensions include aqueous solutions, oils, mucoadhesive gels, and microemulsions (Galindo-Rodriguez et al. 2005). Additionally, nanoparticles incorporated into a conventional tablet (which is not recommended as the integrity of the nanoparticles can be affected under high compression forces/pressures) have been reported, along with the filling of nanoparticles (in dried powder form, with excipients) into hard gelatin capsules (Galindo-Rodriguez et al. 2005).

Oral administration of APIs in nanoparticulate form provides several advantages compared to single-unit oral dosage forms such as capsules (Galindo-Rodriguez et al. 2005). Following the oral administration of nanoparticles, they distribute evenly in the GIT which consequently results in uniform drug absorption, extended drug release and a reduced risk of local irritation (Galindo-Rodriguez et al. 2005).

Comparing nanoparticles with other multiparticulate carriers such as microspheres or pellets, studies indicate that nanoparticles are capable of penetrating the mucus layer to reach the apical membrane of the epithelial cells, whilst microparticles greater than 10 μm in diameter are excluded by the viscous gel layer of the mucus (Galindo-Rodriguez et al. 2005). Furthermore it has been reported that the number of nanoparticles that cross the epithelium is greater than the number of microspheres (Galindo-Rodriguez et al. 2005).

Freeze-dried ODTs consisting of polymeric biodegradable nanoparticles have not been previously reported. Incorporating polymeric biodegradable nanoparticles in freeze-dried ODTs would allow the benefits of both ODTs and polymeric biodegradable nanoparticles to be exploited. As ODTs provide the ease and convenience of administration, whilst polymeric biodegradable nanoparticles can provide extended drug release rates, therefore potentially reducing drug dosage frequency, which could increase patient compliance.

The conventional method of preparing polymeric nanoparticles using the nanoprecipitation/solvent-displacement method, which was first reported by Fessi et al. (1989), is summarised as a flow chart in Figure 7.1.

Additionally, the method of preparing chitosan nanoparticles, using the ionic gelation method, is summarised as a flow chart in Figure 7.2.

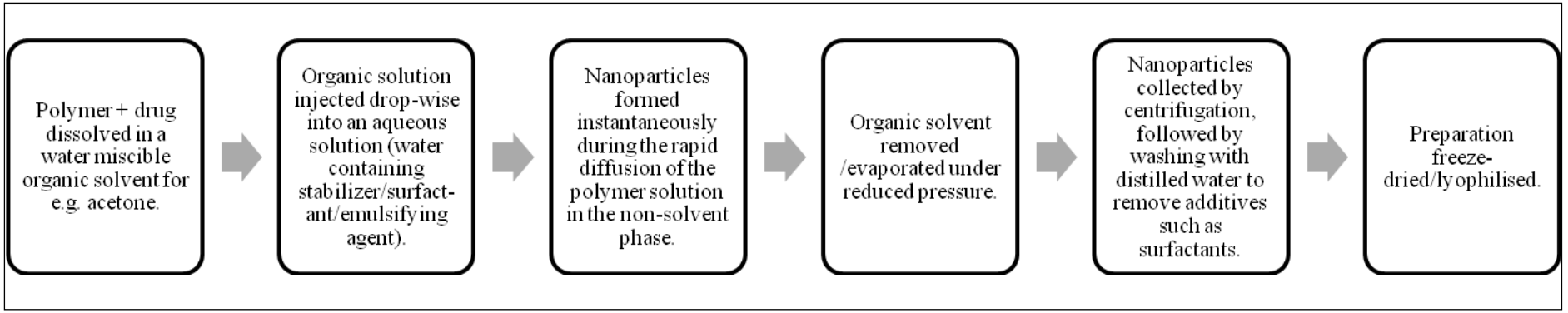


Figure 7.1 A flow chart detailing the conventional nanoprecipitation/solvent-displacement method of preparing polymeric nanoparticles.

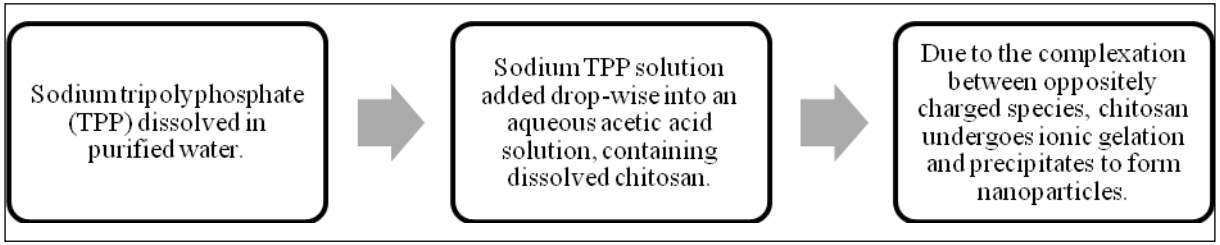


Figure 7.2 A flow chart detailing the ionic gelation method of preparing chitosan nanoparticles.

The aim of this chapter was to incorporate polymeric biodegradable nanoparticles in freeze-dried ODTs using novel methods developed from the nanoprecipitation/solvent-displacement method (shown in Figure 7.1) and ionic gelation method (shown in Figure 7.2), respectively.

The new method, based on the nanoprecipitation/solvent-displacement process, which allows the incorporation of polymeric nanoparticles directly in freeze-dried ODT formulations, is summarised as a flow chart in Figure 7.3. The method therefore combines the preparation of polymeric nanoparticles with the preparation of freeze-dried ODT formulations. As Figure 7.3 shows, the first step in the process involves the solubilisation of the nanoparticle-forming polymer and API in a water miscible organic solvent, *e.g.* acetone. The second step involves the addition of the organic phase to an HPMC or suitably based ODT formulation (aqueous phase), which contains a surfactant/emulsifying agent. Polymeric nanoparticles are formed instantaneously in the ODT formulation, due to the rapid diffusion of the polymer solution in the aqueous phase. The next stage involves the removal of the organic phase, which is evaporated under reduced pressure. The formulation is then dosed and freeze-dried, which results in the preparation of ODTs which consist of polymeric nanoparticles.

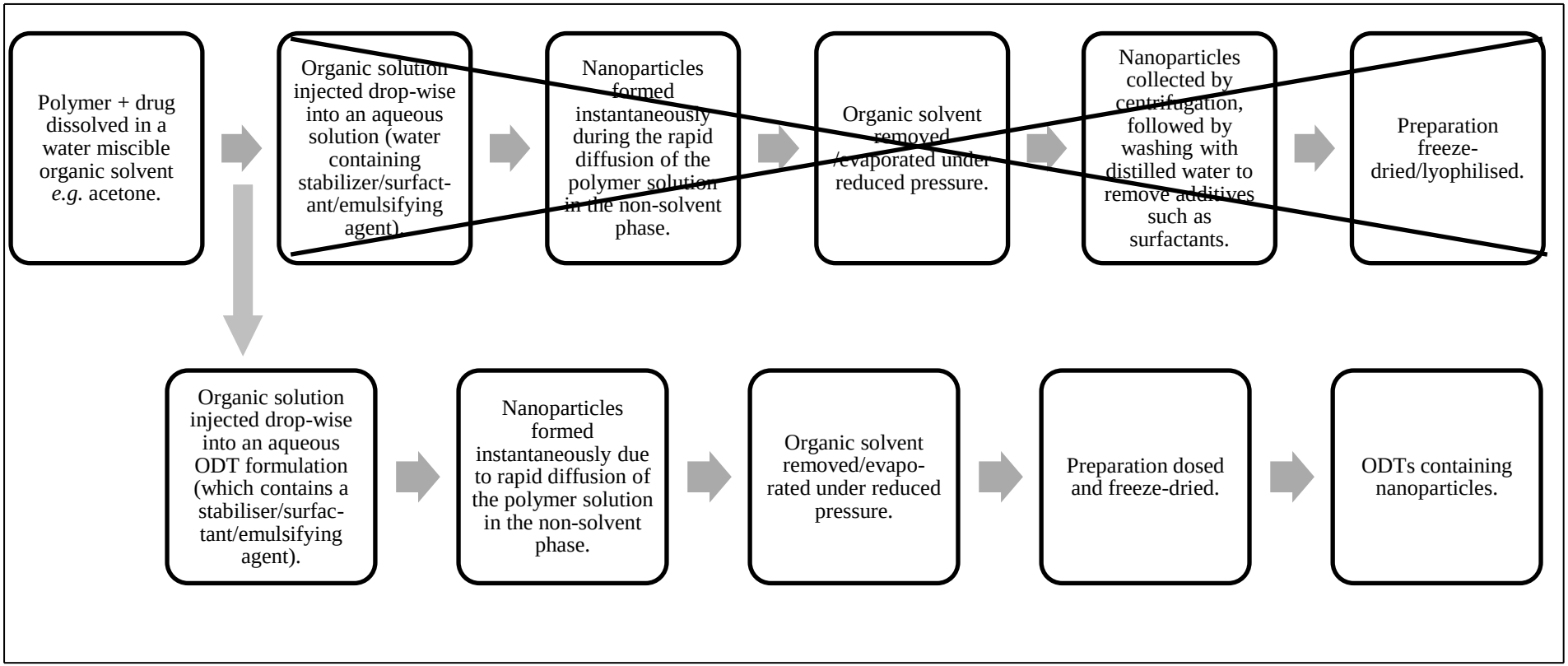


Figure 7.3 A flow chart detailing the modified nanoprecipitation/solvent-displacement method, which allows the direct incorporation of polymeric nanoparticles in freeze-dried ODT formulations.

The novel method, based on the ionic gelation technique, which allows the direct incorporation of chitosan nanoparticles in freeze-dried ODT formulations, is summarised as a flow chart in Figure 7.4. The method therefore combines the preparation of chitosan nanoparticles with the preparation of freeze-dried ODT formulations. As Figure 7.4 shows, the first step involves the solubilisation of sodium tripolyphosphate in HPMC or suitably based ODT formulation. This solution is then added to an aqueous acetic acid solution, which contains dissolved chitosan. Chitosan nanoparticles are formed due to the complexation between oppositely charged species. The formulation is then dosed and freeze-dried, which results in the preparation of ODTs which consist of chitosan nanoparticles.

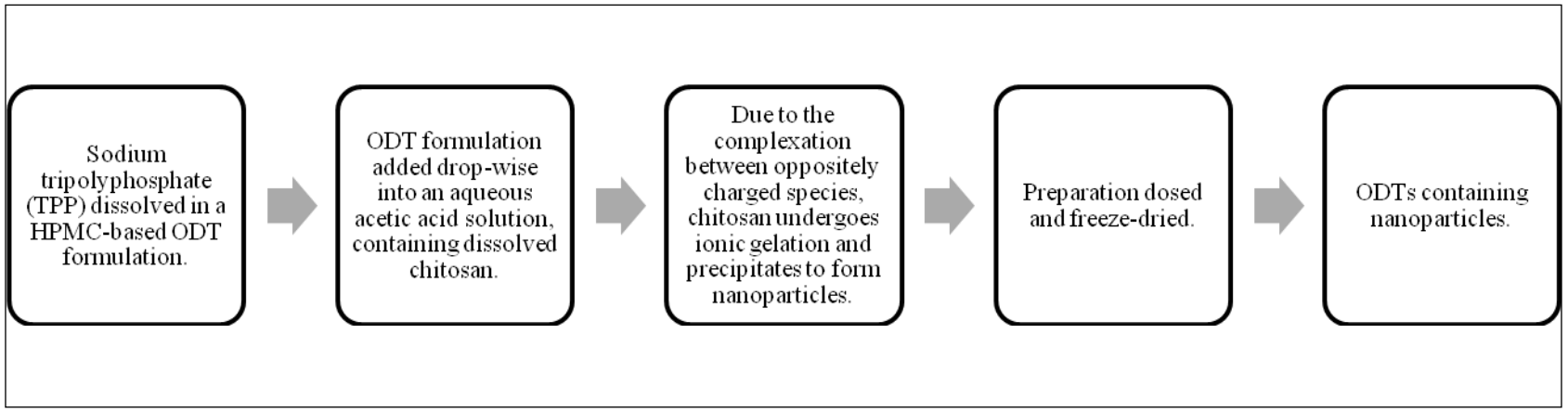


Figure 7.4 A flow chart detailing the modified ionic gelation method, which allows the direct incorporation of chitosan nanoparticles in freeze-dried ODT formulations.

7.2 Materials

Polyethylene oxide (Polyox™ WSR N10, Lot No.: DT353617) and hydroxypropyl methylcellulose (Methocel™ E3LV, Lot No.: DT220342) were supplied by Colorcon Ltd. (Dartford, UK). The following materials were supplied by Sigma-Aldrich (Poole, UK): polycaprolactone (PCL), chitosan (medium molecular weight), Pluronic® F127, sodium tripolyphosphate, poly(DL-lactide-*co*-glycolide) (50:50) (PLGA), mannitol and gelatin (type B, 75 bloom). The following materials were supplied by Fisher Scientific (Loughborough, UK): acetone, acetic acid, hydrochloric acid and methanol. The APIs: rizatriptan benzoate and piroxicam, were supplied by Discovery Fine Chemicals (Wimborne, UK) and Scientific Laboratory Supplies Ltd. (Nottingham, UK), respectively.

7.3 Methods

7.3.1 Preparation of Freeze-Dried Tablets Consisting of Nanoparticles

For the preparation of Methocel™ E3LV-based formulations, mannitol was firstly dissolved in half the required amount of water which was then heated to around 90ºC under stirring, followed by the addition of HPMC. Cold water was then added to solubilise the HPMC and form the final solutions.

For the preparation of the optimised formulation from the DOE study, firstly, the required amount of mannitol and polyethylene oxide were added to half the required amount of water (at ambient temperature) under stirring, until fully dissolved. These solutions were then heated to around 90ºC under stirring, followed by the addition of HPMC. Cold water was then added to solubilise the HPMC and form the final solutions.

For the preparation of gelatin-based formulations, gelatin was dissolved in double-distilled water at about 40°C under stirring, followed by the addition of mannitol to form a solution.

In order to prepare the formulations consisting of PCL or PLGA nanoparticles, the required amounts of PCL or PLGA was added to the required volume of acetone, and was dissolved with the aid of a sonicator. This organic solution was added drop-wise into the appropriate ODT formulation which consisted of Pluronic® F127, under magnetic stirring. The organic solvent was then removed from the preparation under reduced pressure.

In order to prepare the formulations consisting of chitosan nanoparticles, the required amount of sodium tripolyphosphate was firstly dissolved in the appropriate ODT formulation. The ODT formulation was then added drop-wise into an acetic acid solution consisting of dissolved chitosan under magnetic stirring.

1.5g of the resulting preparations were dosed into a tablet mould, frozen at -70°C for a minimum of sixty minutes and freeze-dried (ADVANTAGE, Freeze-Dryer, VIRTIS), according to the following regime; primary drying for forty eight hours at a shelf temperature of -40°C, secondary drying for ten hours at a shelf temperature of 20°C, and vacuum pressure of 6.67 Pa (50 mTorr).

Using the novel method described in Figure 7.3, the following formulations were prepared in order to incorporate PCL and PLGA nanoparticles:

- 5 % w/w Methocel™ E3LV with 30 % w/w (of dried tablet weight) mannitol.
- 10 % w/w Methocel™ E3LV with 30 % w/w (of dried tablet weight) mannitol.
- 5 % w/w gelatin with 30 % w/w (of dried tablet weight) mannitol.
- Optimised formulation from the DOE study.

These formulations were selected in order to establish if the method shown in Figure 7.3 could be applied with a range of different formulations, i.e. HPMC and gelatin-based, to successfully form and incorporate PCL and PLGA nanoparticles.

7.3.2 Tablet Hardness

The hardness of the tablets was investigated using a texture analyzer (QTS 25: Brookfield, Essex, UK) equipped with a 25 kg load cell. The instrument was calibrated by standard weights of 500 g and 5 kg. The tablets were placed in a holder with a cylindrical hole. The hardness was taken as the peak force after 1 mm penetration of a 5 mm diameter probe at a rate of 6 mm/min. Three measurements were taken for each formulation, and the mean ± standard deviation is reported.

7.3.3 Disintegration Time of the Tablets

The disintegration time of the tablets was determined using a USP disintegration tester (Erweka, ZT3). 800 mL of double distilled water, which was kept at 37 ± 2ºC, was used as the medium and the basket was raised and lowered at a fixed rate of 30 rpm. Three tablets were evaluated from each formulation, and the mean values ± standard deviation is reported.

7.3.4 Particle Size and Zeta Potential (Particle Charge) Analysis

Particle sizes of the formulations were determined using a ZetaPlus particle size and zeta potential analyser (Brookhaven Instruments Corporation, New York, USA). 50 µL of sample of each formulation was added to 1.5 mL of double-distilled water in a cuvette, and analysed for particle size. The reported particle size values are reported as the mean ± standard error of three measurements.

Zeta potential of the formulations was determined using a ZetaPlus particle size and zeta potential analyser (Brookhaven Instruments Corporation, New York, USA). 50 µL of sample of each formulation was added to 1.5 mL of double-distilled water in a cuvette, followed by the insertion of a zeta potential probe. Analysis of the zeta potential of the formulations was then carried out. The reported zeta potential values are reported as the mean ± standard error of 10 measurements.

7.3.5 *In Vitro* Tablet Dissolution Testing

The *in vitro* dissolution testing of rizatriptan benzoate tablets was carried out using dissolution apparatus 2 (paddle apparatus) (Erweka DT600 dissolution bath, Dorset, UK). Dissolution testing was performed at 37ºC, in a medium consisting of 900 mL water (deaerated), with the paddle rotating at 50 rpm. Samples were withdrawn at 5 minute intervals up to 30 minutes, and were analysed by UV spectroscopy (Jenway 6405 UV/Vis. Spectrophotometer, Stone, UK) at 225 nm. The samples were then immediately returned to the dissolution vessel, in order to maintain sink conditions. Three tablets were analysed for both the nanoparticle-free and nanoparticulate formulations, and the mean ± standard deviation values are reported.

In terms of the *in vitro* dissolution testing of piroxicam tablets, this too was performed using dissolution apparatus 2 (paddle apparatus). Dissolution testing was performed at 37ºC, in a medium consisting of 900 mL 0.1 M hydrochloric acid, with the paddle rotating at 50 rpm. Samples were withdrawn at 5 minute intervals up to 90 minutes, and were analysed by UV spectroscopy (Jenway 6405 UV/Vis. Spectrophotometer, Stone, UK) at 242 nm. The samples were then immediately returned to the dissolution vessel, in order to maintain sink conditions. Three tablets were analysed for both the nanoparticle-free and nanoparticulate formulations, and the mean ± standard deviation values are reported.

7.3.6 Determination of Drug Entrapment Efficiency and Drug Recovery/Dose Uniformity

The drug entrapment efficiency of rizatriptan benzoate nanoparticulate formulations was determined, by firstly reconstituting a tablet with double-distilled water. This nanosuspension underwent centrifugation (Beckman Coulter Avanti® J-E Centrifuge, High Wycombe, UK) at 15,000 rpm for 30 minutes, in order to separate the entrapped and non-entrapped drug. The resulting supernatant containing the dissolved free drug was analysed by UV spectroscopy (Jenway 6405 UV/Vis. Spectrophotometer, Stone, UK) at 225 nm. The drug entrapment efficiency was determined using Equation 7.1. Three tablets were analysed and the mean values ± standard deviation are reported.

$$\text{Drug Entrapment Efficiency} = \frac{\text{Total Amount of Drug} - \text{Free Amount of Drug}}{\text{Total Amount of Drug}} \times 100$$

(Equation 7.1)

The drug entrapment efficiency of piroxicam nanoparticulate formulations was determined, by firstly reconstituting a tablet with 0.1 M HCl. This nanosuspension underwent centrifugation (Beckman Coulter Avanti® J-E Centrifuge, High Wycombe, UK) at 15,000 rpm for 30 minutes, in order to separate the entrapped and non-entrapped drug. The resulting supernatant containing the dissolved free drug was analysed by UV spectroscopy (Jenway 6405 UV/Vis. Spectrophotometer, Stone, UK) at 242 nm. The entrapment efficiency was determined using Equation 7.1. Three tablets were analysed and the mean values ± standard deviation are reported.

The drug recovery/dose uniformity of piroxicam tablets was determined by dissolving a tablet in 50 mL of methanol. A sample was taken and analysed by UV spectroscopy (Jenway 6405 UV/Vis. Spectrophotometer, Stone, UK) at 333 nm. Three tablets were analysed and the mean values ± standard deviation are reported.

7.3.7 Stability Study of Piroxicam Tablets

The stability of piroxicam tablets (both nanoparticle-free and nanoparticle-containing tablets) were assessed over a period of 45 days in conditions of; 25ºC/60% RH and 40ºC/75% RH, respectively. At time points of day; 0, 30 and 45, tablets were removed from the Firlabo SP-BVEHF (Meyzieu, France) stability cabinets and analysed. At each time point, piroxicam nanoparticle-free tablets were analysed for; general appearance,

drug recovery/dose uniformity, tablet hardness and tablet disintegration time. Whilst for piroxicam nanoparticle-containing tablets, at each time point the tablets were examined for; general appearance, tablet hardness, tablet disintegration time, and the particle size and zeta potential of the nanoparticles. During the stability study, the tablets were packaged in 100 mL amber coloured polyethylene terephthalate (PET) screw-cap bottles.

7.3.8 Statistical Analysis

Statistical analyses were performed using GraphPad Prism 5 statistical analysis software. One-way analysis of variance (ANOVA) was the statistical analysis test performed, using the Tukey post test. A significance level of $p < 0.05$ (95% confidence interval) was judged as being statistically significant.

7.4 Results and Discussion

7.4.1 Incorporating PCL and PLGA Nanoparticles in Freeze-Dried ODTs

The prepared tablets for both PCL and PLGA nanoparticles, were characterised for hardness and disintegration time. The particle size and zeta potential of the nanoparticles of the various formulations were also determined, following reconstitution of the tablets. The characterisation results of the formulations are shown in Table 7.1.

Table 7.1 Characterisation results for the four formulations, for both PCL and PLGA nanoparticle-containing formulations. The results shown are the mean values ± standard deviation, following three measurements. The zeta potential results shown are the mean values ± standard error, following ten measurements.

Formulation	Particle Size (nm)	Zeta Potential (mV)	Tablet Hardness (Newtons, N)	Tablet Disintegration Time (seconds, s)
5% Methocel with 30% Mannitol – PCL Nanoparticles	291.6 ± 10.5	-2.73 ± 2.97	0.90 ± 0.37	66.00 ± 9.54
10% Methocel with 30% Mannitol – PCL Nanoparticles	417.5 ± 67.5	-3.96 ± 2.51	8.23 ± 3.50	55.56 ± 23.71
5% Gelatin with 30% Mannitol – PCL Nanoparticles	197.9 ± 31.3	-7.04 ± 1.70	9.29 ± 5.39	76.33 ± 47.14
Optimised Formulation from the DOE Study – PCL Nanoparticles	383.5 ± 129.3	-1.70 ± 1.36	5.48 ± 2.63	65.33 ± 17.44
5% Methocel with 30% Mannitol – PLGA Nanoparticles	248.4 ± 36.7	-12.14 ± 3.76	0.98 ± 0.07	54.55 ± 25.55
10% Methocel with 30% Mannitol – PLGA Nanoparticles	220.4 ± 55.8	-3.36 ± 7.01	8.18 ± 0.80	103.78 ± 70.32
5% Gelatin with 30% Mannitol – PLGA Nanoparticles	211.3 ± 54.8	-8.74 ± 1.39	7.67 ± 2.38	82.22 ± 9.15
Optimised Formulation from the DOE Study – PLGA Nanoparticles	396.7 ± 24.2	-3.06 ± 0.94	5.24 ± 2.37	86.33 ± 40.71

The characterisation results for the four formulations, for both PCL and PLGA nanoparticle-containing preparations indicated that the new method (shown in Figure 7.3) successfully produced ODTs which consisted of polymeric nanoparticles. Intact and robust tablets were formulated using this method. In terms of the tablets consisting of PCL nanoparticles, between the four formulations, they exhibited hardness and disintegration time which ranged from 0.90 ± 0.37 to 9.29 ± 5.39 N and 55.56 ± 23.71 to 76.33 ± 47.14 s, respectively.

Reconstituting these tablets revealed that the nanoparticles exhibited a particle size which ranged in size from 197.9 ± 31.3 to 417.5 ± 67.5 nm, between the four formulations. In terms of the zeta potential of the nanoparticles, between the four formulations, values ranged from -1.70 ± 1.36 to -7.04 ± 1.70 mV.

For the formulations consisting of PLGA nanoparticles, the tablets ranged in hardness from 0.98 ± 0.07 to 8.18 ± 0.80 N, between the four formulations. In terms of tablet disintegration time, the values ranged from 54.55 ± 25.55 to 103.78 ± 70.32 s.

Reconstituting the tablets which consisted of PLGA nanoparticles, revealed that the nanoparticles exhibited particle size and zeta potential which ranged from 211.3 ± 54.8 to 396.7 ± 24.2 nm and -12.14 ± 3.76 to -3.06 ± 0.94 mV, respectively, between the four formulations.

The results shown in Table 7.1 revealed that with all four ODT formulations, which included both PCL and PLGA nanoparticles, the nanoparticles exhibited particle sizes in the nanometre range. This can be attributed to the rapid diffusion of the organic phase into the ODT formulations, which decreases the interfacial tension between these two components (Rao and Geckeler, 2011). This subsequently increases the surface area of the organic phase, which forms small droplets of organic solvent leading to the precipitation of PCL and PLGA resulting in nanoparticle formation (Rao and Geckeler, 2011).

A number of formulation parameters can influence the particle size of nanoparticles, such as; stabiliser concentration (Hans and Lowman, 2002), polymer concentration, molecular weight of the polymer and the method of organic phase addition into the aqueous phase (Rao and Geckeler, 2011). As a result, the particle size of nanoparticles can be controlled by adjusting these parameters accordingly.

The novel method (shown in Figure 7.3) has shown to successfully form nanoparticles in the four formulations studied. The four formulations have shown to be compatible with this method, as the organic phase is capable of diffusing rapidly into the formulations, and subsequently form nanoparticles.

Evaluating the zeta potential of the nanoparticles of the four formulations, which included both PCL and PLGA nanoparticles, revealed that the zeta potential of the nanoparticles were near neutral in charge. The zeta potential of nanoparticles is related to the nanoparticle-forming polymer. PCL is non-ionic in nature and therefore neutral charged nanoparticles were expected to be formed. However as PLGA is anionic, the zeta potential of the prepared nanoparticles was expected to be more negative in nature. In order to modify the zeta potential of PLGA nanoparticles, adjusting the concentration of the nanoparticle-forming polymer would be one formulation approach.

7.4.2 Incorporating Chitosan Nanoparticles in Freeze-Dried ODTs

Using the method described in Figure 7.4, the following formulations were prepared, in order to incorporate chitosan nanoparticles:

- 5 % w/w Methocel™ E3LV with 30 % w/w (of dried tablet weight) mannitol.
- 10 % w/w Methocel™ E3LV with 30 % w/w (of dried tablet weight) mannitol.
- 5 % w/w gelatin with 30 % w/w (of dried tablet weight) mannitol.
- Optimised formulation from the DOE study.

As with the formulations consisting PCL and PLGA nanoparticles, these formulations were selected in order to establish if the method shown in Figure 7.4 could be applied to a range of different formulations, to successfully form and incorporate chitosan nanoparticles.

Of the four formulations which were attempted, only the 5% w/w Methocel™ formulation proved successful in preparing tablets. The prepared tablets of this formulation were intact, which exhibited sufficient robustness to withstand manual handling, which were easily removable from the tablet moulds.

In terms of the 10% w/w Methocel™ formulation and the optimised formulation from the DOE study, these formulations proved too viscous to be added drop-wise into the

aqueous acetic acid solution containing dissolved chitosan, and therefore the final formulations could not be prepared.

The 5% w/w gelatin formulation was not deemed suitable for this particular application due to the acidic nature of the preparation method (as chitosan is dissolved aqueous acetic acid), and the instability of gelatin in acidic conditions (Lund, 1994 and Northrop, 1921).

The prepared tablets of the 5% w/w Methocel™ formulation were characterised in terms of hardness and disintegration time. The particle size and zeta potential of the nanoparticles were also studied, following reconstitution of the tablets.

In terms of hardness, the tablets exhibited a mean hardness of 2.34 ± 0.20 N. Tablet disintegration time analysis showed that the tested tablets did not disintegrate up to three minutes of testing possibly due to the water-insoluble nature of chitosan (Agnihotri et al. 2004). Consequently, the tablets could not be reconstituted in order to evaluate the particle size and zeta potential of the chitosan nanoparticles. However, prior to dosing and freeze-drying the tablets, the particle size and zeta potential of the nanoparticles were evaluated. The mean particle size of the nanoparticles was 428.7 ± 23.4 nm, which displayed a mean zeta potential of 9.01 ± 1.27 mV.

7.4.3 Incorporation of Rizatriptan Benzoate Nanoparticles in Freeze-Dried ODTs

Having successfully developed a new method of preparing ODTs with *in situ* formation of nanoparticles and its suitability to a wide range of formulation excipients, the next phase of the investigation was to determine the impact of drug loading on formulation and release kinetics from the dosage form.

Rizatriptan benzoate is a 5-hydroxytryptamine$_1$ ($5HT_1$) receptor agonist, which is used in the treatment of acute migraine (Joint Formulary Committee, 2003), and is available as a freeze-dried ODT dosage form, under the name Maxalt-MLT® (which is available as a 5 or 10 mg dose).

In this particular study, rizatriptan benzoate was encapsulated in PCL nanoparticles, in order to ascertain the influence of inclusion of excipients for nanoparticle preparation and to provide a sustained/extended release/dissolution of the API. Both, nanoparticle-containing and nanoparticle-free tablets were prepared (both at doses of 5 mg, in 10%

w/w Methocel™-based formulations), characterised and their dissolution profiles compared.

Following the preparation of the nanoparticle-containing tablets, the tablets were characterised for encapsulation-efficiency of the API, particle size and zeta potential of the nanoparticles, and the dissolution profile of the API.

The encapsulation-efficiency of the API was calculated as being 1.8 %. This low encapsulation-efficiency can be attributed to the highly water-soluble nature of the API (Avachat et al. 2012). It has been widely reported that it is difficult to encapsulate hydrophilic APIs using the nanoprecipitation method of preparing nanoparticles (Peltonen et al. 2004, Govender et al. 1999 and Barichello et al. 1999). A number of reasons have been reported for the poor encapsulation of hydrophilic APIs. Peltonen et al. (2004) reported the low affinity the hydrophilic API has for the nanoparticle-forming polymer which results in low diffusion of the drug into the polymeric network. Peltonen et al. (2004) additionally reported that interactions between the polymer and API are weak, and that the API has a tendency to move from the organic phase to the aqueous phase. Govender et al. (1999) reported that the poor encapsulation of water-soluble APIs is due to the rapid migration of the APIs into the aqueous phase. Reconstitution of the nanoparticle-containing tablets revealed that the nanoparticles exhibited a mean particle size of 264.8 ± 11.4 nm, with a mean zeta potential of -3.68 ± 0.48 mV.

Figure 7.5, shows the dissolution profile of rizatriptan benzoate from nanoparticle-containing and nanoparticle-free tablets.

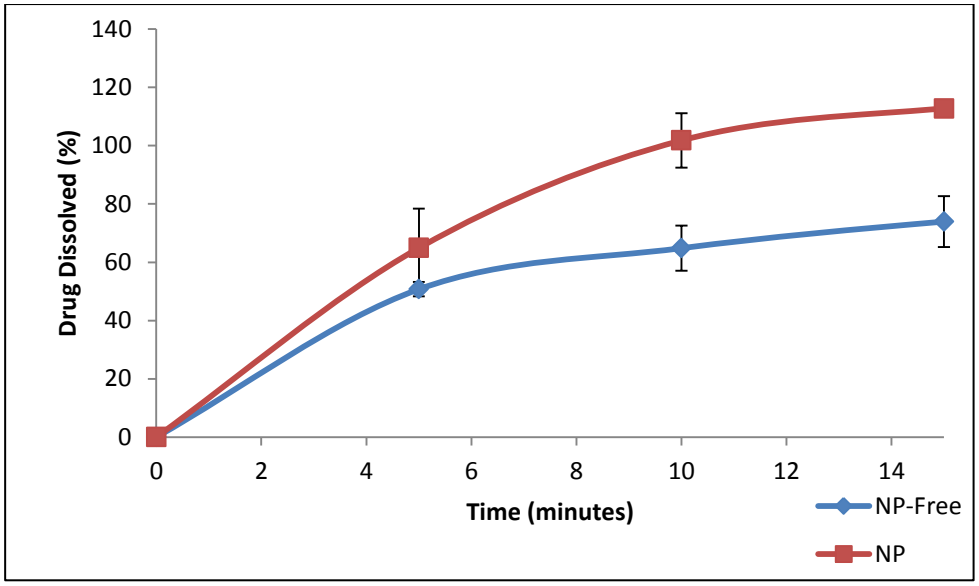


Figure 7.5 A comparison of the dissolution profile of rizatriptan benzoate from nanoparticle-free (NP-Free, shown in blue) and nanoparticle-containing (NP, shown in red) tablets. Each formulation was tested three times and the mean values ± standard deviation is shown.

The results show that after 10 minutes of dissolution testing, 101.81 ± 9.33% of rizatriptan benzoate dissolved from the nanoparticle-containing tablet (NP), whilst for the nanoparticle-free tablet (NP-Free), only 64.83 ± 7.74% of the API was released. The results therefore revealed that although it was hoped that attempting to encapsulate the API in nanoparticles would result in sustained/extended release/dissolution of the API, it was actually observed that the nanoparticle-containing tablets displayed improved/increased drug dissolution, compared to the nanoparticle-free tablets.

In an attempt to modify the release/dissolution of the API, the next stage of the investigation was focussed on increasing the encapsulation-efficiency of the API. The effects of several formulation parameters have been previously investigated/reported, in an effort to increase the encapsulation-efficiency of hydrophilic APIs using the nanoprecipitation method, such as; amount of model API, solvent selection, electrolyte addition, and pH modification (Peltonen et al. 2004). Similarly, Govender et al. (1999) reported investigating aqueous phase pH, changing the form of the API and the inclusion of fatty-acid excipients in the formulation to promote loading of hydrophilic drug candidates

Peltonen et al. (2004) reported that pH adjustment was the most effective way of increasing drug loading of sodium cromoglycate from 10-15% (without pH change) to

70% (following pH adjustment). Furthermore, Govender et al. (1999) reported that modifying pH from 5.8 to 9.3 increased drug entrapment from 11.0 to 58.2%.

In this particular study, the pH of the aqueous phase of the formulation was adjusted from 8.07 to 10.48, in an effort to increase the encapsulation of rizatriptan benzoate. The prepared pH adjusted nanoparticle-containing tablets were characterised for; encapsulation efficiency of the API, tablet hardness, tablet disintegration time, particle size and zeta potential of the nanoparticles, and the dissolution profile of the API. The nanoparticle-free tablets were characterised for; tablet hardness, tablet disintegration time, and the dissolution profile of the API.

Evaluating the encapsulation-efficiency of the API from the pH adjusted nanoparticle-containing tablets, the results revealed a mean encapsulation-efficiency of 3.30 ± 4.10%. This value therefore showed that adjusting the pH of the aqueous phase during the preparation of the formulation did not result in increasing the encapsulation-efficiency of the API, when compared to the original nanoparticle-containing formulation. This low value suggested that the API migrates rapidly into the aqueous phase, when introduced into water, during the determination of encapsulation-efficiency (Govender et al. 1999).

Assessing the hardness of the tablets from the pH adjusted nanoparticle-containing formulation, revealed a mean value of 11.60 ± 2.53 N, whilst the nanoparticle-free tablets had a hardness value of 13.70 ± 1.83 N ($p > 0.05$).

The assessment of the dissolution profile of rizatriptan benzoate from pH adjusted nanoparticle-containing tablets, is shown below in Figure 7.6, and is compared with the dissolution profile of the API from nanoparticle-free tablets.

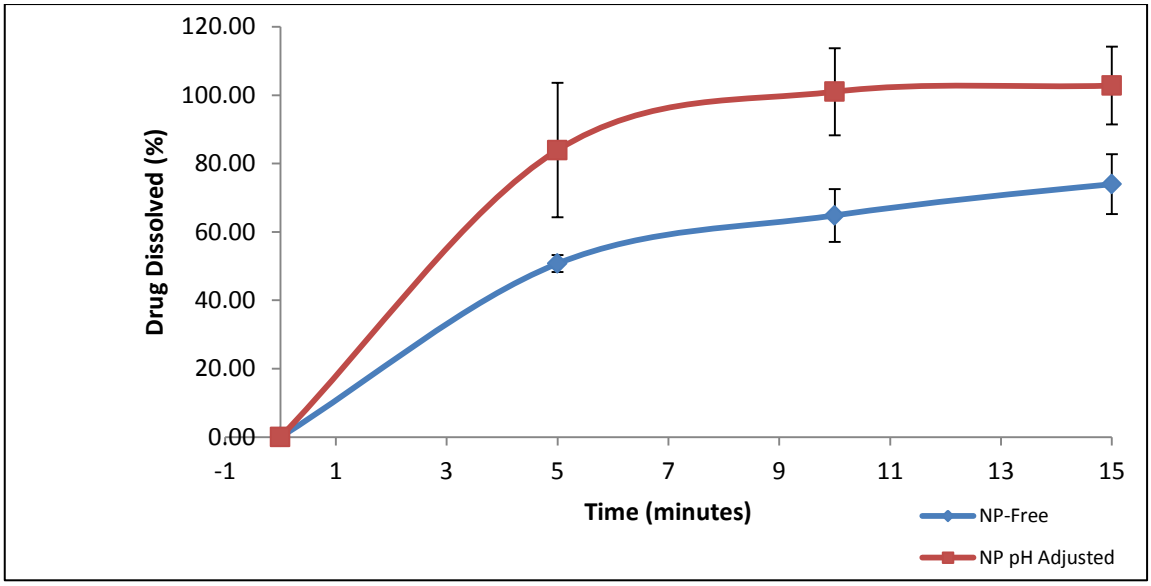


Figure 7.6 A comparison of the dissolution profile of rizatriptan benzoate from nanoparticle-free (NP-Free, shown in blue) and pH adjusted nanoparticle-containing (NP pH Adjusted, shown in red) tablets. Each formulation was tested three times and the mean values ± standard deviation is shown.

The results show that after 10 minutes of dissolution testing, 101.01 ± 12.72% of the API was released from the pH adjusted nanoparticle-containing tablets, whilst only 64.83 ± 7.74% of the API dissolved from the nanoparticle-free tablets. These results are in agreement with the dissolution results shown in Figure 7.5, where it was also shown that rizatriptan benzoate displayed increased dissolution from nanoparticle-containing tablets, in comparison with nanoparticle-free tablets.

The increased dissolution of rizatriptan benzoate from the nanoparticle-containing tablets, in comparison with the nanoparticle-free tablets, could be due to the presence of Pluronic® in the nanoparticle-containing tablet formulations. Pluronic®, which is a synonym for poloxamer, is a surfactant composed of a block copolymer of ethylene oxide and propylene oxide, and has applications in pharmaceutical formulations as a dispersing, emulsifying and solubilising agent (Rowe, 2012). Pluronic® was therefore incorporated in the nanoparticle-containing tablet formulations, to function as a surfactant, in the preparation of the nanoparticles.

It is possible that the observed increase in dissolution of rizatriptan benzoate from the nanoparticle-containing tablets, compared to the nanoparticle-free tablets, could be attributed to the dispersing effect of Pluronic®. The dispersing effect of Pluronic® could also explain why the tablets of the pH adjusted nanoparticle-containing formulation, exhibited a shorter disintegration time than the tablets of the nanoparticle-free

formulation. Tablets from the nanoparticle-free formulation displayed a disintegration time of 79.33 ± 14.01 s, whilst tablets from the pH adjusted nanoparticle-containing formulation displayed a disintegration time of 47.67 ± 5.86 s ($p < 0.05$).

7.4.4 Incorporation of Piroxicam Nanoparticles in Freeze-Dried ODTs

Piroxicam is a non-steroidal anti-inflammatory drug, which is used in the treatment of rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis (Joint Formulary Committee, 2003). It is available as a freeze-dried ODT dosage form, under the name Feldene™ Melt, which is available as a 20 mg dose.

In this particular study, piroxicam was studied for encapsulation in PCL nanoparticles, in order to assess the sustained/extended release/dissolution of the API. Both, nanoparticle-containing and nanoparticle-free tablets were prepared (both at doses of 20 mg, in 10% w/w Methocel™-based formulations), in order to compare their dissolution profiles of the API.

The encapsulation-efficiency of the API was calculated as 96.83 ± 1.61 %. This high encapsulation-efficiency of piroxicam was observed, as the nanoprecipitation/solvent displacement method is applicable to lipophilic APIs, due to the miscibility of the organic solvent (in which piroxicam is soluble in) with the aqueous phase (in which piroxicam is practically insoluble in) (Barichello et al. 1999). Reconstitution of the nanoparticle-containing tablets, revealed that the nanoparticles exhibited a mean particle size of 898.8 ± 501.7 nm, with a mean zeta potential of 4.32 ± 1.85 mV.

Figure 7.7 (below), shows the dissolution profile of piroxicam from both nanoparticle-containing and nanoparticle-free tablets.

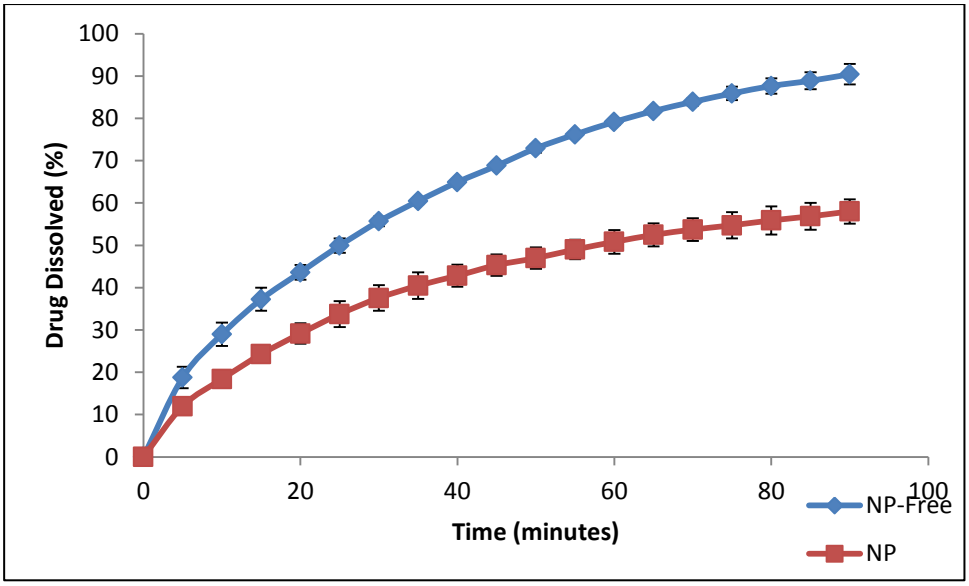


Figure 7.7 A comparison of the dissolution profile of piroxicam from nanoparticle-free (NP-Free, shown in blue) and nanoparticle-containing (NP, shown in red) tablets. Each formulation was tested three times, and the mean values ± standard deviation is shown.

The results revealed that after 90 minutes of dissolution testing, 90.40 ± 2.43% of piroxicam dissolved from the nanoparticle-free tablets, whilst for the nanoparticle-containing tablets, only 58.00 ± 2.86% of the API dissolved. The results therefore showed that a sustained/extended release/dissolution of piroxicam was achieved, by incorporating piroxicam nanoparticles in freeze-dried ODTs.

Tablet hardness and disintegration time of both formulations were also evaluated. In terms of hardness, tablets from the nanoparticle-free formulation displayed a value of 12.34 ± 0.69 N, which was comparable to the hardness of the tablets from the nanoparticle-containing formulation, 12.34 ± 1.52 N ($p > 0.05$). These comparable results indicated that preparing nanoparticle-containing tablets using the method shown in Figure 3 did not compromise tablet hardness.

Investigating tablet disintegration time, the results showed that tablets with nanoparticle-free formulation exhibited a value of 75.00 ± 13.34 s, whilst tablets from the nanoparticle-containing formulation displayed a value of 38.00 ± 3.61 s ($p < 0.05$). These results therefore showed that freeze-dried ODTs consisting of polymeric nanoparticles, displayed a shorter disintegration time than the conventional nanoparticle-free freeze-dried ODTs.

7.5 Stability Study of Piroxicam Tablets

Following piroxicam demonstrating a sustained/extended release/dissolution by incorporating its nanoparticles in freeze-dried ODTs, both nanoparticle-free and nanoparticle-containing tablets of this API underwent a 45 day stability assessment to assess their stability.

The results from the stability study for the nanoparticle-free piroxicam tablets are shown in Table 7.2. The results revealed that no significant change in drug recovery/dose uniformity occurred with the tablets stored at 25ºC/60% RH ($p > 0.05$). However, a significant change was observed with the tablets stored at 40ºC/75% RH, as the drug recovery/dose uniformity observed after 45 days, was significantly different to that observed at 0 and 30 days, respectively ($p < 0.05$). This could suggest that piroxicam was unstable in these conditions, due to the high temperature and high humidity of the storage conditions. The results from the stability study for the nanoparticle-containing piroxicam tablets are shown in Table 7.3.

Table 7.2 Stability study results of the characterisation of the nanoparticle-free piroxicam tablets, stored at 25ºC/60% RH and 40ºC/75% RH, respectively. The results shown are the mean values ± standard deviation, following three measurements.

	Time Point (Days)					
	0		30		45	
Parameter	**25ºC/75 % RH**	**40ºC/75 % RH**	**25ºC/75 % RH**	**40ºC/75 % RH**	**25ºC/75 % RH**	**40ºC/75 % RH**
Drug Recovery/Dose Uniformity (%)	92.71 ± 5.09	90.21 ± 4.69	101.04 ± 18.31	93.13 ± 4.37	90.42 ± 3.77	107.50 ± 3.80
Tablet Hardness (N)	18.34 ± 3.13	15.74 ± 1.47	9.37 ± 2.30	10.80 ± 0.91	12.41 ± 2.97	6.62 ± 0.39
Tablet Disintegration Time (s)	55.33 ± 20.60	61.00 ± 6.00	34.00 ± 6.56	42.33 ± 5.69	166.33 ± 184.87	81.33 ± 84.68

Table 7.3 Stability study results of the characterisation of the nanoparticle-containing piroxicam tablets, stored at 25ºC/60% RH and 40ºC/75% RH, respectively. The results shown are the mean values ± standard deviation, following three measurements. The zeta potential results shown are the mean values ± standard error, following ten measurements.

	Time Point (Days)					
	0		30		45	
Parameter	**25ºC/60 % RH**	**40ºC/75 % RH**	**25ºC/60 % RH**	**40ºC/75 % RH**	**25ºC/60 % RH**	**40ºC/75 % RH**
Tablet Hardness (N)	19.37 ± 2.76	15.93 ± 5.32	7.73 ± 1.67	7.00 ± 3.73	16.95 ± 1.11	9.42 ± 1.85
Tablet Disintegrati-on Time (s)	41.33 ± 0.58	45.00 ± 3.61	10.67 ± 1.53	9.67 ± 0.58	14.67 ± 1.53	14.33 ± 1.15
Particle Size (nm)	727.3 ± 127.5	715.2 ± 119.0	615.9 ± 32.9	397.3 ± 58.8	472.0 ± 26.1	508.4 ± 25.7
Zeta Potential (mV)	-4.70 ± 0.90	-4.39 ± 1.16	-11.05 ± 2.12	-3.00 ± 1.04	-9.43 ± 0.63	-11.17 ± 2.25

In terms of tablet hardness, the results revealed that this property changed significantly during the 45 days of stability assessment, for both the tablets stored at 25ºC/60% RH and 40ºC/75% RH, respectively ($p < 0.05$). As with the tablets stored at 25ºC/60% RH, the hardness of the tablets after 0 days were significantly greater to those after 30 days ($p < 0.05$). Whilst with the tablets stored at 40ºC/75% RH, the hardness of the tablets decreased over time ($p < 0.05$), suggesting deterioration in the physical stability of the tablets. The deterioration in the physical stability of the tablets over time could possibly be attributed to the high humidity, plasticising the tablet which results in the deterioration of the physical properties of the tablets. It has been reported previously that once tablets reach a certain level of moisture content, the physical properties of the tablets can deteriorate (Nakabayashi et al. 1980).

The tablet disintegration time results revealed that no significant change was observed after 45 days of storage, for the tablets stored at both conditions ($p > 0.05$).

No noticeable change in the general appearance of the nanoparticle-free tablets was observed, during and after the stability study, for the tablets stored at both conditions.

The results from the stability study for the nanoparticle-containing piroxicam tablets, shown in Table 7.3, revealed that in terms of the hardness of the tablets stored at 25ºC/60% RH, this property changed significantly during the 45 days of stability assessment ($p < 0.05$). The tablets analysed after 30 days of storage exhibited a significantly lower hardness than those analysed after 0 and 45 days, respectively ($p < 0.05$). As the hardness of the tablets did not deteriorate over time, i.e. the hardness of the tablets analysed after 45 days were not lower than those after 30 days, it appeared that there was no physical stability issue with these tablets.

No significant change in the hardness of the tablets stored at 40ºC/75% RH was observed after 45 days of stability assessment ($p > 0.05$).

The results for the disintegration time of the tablets stored at both storage conditions indicated that this property changed significantly during the 45 day stability study ($p < 0.05$) as the disintegration time of the tablets analysed after 30 and 45 days, respectively, were significantly lower than those analysed at day 0 ($p < 0.05$). As tablet disintegration testing involves human judgment to determine when a tablet has disintegrated, variability in the results will exist. For this reason, it appeared that the

significant changes observed with tablet disintegration time during the stability study, was not related to the stability of the tablets.

In relation to the particle size of the nanoparticles of the tablets stored at both storage conditions, this property displayed significant changes during the 45 days of stability assessment ($p < 0.05$) as the particle size of the nanoparticles of the tablets analysed after 45 days of storage, were significantly smaller than those analysed at day 0 ($p < 0.05$). As discussed earlier, one factor which influences the particle size of nanoparticles is the method of organic phase addition into the aqueous phase. As the preparation of these formulations was performed manually, the rate of organic phase addition was not constant. Consequently, variability in the particle size of the nanoparticles was expected. Therefore the significant changes observed with the particle sizes of the nanoparticles in the stability study, appeared not be related to the stability of the nanoparticles.

This could possibly be related to the zeta potential results, where it was found that analysing the zeta potential of the nanoparticles for the tablets stored at both storage conditions, revealed that this property changed significantly during the stability study ($p < 0.05$).

No noticeable change in the general appearance of the nanoparticle-containing tablets was observed, during and after the stability study, for the tablets stored at both conditions.

7.6 Conclusions

A novel method has been developed, which is based on the nanoprecipitation/solvent-displacement method of preparing polymeric nanoparticles, which allows the direct incorporation of polymeric nanoparticles in freeze-dried ODT formulations. The method was used to prepare PCL (non-ionic) and PLGA (anionic) nanoparticles, which were subsequently incorporated in both Methocel™ and gelatin-based freeze-dried ODT formulations, forming intact and robust tablets.

A novel method has also been developed, which is based on the ionic gelation method of preparing chitosan (cationic) nanoparticles, which allows the direct incorporation of these nanoparticles in freeze-dried ODT formulations. The new method produced chitosan nanoparticles, which were subsequently incorporated in a 5% w/w Methocel™-

based freeze-dried ODT formulation, forming intact tablets. However, due to the water-insoluble nature of chitosan, the prepared tablets did not show signs of disintegration during testing. A potential formulation strategy to overcome this, could involve investigating water-soluble forms of chitosan.

The APIs; rizatriptan benzoate (water-soluble) and piroxicam (practically insoluble in water), were encapsulated in PCL nanoparticles, in order to develop a sustained/extended release/dissolution of the API from ODTs. Due to the water-soluble nature of rizatriptan benzoate, a very low encapsulation-efficiency of the API was observed (< 5 %), and as a result a sustained/extended release/dissolution of the API was not observed. In fact an increase in drug dissolution was observed with the nanoparticle-containing formulation tablets, compared to the nanoparticle-free formulation tablets. Tablet hardness was comparable between the two formulations, and in terms of tablet disintegration time, nanoparticle-containing tablets exhibited a shorter disintegration time.

Piroxicam on the other hand had a high encapsulation-efficiency (> 95 %) and the dissolution results of the nanoparticle-containing tablets revealed a sustained/extended release/dissolution of the API, compared to the tablets of the nanoparticle-free formulation. Tablet hardness was comparable between the two formulations, and in terms of tablet disintegration time, nanoparticle-containing tablets exhibited a shorter disintegration time.

The stability study results for the nanoparticle-free piroxicam tablets revealed potential API and physical stability issues with the tablets stored at 40ºC/75% RH.

Chapter Eight: Summary and Implications of Research Findings

8.1 The Influence of Formulation and Manufacturing Process Parameters on the Characteristics of Lyophilised Orally Disintegrating Tablets

Gelatin is a widely-studied excipient used in the formulation of freeze-dried ODTs. Studies have shown that the swelling behaviour and solubility of gelatin are pH-dependent. Additionally, the swelling behaviour of gelatin is influenced by ionic strength. Despite the extensive literature on the properties of gelatin, no work has been reported previously which attempted to exploit its properties in order to modify the behaviour of freeze-dried ODTs. The aim of this study was to further understand the properties of gelatin, and its implications on the performance of freeze-dried ODTs.

The results from the study revealed that the disintegration time of gelatin-based freeze-dried ODTs can be reduced by adjusting the pH of the formulation. This was likely due to an increase in tablet porosity (possibly due to the swelling behaviour gelatin) and an improvement in gelatin solubility. Meanwhile, adjusting the ionic strength of gelatin-based formulations has shown to influence tablet porosity, again this was possibly attributed to the swelling behaviour of gelatin.

This study has shown than an appreciation and understanding of the properties of gelatin has allowed the formulation and process of preparing gelatin-based formulations to be modified, in order to exploit the properties of gelatin and subsequently adjust the characteristics of the tablets.

8.2 Investigating the Application of Methocel™ (Hydrophilic Cellulose Ethers) as Binders in Freeze-Dried ODTs

As discussed earlier, gelatin is used extensively in the preparation of freeze-dried ODTs. As gelatin is derived from animal-sources a number of ethical issues surround its use as an excipient in pharmaceutical preparations. Consequently, certain groups of the population restrict gelatin from their diet, including when gelatin is used in their medication. For this reason extensive research has been published, investigating alternative materials to use as a replacement to gelatin. Certain grades of Methocel™ have been reported to be used as binders in the preparation of freeze-dried ODTs, however, an extensive and comprehensive evaluation of the use of Methocel™ has not been previously reported.

The aim of this investigation therefore was to undertake a systematic and comprehensive evaluation of Methocel™ as a binder in freeze-dried ODTs. Both low

and high viscosity grades of Methocel™ were evaluated, together with both methylcellulose and HPMC grades.

The results from the study revealed that low viscosity grades, in particular; Methocel™ E3LV and K3LV, appeared most suitable as binders in freeze-dried ODTs, as high concentrations could be used to form robust tablets. It was found that increasing Methocel™ concentration resulted in a general increase in tablet hardness and disintegration time, respectively. The higher viscosity grades did not appear suitable as binders, as only low concentrations could be used, and as a result robust tablets were not prepared. Performing a systematic and comprehensive evaluation identified which grades were most suitable and also which concentrations were appropriate.

8.3 Investigating the Application of Polyox™ (Synthetic Polyethylene Oxide) in Freeze-Dried ODTs

Polyox™ is a highly versatile polymer, as it is available in a number of grades which differ in molecular weight. Consequently, Polyox™ has a number of applications in pharmaceutical preparations, such as a tablet binder, viscosity increasing agent and mucoadhesive agent. Due to the tremendous versatility of Polyox™, a systematic and comprehensive evaluation of its applications as an excipient in freeze-dried ODTs was conducted.

The results from the study revealed that the lowest viscosity grade, Polyox™ N10, was most suitable as a binder, as tablets were produced of satisfactory hardness and disintegration time. The higher viscosity grades, due to their highly viscous and cohesive nature, appeared potentially suitable as excipients which could increase the retention of ODT formulations in pre-gastric regions. By increasing the retention of ODT formulations in the pre-gastric regions, this opens up the possibility for pre-gastric absorption of APIs, which could provide clinical/therapeutic benefits to patients.

8.4 Investigating the Application of Kollicoat® IR as a Binder in Freeze-Dried ODTs

Kollicoat® IR as a co-polymer, has been reported widely as a versatile material able to fulfil various functions, such as a binder, and as a hydrophilic excipient in solid dispersions preparations. Additionally, Kollicoat® IR displays a number of physico chemical properties which could be utilised in order for it to function as a binder in freeze-dried ODTs, such as its high aqueous solubility and low viscosity. For this

reason, a systematic and comprehensive evaluation of Kollicoat® IR was performed in order to exploit its favourable properties.

Data from the study indicated that Kollicoat® IR functions as a binder, and its function is concentration dependent. Kollicoat® IR at concentrations above 10% w/w, forms robust tablets which demonstrate satisfactory disintegration times. In fact it was found that increasing Kollicoat® IR concentration, resulted in an increase in tablet hardness and disintegration time, respectively. Additionally, Kollicoat® IR-based formulations can be prepared at room/ambient temperature, which makes formulation preparation highly efficient and convenient.

8.5 A Design of Experiments (DOE) Study Evaluating the Role of Excipients on the Properties of Freeze-Dried ODTs

The use of DOE – factorial design is a highly useful tool in the development of pharmaceutical formulations. Using DOE – factorial design in the development of pharmaceutical formulations provides a wealth of information about the formulation excipients, and their role, both individually and interactively, on the properties of the formulation. Additionally, formulations can be optimised in order for the formulation to exhibit desired properties. A DOE study was conducted in order investigate the role of; Methocel™ E3LV, Polyox™ N10 and mannitol concentrations, on; formulation viscosity, tablet hardness, tablet disintegration time and *in vitro* oral retention time.

The results from the study revealed that formulation viscosity was primarily influenced (significant positive effect) by Polyox™ N10 and Methocel™ E3LV, and in fact a synergistic-interactive effect was observed between these two excipients. Methocel™ E3LV and mannitol were most influential (significant positive effect) on tablet hardness. In terms of tablet disintegration time and *in vitro* oral retention time, mannitol was found to exhibit a significant negative effect.

An optimised formulation was identified which exhibited desired properties, relating to tablet hardness, tablet disintegration time and *in vitro* oral retention time. Assessing the *in vitro* oral retention of nystatin, demonstrated that the *in vitro* oral retention of APIs can be modified based on the composition of the formulation.

The results from this study suggest that the retention of formulations in the mouth and other pre-gastric regions could potentially be optimised, in order to treat localised medical conditions or encourage pre-gastric absorption of APIs.

8.6 Incorporation of Nanoparticles in Freeze-Dried ODTs

Polymeric nanoparticles possess a number of unique properties as drug delivery systems. Polymeric nanoparticles have the capability of releasing APIs over extended periods of time, and the ability of encapsulating a wide range of APIs. Incorporating polymeric nanoparticles in freeze-dried ODTs would allow the benefits of both ODTs as a dosage form and polymeric nanoparticles as drug delivery devices to be experienced by patients, simultaneously. The aim of this study was to formulate ODTs consisting of polymeric nanoparticles, using novel methods.

The results from the study revealed that novel methods have been developed, that allows the direct incorporation of polymeric nanoparticles in freeze-dried ODTs. A method based on the nanoprecipitation/solvent-displacement method of preparing polymeric nanoparticles has shown to incorporate PCL and PLGA nanoparticles, directly into gelatin and Methocel™-based ODT formulations, forming intact and robust tablets. A novel method based on the ionic gelation method of preparing chitosan nanoparticles, has shown to incorporate these nanoparticles directly into a Methocel™-based ODT formulation, forming intact tablets. However, due to the water-insoluble nature of chitosan, the tablets did not disintegrate during testing. Further research is required in this area.

The APIs; rizatriptan benzoate and piroxicam were studied as model drugs for encapsulation in PCL nanoparticles in order to exhibit a sustained release of the API. Due to the water-soluble nature of rizatriptan benzoate, a low encapsulation-efficiency was observed, consequently, a sustained release of this API was not observed. Further research is required to increase the encapsulation-efficiency of water-soluble APIs.

In terms of piroxicam, a high encapsulation-efficiency was observed. The formulation displayed a sustained release of the API, additionally, the disintegration time of the formulation was shorter than that of the nanoparticle-free formulation.

The stability study results for the nanoparticle-free tablets revealed potential API and physical stability issues with the tablets stored at 40ºC/75% RH.

The results from this study are significant, as freeze-dried ODTs have been developed which consist of polymeric nanoparticles, prepared using novel methods. The method based on the nanoprecipitation/solvent-displacement technique, has shown to produce

a sustained release formulation of piroxicam. A sustained release ODT formulation could potentially improve patient compliance with their medication programs, due to a reduction in the frequency of medication administration.

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