DESIGN AND CHARACTERISATION OF ORALLY DISSOLVING FILMS AS A POTENTIAL NEW DOSAGE FORM FOR PAEDIATRICS

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Thesis summary

Orally dissolving films (ODFs) have received much attention as potential delivery systems for oral administration of drugs to paediatric patients. With their unique properties and advantages, the technology offers improved patient compliance and wider acceptability, eliminated fear of choking, ease of administration and dosing convenience, without the requirement of water. This research focused on the formulation of ODFs with suitable physico-chemical and clinical properties as a potential dosage form for paediatric use.

Initial studies focused on screening different film-forming materials used for the preparation of orally dissolving films in order to optimise and propose suitable polymers and plasticisers with a suitable manufacturing technique. Kollicoat Protect was a selected candidate for further studies, due to its excellent film forming capacity with rapid disintegration.

The work also sought to improve the loading capacity, taste masking and drug content uniformity of both hydrophilic (dexchlorpheniramine malate) and hydrophobic (glipizide) drugs into ODFs, especially for poorly water soluble drugs, through complexation with cyclodextrins (CDs) and incorporation with nanoparticles. Results demonstrated that CD complexation showed improvement in the solubility profile of glipizide, whilst drug loading efficiency and drug content uniformity only improved at low doses, based on the limited cavity sizes. Nonetheless, the application of nanoparticles achieved good drug loading efficiency for glipizide at higher doses. In contrast, the loading capacity and other physico-chemical properties of dexchlorpheniramine maleate loaded films remain flexible.

Further, method development to optimise the determination of disintegration time of ODFs proved that the media and media volume has no effect on disintegration time using either beaker or the texture analyser method, but the analyser method demonstrated to be more suitable for quality control setting of ODFs. Of the stability performance of ODFs, films packed with the prototype packaging remained stable over the period of time studied at both long term and accelerated conditions, which indicated their robust and clinical use through the product developmental stages.

Keywords: Orally dissolving films, paediatric**,** Kollicoat protect, mechanical property, drug content uniformity, drug loading, poorly soluble drugs, cyclodextrins and nanoparticles.

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List of Publications

Related abstracts, posters and presentations

Oral Presentations

1) Orally dissolving film loading of poorly water-soluble drugs by cyclodextrin complexation. *Strathclyde- Aston 2nd Workshop, Glasgow, July 2015*.

2) Design and characterisation of antihistamine loaded orally dissolving films as a potential new dosage form. *11th biennial meeting of the Globalization of Pharmaceutics Education Network (GPEN), Lawrence, Kansas, September 2016.*

Conference Proceedings

1) Anjali Sonpal, **Thu Pham** and Daniel J. Kirby**.** Improving drug content uniformity in Orally Dissolving Films. *UK Pharm Sci, University of Strathclyde*, 2016.

2) **Thu Pham**, David Terry, Deborah Lowry, and Dan J. Kirby. Evaluation of dexchlorpheniramine maleate loaded orally dissolving films. *Aston Post Graduate Research Day, Aston University, June 2016*.

3) **Thu Pham**, David Terry, Deborah Lowry, and Dan J. Kirby. Comparative studies on a novel testing method for the determination of disintegration time of orodispersible films. *43th Annual Meeting and Exposition of Controlled Release Society, Seattle, 2016.*

4) **Thu Pham**, David Terry, Deborah Lowry, and Dan J. Kirby. Physicochemical characterisation of fast dissolving films with enhanced bioavailability properties of poorly soluble drug by cyclodextrin derivatives. *UK Pharm Sci, Nottingham, 2015.*

5) **Thu Pham**, David Terry, Deborah Lowry, and Dan J. Kirby. Improved loading of a poorly water soluble drug in Orally Dissolving Films through cyclodextrin inclusion complex. *42th Annual Meeting and Exposition of Controlled Release Society, Edinburgh, 2015*.

6) **Thu Pham**, David Terry, Deborah Lowry, and Dan J. Kirby. Characterisation and phase- solubility studies of orally dissolving films loading with inclusion complex of glipizide with cyclodextrin derivatives. *UKICRS, Nottingham, 2015*.

7) **Thu Pham**, David Terry, Yvonne Perrie, Defang Ouyang. Development of Oral Dissolving Films Using Sodium Alginate as Film Former. *APS, Hertfordshire, 2014.*

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Awards/ Poster Prizes

1) Post Graduate Research Day of Aston University, School of Life and Health Science- Poster Prize Award, *June 2016.*

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Abbreviation lists

^{0}C	Degree celsius
ANOVA	Analysis of variance
API	Active pharmaceutical ingredient
BCS	Biopharmaceutical Classification System
BNF	British National Formulary
BPCA	Best Pharmaceutical Children Act
CDs	Cyclodextrins
cm	centimetre
DCM	Dexchlorpheniramine malate
DSC	Differential Scanning Calorimetry
EMA	European Medicine Agency
EUPFi	European Paediatric Formulation Initiative
FDA	Food & Drug Administration
FDD	Fast drug delivery
FTIR	Fourier Transform Infrared
GIT	Gastrointestinal tract
GMP	Good Manufacturing Practice
HPC	Hydroxylpropyl cellulose
HPCD	Hydroxypropyl β-cyclodextrin
HPMC	Hydroxypropylmethylcellulose
HPLC	High Pressure Liquid Chromatography
ICH	International Conference on Harmonisation
IR	Immediate Release
KP	Kollicoat protect

Metformin HCl	Metformin Hydrochloride
Mins	Minutes
mL	millilitre
Mwt	Molecular weight
nm	Nanometre
ODFs	Orally dissolving films
ODTs	Orally disintegrating tablets
PCL	Polycaprolactone
PDI	Polydispersity Index
PEG	Polyethylene glycol
PVA	Polyvinyl alcohol
PVP	Polyvinyl pyrrolidone
RH	Relative Humidity
RSD	Relative Standard Deviation
s	seconds
SA	Sodium alginate
SD	Standard Deviation
SEM	Scanning electron microscope
STEP	Safety and Toxicity Excipients for Paediatrics
Tg	Glass transition temperature
µm	micrometre
UV	Ultraviolet
USP	United States Pharmacopeia
v/v	volume/ volume
WHO	World Health Organisation

Chapter 1
General Introduction

1. Introduction

1.1 Drug delivery to the oral cavity

It is no surprise that the oral route is one of the most widely accepted ways of administering drugs to the body, due to ease of ingestion, convenience, pain avoidance and high level of patient compliance (Desu *et al.*, 2013). In addition. the oral cavity (mouth) offers an accessible gateway for the delivery of an active pharmaceutical ingredient (API), either *via* the saliva or absorbing *via* the oral muscosa to achieve local or systemic administration (Rathbone *et al.*, 1994). The cavity provides a large surface area (100- 200 cm^2), with an extent of smooth muscle, a relatively immobile mucosa and low enzymatic activity compared to the gastro- intestinal tract (Khafagy *et al.*, 2007).

1.1.1 Anatomical structure of oral cavity

The oral cavity is structured by the cheek, the hard and soft palate and the tongue (Fig. 1.1). The total surface area of the human oral cavity is approximately 200 cm^2, which comprises of 20% surface area of teeth and the remainder attributed to the oral mucosa (Wilson, 2005),which are classified into three sections in the oral cavity, depending on the deviation of thickness and nature of the mucosa lining. The surface of the oral mucosa is composed of stratified squamous epithelium, which is separated by a further, underlying basement layer, called the lamina propria and submucosa; these layers contain blood vessels, sensory receptors and nerves. The lining mucosa, which is non-keratinised, covers the soft palate and other buccal regions, whereas the masticatory mucosa, which are composed of keratinised cells, encompasses the hard palate and gingival. The

keratinised mucosa contains ceramides and acylceramides, which are believed to relate to the barrier function, while only a small amount of ceramides are present in the lining mucosa (Shojaei, 1998). The saliva is secreted by three major salivary glands – the parotid, submandibular and sublingual glands from the oral cavity– and is influenced either directly or indirectly by sympathetic and parasympathetic nerves (Smart, 2005). The pairs of parotid and submandibular glands produce mainly serous fluid, whereas the sublingual glands generate only mucus containing saliva. Saliva maintains the health of oral tissues, as well as functioning as a lubricant for swallowing and a buffering agent, in addition to being responsible for the clearance of many drugs; antibacterial activity, taste and digestion (Humphrey and Williamson, 2001, de Almeida *et al.*, 2008).

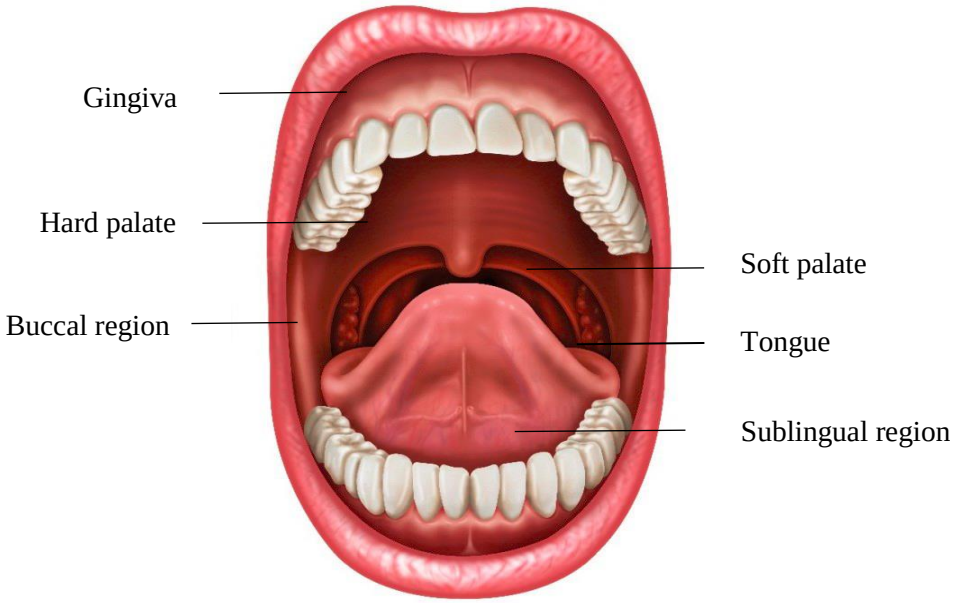


Figure 1.1- Anatomy of the oral cavity. Adapted from (Robinson and Mickelson, 2006)

1.1.2 Saliva composition, flow and functions

Since the oral cavity is the main site for drug delivery for oral dosage forms, the saliva composition contributes to the rate of diffusion of drug molecules. Saliva plays an important role in drug absorption, by rapidly providing the aqueous environment to facilitate drug action. Saliva fluid is a mixture made of a large extent of water (approximately 99 %) containing a variety of electrolytes, such as sodium, potassium, chloride and bicarbonate, as well as other digestive enzymes, proteins and antimicrobial constituents (Kaufman and Lamster, 2002, de Almeida *et al.*, 2008). Different types of enzymes, such as lipase, α-amylase and lysozyme help to stimulate digestion in the human oral cavity (Wilson, 2005, Walsh and Mills, 2013).

However, the salivary flow rate and its composition changes throughout the lifespan of a human; the flow rate of saliva increases in children up to 6 years of age, but the mean electrolyte content increases with a decline in saliva flow after that stage. A range of unstimulated saliva flow rates in children is generally from 0.22- 0.82 mL/min and 0.33-1.42 mL/min in adults (Rotteveel *et al.*, 2004, Lam *et al.*, 2014). On the other hand, pH is also important factor to drug absorption; the pH of the oral cavity in healthy children is 6.6, which is slightly lower than that of adults. In addition, diet and saliva flow rate can also have an impact on pH level and, hence, the absorption of drugs (Aps and Martens, 2005).

1.1.3 Routes of drug transport

Drugs penetrate across cells from saliva by passive diffusion or by an active transport mechanism (Kaufman and Lamster, 2002, Bosch, 2014). Passive diffusion is the major mechanism by which drug molecules transport via two main pathways: the transcellular (crossing thorough the cell membrane) and paracellular (passing spaces between cells) routes (Figure 1.2) (Rossi *et al.*, 2005, Sudhakar *et al.*, 2006). Lipophilic molecules are subject to transport via the transcellular pathway, as they can easily permeate the lipophilic cell membranes, whereas hydrophilic and small molecules pass the lipid layers of membranes by being transported paracellularly. However, drug transport mechanisms can dominate simultaneously by the two pathways, with one route performing predominant permeation (Rossi *et al.*, 2005).

Yet, the route and the rate of drug absorption via permeable membranes may be influenced by other physio-chemical factors, including the drug concentration on the surface of mucosa, the molecular weight of molecules, the delivery vehicle, the pH of saliva and the saliva flow (Reddy *et al.*, 2011, Hooda *et al.*, 2012). For example, the absorption of hydrophilic molecules are highly dependent on molecular size (smaller molecules diffuse more readily) and low acid dissociation constant (pKa) of drug, where the pH value of saliva is prone to alter the drug ionisation (ionised molecules are polar) and, hence, facilitating the diffusion.

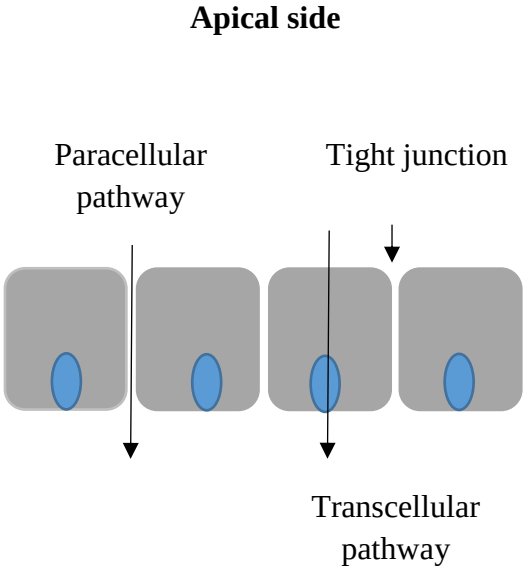


Figure 1.2- Schematic representation of paracellular and transcellular transport. Adapted from (Andrade *et al.*, 2011).

1.2 Paediatric dosage forms

1.2.1 Medicines for paediatric use

The improvement of medicines for paediatric use can be a challenging task, due to poor availability in the drug development process and the lack of understanding of appropriate dosage forms. Besides that, numbers of unlicensed and off- label medicines used for paediatrics are often given by healthcare professionals, which arise with risk due to lack of information, as a consequence of a relatively small market share, leading to a lack of financial incentives, as well as challenging clinical trial methodology and recruitment, for

what is a heterogeneous patient population (Ivanovska *et al.*, 2014). Indeed, the incidence of use of off-label (prescribed outside the terms of the product license) or unlicensed (where no license exists) medicine is estimated to range from around 11 % in general practice (McIntyre *et al.*, 2000), increasing up to 36% in general paediatric hospital admission (Turner *et al.*, 1998) and as much as 90% in neonatal intensive care units (Conroy *et al.*, 1999). Besides that, the use of unlicensed and off-label medicines has the potential to produce adverse drug reaction in babies and children, with severe effects including hypotension, difficulty in breathing, or prolonged sedation (Nunn, 2003, Choonara, 2004).

Recently, the Committee for Medicinal Products for Human Use released the reflection paper "Formulation of choice for the paediatric population" (EMA, 2006), which classifies the paediatric population into six developmental stages: preterm newborn infants, term newborn infants (from 0 to 27 days), infants / toddler (from 1 month to 23 months), children (from 2 to 11 years old) and adolescents (from 12 to 18 years old) (Figure 1.3). According to this report, the EMA (European Medicine Agency) have proposed a new safety and acceptability guideline for formulation suitability for each age group, including the indication and route of administration (Drakulich, 2009). Since children grow up through different stages of growth and development, not only dosage form, but also the dose regimen, acceptability and palatability must be considered. Moreover, the EMA recently published a guideline on pharmaceutical development of medicines for paediatric uses in 2013, which further enhances the balance between clinical prediction and regulatory assessments of paediatric medicines, in terms of facilitating the development and accessibility of medicinal products in the numerous of

paediatric population with target age group as proposed in marketing- authorisation application (EMA, 2013). Importantly, the regulation of medicinal products should be aims for safety and efficacy, which is a critical factor to protect the wellbeing of the children (Salunke *et al.*, 2012). In an attempt to rectify this worrying situation, The Best Pharmaceuticals for Children Act (BPCA) (2002) took the lead to encourage the pharmaceutical industry to perform paediatric studies to improve labelling for patented drug products used in children (Christensen, 2012). The European Paediatric Regulation (2006), which came into force in 2007, went one step further and has changed the landscape of accessibility of medicine products via research and development to enhance the safety and quality of medicine for children as it is very important for paediatric development. These regulatory approaches to improve paediatric medicines sparked a range of research initiatives, such as the European Paediatric Formulation Initiative (EUPFi), which has developed an online Safety and Toxicity Excipients for Paediatrics (STEP) database to capture the specific need for potential users from the input of industry and researchers (Drakulich, 2009, Salunke *et al.*, 2012).

Figure 1.3- Illustration of different developmental stages in paediatric groups. Adapted from (Children, 2013).

Nevertheless, age-adapted drug formulations represent a particular challenge in drug development for paediatrics; the paediatric formulations are required to deliver variable doses of APIs to children with different ages and body weight (Nunn and Williams, 2005), whilst children in different parts of the world have different requirements for medicines. For examples, young children from poor countries suffering from the malnutrition are associated with paracetamol hepatotoxicity, therefore it is necessary to deliver the suitable medicine products for children (Vitols, 2003).

Administration of the APIs via the oral route is the most popular choice because of their extensive advantages, including low manufacturing cost, ease of administration and pain free (Batchelor and Marriott, 2015). Oral dosage forms are generally available in solid (e.g. tablets, capsules) or liquid (e.g solutions, suspensions and emulsions); however, there are obstacles for young patients to accept the oral medicines as results of swallowing difficulty or chewing problems in patients. It estimated that 50% of the specific target groups, particularly young children, the elderly and patients suffering from a variety of disorder such as stroke or neurological problems, experience difficult in swallowing, which leads to a high incidence of non-compliance and delivery of non-effective therapy (Schiele *et al.*, 2012).

In addition, due to the difficulty in swallowing solid dosage forms, particularly for the younger age groups, currently most oral paediatric medicines are liquid dosage forms. However, liquid formulations generally require more excipients than solid dosage forms due to their poor stability. Volume is an important factor for the liquid formulation; the dose volume of liquid formulations should ideally be prescribed at less than 5 mL for

children under 5 years of age and no more than 10 mL for children from 5 years and older (Batchelor and Marriott, 2015). Moreover, the volume is not only important for the palatability of the formulation, but also for the accurate measurement of the appropriate dose to children. There are also limitations on choice and concentration of excipients for paediatric patients; for example, alcohol is not suitable in paediatric formulation because of its toxicity to young children (Walsh, 2012). On the other hand, the taste masking techniques in drug dosage forms (e.g. sweeteners and flavours) is very important for good patient compliance, although the selection of flavouring agents in the formulations depends on the physiochemical properties of drugs. Moreover, disease types and regional preferences should also be taken into account for treatment weight (Nunn and Williams, 2005). Furthermore, packaging of paediatric products is also important for their chemical and physical stability. They need to be free from any source of contamination with child-protection packaging (Maldonado and Schaufelberger, 2011).

1.2.2 Challenges and considerations for paediatric formulations

First of all, the doses of drugs for children in different age populations is variable; children are not small adults and they go through different stages of growth and development. Thus, they differ from adults in many aspects, such as body development, drug disposition and metabolism and taste preferences (McNally and M.Railkar, 2009). For example, infants have slower gastrointestinal movement, less protein binding and slower drug metabolism than adults. In addition, the total body water between children and adults is also different; neonates have 80% body water, whereas this is just 50-60% in adults. Moreover, different paediatric subpopulations have different pharmacokinetic and

pharmacodynamics profiles, as they vary with the broad ranges of age and body size/ weight of the paediatric population. Therefore, it is a significant challenge for formulation scientists to develop an effective medicine suitable for all paediatric subpopulations (Stephenson, 2005).

What's more, the excipient selection in paediatric formulation is different from formulations developed for adults. It depends on the functionality and safety profile across different populations, illness conditions and treatment duration (Walsh, 2012). It is well known that the dosage forms will noticeably impact on the choice of excipients. Accordingly of the age differences, the excipients are generally associated with potential toxicological risk in different age populations; for instance, ethanol and propylene glycol are widely-used excipients in adult formulations, yet they are restricted for use in newborns and infants (Zuccotti and Fabiano, 2011). As previously mentioned, the liquid formulations are the most preferred dosage form for babies and children under aged of six, yet stability becomes another challenge for liquid formulations. Furthermore, to improve the shelf life of liquid dosage forms, there are additional concerns associated with large amount of excipients in the paediatric formulations (Jenny Walsh, 2012); larger amounts of excipients may cause young children to be exposed to potential toxicities. As mentioned before, excipients such as propylene glycol and benzyl alcohol – an excipient that can potentially be fatal for neonates – remain the excipients of choice for liquid formulation (Allegaert, 2013).

Furthermore, taste masking of paediatric medicines can also be challenging, especially for high solubility drugs, which may dissolve rapidly in the mouth (Cram *et al.*, 2009).

Paediatric palatability of oral medications is also a significant challenge in the early stage of formulation development; the use of sweeteners and flavouring agents are commonplace and crucial in paediatric formulations, in order to improve drug palatability and children compliance. However, these excipients have been shown to cause adverse effects (e.g. dental caries and laxative effect), whilst also potentially causing some allergic reactions with unknown reasons (Fabiano *et al.*, 2011).

1.2.3 Alternative paediatric dosage forms

There exists some comprehensive guidance focusing on formulation preferences within the paediatric population, stating that the ideal specification of the formulation should: (i) reduce dose frequency; (ii) minimise the size of dosage forms; (iii) ensure convenience and ease of administration; (iv) display good taste and (v) contain safe excipients (Batchelor and Marriott, 2015, Preis, 2015). The use of "disperse systems" has been established as the strategical reconstitution of solid oral dosage forms for paediatric formulation. Multi- particulate systems, such as granules and pellets, are useful for paediatrics, which can be administrated directly into the mouth or even mixed with food, therefore providing ease of swallowing and dose flexibility. Nevertheless, there is an issue of incomplete ingestion and, therefore, reduced dose, whilst stability matters also need to be reconsidered (Nunn and Williams, 2005).

Mini tablets are also an innovative formulation development, with their tablet diameter of less than 3 mm, which have demonstrated fair acceptability from young children (Thomson *et al.*, 2009). Other studies found that the young children prefer to accept mini tablets (2 mm in diameter) to the syrup formulation (Klingmann *et al.*, 2013).

Chewable tablets are another dosage form that are formulated to be mechanically processed in the mouth to enhance the disintegration/ or dissolution of the API. Literature reports suggested that chewing patterns and efficiency are recorded in children from the age three and over (Liu *et al.*, 2014).

Oral disintegrating tablets, are also promising approach for paediatrics that are designed to disintegrate quickly within the oral cavity without the need of water (Slavkova and Breitkreutz, 2015). However, the tablet formulation is dependent on the properties of APIs and taste masking of bitter drugs is necessary with the careful choice of excipients. Yet, they are still limited by the dose rigidity, integrity during transportation or handling, and, although a low risk of choking and aspiration (particle inhalation), the administration of ODTs might be obtrusive for young children (Lam *et al.*, 2014, Batchelor and Marriott, 2015).

1.3 Orally dissolving films- A novel potential approach

As a consequence of the issues stated above, particularly with regards to the inability of children to swallow traditional oral solid dosage forms, in order to aid patient compliance and convenience, the expansion of orodispersible products has emerged, with dosage forms of different types, such as oral disintegrating tablets and oral lyophilisates. First developed in the late 1970s, the fast-dissolving drug delivery system is a pioneering technology, providing an alternative oral dosage form for paediatric, geriatric and non-compliant patients with difficulties in swallowing or fear of choking (Bala *et al.*, 2013). The incorporation of various water soluble drugs into oral disintegrating films (ODFs)

has been reported (Nishimura *et al.*, 2009, Shimoda *et al.*, 2009). This new drug delivery system gained advantageous potential in the oral route of administration, as they provide patients the medicine without the need of swallowing, provide rapid dissolution in a small amount of saliva without the consumption of water.

1.3.1 Types of Oral Films

Depending on the design of formulation, the application areas and disintegration time, different types of oral films are applicable. Mucoadhesive films and oral patches were introduced in the market as buccal sustained drug delivery systems, specifically designed for the buccal or sublingual region, whereas the fast dissolving film is placed on the tongue. The characteristics of these films are summarised in Table 1.1 (Nagaraju *et al.*, 2013).

Table 1.1- Properties of different types of ODFs. Adapted from (Bala *et al.*, 2013, Nagaraju *et al.*, 2013).

Properties	Fast release films	Mucoadhesive films	Mucoadhesive sustained wafers
Area (cm^2)	2-8	2-7	2-4
Thickness (μm)	20-70	50-500	50-250
Structure	Single layer	Single or multiple	Multiple layer
Components	Soluble polymers	Soluble polymers	Insoluble polymers
Drug phase	Solid solution	Solid or suspension	Solid and/ or suspension
Delivery region	Tongue	Gingival or buccal region	Oral cavity region
Dissolution time	30s	Few minutes	8-10h
Location of action	Systemic or local	Systemic or local	Systemic or local

1.3.2 Why oral films? Special features

ODFs have acquired great importance in the pharmaceutical industry, due to their unique properties and advantages. They undergo rapid disintegration in the presence of salivary fluids of the oral cavity within a few seconds, without the requirement of water. Their characteristics offer tremendous advantages in terms of improved patient compliance, rapid onset of action, no risk of choking, increased bioavailability and ease of administration and portability (Dixit and Puthli, 2009, Irfan *et al.*, 2015). Regarding paediatric formulations, oral film delivery system can be beneficial for the small size of

dosage form and for those who require accurate doses for monitoring in disease states suitable for children at all ages. Furthermore, ODFs offer advantages over other dosage forms, where they are more stable and resistant compared to ODTs, which are fragile and brittle. In addition, other advantages over ODTs is providing a larger surface area for faster disintegration in the oral cavity with great flexibility and eliminated fear of swallowing tablets (Hirani *et al.*, 2009). In contrast, although liquid dosage forms are flexible and provide ease of administration, accurate measuring is often problematic, especially without a dedicated measuring device, whilst poor stability of the liquid formulation is the major limiting factor (Borges *et al.*, 2015).

1.3.3 Marketed oral dissolving films and future potentials

Thin-film strip technology exploits a range of water soluble polymers to incorporate various APIs, making the medicines more flexible, robust and stable. The first ODFs were commercialised in the market as breath fresheners and healthcare products (Listerine Cool Mint Pocket Packs by Pfizer). Following the success of Listerine pocket packs, they introduced the Triaminic and Theraflu brands in strip forms for cold suppression. Prestige pharmaceutical company launched the first therapeutic oral thin film, Chloraseptic® relief strips, with benzocaine in 2003, which was used for the treatment of sore throat. In recent times, ondansetron containing oral fast dissolving films, Zuplenz®, was approved by FDA for vomiting prevention of cancerous patients before chemotherapy, with each film containing either 4 mg or 8 mg API, which dissolves within 20 seconds, thereby allowing ondansetron to be easily taken by dysphagia patients (FDA). It was prescribed as 8 mg films twice daily for paediatric patients aged 12 or older, whilst paediatric patients less

than 12 years old can take 4 mg oral films 3 times a day. Currently, there are a wide range of commercial oral fast dissolving films available in the global market, as summarised in Table 1.2.

Table 1.2- Lists of marketed ODFs.

Products	Ingredients	Mode of action	Company
Listerine Cool Mint Pocket Packs	Cool mint	Mouth fresheners	Pfizer
Minerals and nutraceutical films	B6, B12, vitamin C	Vitamin supplements	Paladin Labs (Bioenvelop)
Energy booster	Caffeine, green tea extract	Boost energy levels	Biofilm
Donepezil Rapidfilm ®	Donepezil Hydrochloride (5 mg and 10 mg).	Management of Alzheimer's type.	Labtec GmbH
Ondansetron Rapidfilm ®	Ondansetron 4mg & 8mg	Prevention of nausea and vomiting after post chemotherapy	Strativa Pharmaceuticals

Onsolis (fentanyl buccal soluble film)	Fentanyl citrate 200 mcg, 400 mcg, 600 mcg, 800 mcg, 1200 mcg	Pain relief for cancer patients	Biodelivery Sciences
Triaminic Thin Strips Cough & Runny Nose	Diphenhydramine (12.5mg)	Anti- allergic	Novartis Consumer Health
Day Time Triaminic Thin Strips® Cold & Cough	Dextromethorphan 3.67 mg (equivalent to 5 mg Dextromethorphan HBr), Phenylephrine HCl 2.5 mg,	Nasal decongestant	Novartis Consumer Health
Theraflu Thin Strips Long Acting Cough	Dextromethorphan HBr (15 mg)	Cough suppressant	Novartis Consumer Health
Suppress Herbal Cough Relief Strips	Menthol (2.5 mg)	Cough suppressant	Innozen Inc
Chloraseptic relief strips	Benzocaine/menthol (3mg/3mg)	Sore throat	Prestige Brands
Snoreeze® strips	Peppermint oil	Reduce snoring	Passion for Life Healthcare

1.3.4 Patented technologies

The development in the field of fast dissolving products, especially for ODFs, has been gaining attention and is well accepted by consumers. Some of the patent technological platforms of the orally dissolving films are summarised in Table 1.3 (Siddiqui *et al.*, 2011, Nagaraju *et al.*, 2013, Borges *et al.*, 2015).

Table 1.3- ODF patented technologies platform.

Patented technology	Technological platform
Soluleaves™	A wide range of flavours, vitamin and APIs incorporated into edible thin films for slow release once adhere on the oral mucosa.
WaterTab™	A designed technology for precise dose of API loaded into pre-manufactured body of indigestive strips to prevent heat and moisture exposure, thus enhancing product stability. It can be used in oral or topical administration.
Foamburst™	A special patent derived from Soluleaves technology for capsule made from foam film, where a honeycombed structure is formed, which deliver flavours for quick dissolution with a good mouth sensation.
RapidFilm®	A patent developed by Labtec GmBH that use non-adhesive, water- soluble polymers to incorporate drug (maximum 30 mg) for quick release in the mouth.
Bio-erodible muco-adhesive (BEMA®)	The technology is designed for multilayer bioerodible films that adhere to the oral mucosa with the unidirectional deliver of APIs from the backing layer for quick onset of action.

1.3.5 Limitations for ODFs

One of the issues with the ODFs manufacture is their low dose capacity (less than 40 mg API), since the small, thin size is the limiting factor, therefore restricting their use to potent drugs that are clinically efficacious at lower doses (Dixit and Puthli, 2009). Combinations of more than one drug, especially for poorly water soluble compounds, is also a challenging task that could have an impact on the disintegration time and dissolution profile of ODFs (Jadhav *et al.*, 2013). Taste masking of bitter drugs is also a prerequisite step for ODFs, as films remain in contact with oral mucosa by the hydrolysis of saliva. In addition, incorporation of taste masking agents may affect the physical properties of ODFs. Another critical issue is to obtain a uniform dose for the individual unit of films where they are cut into desired sizes and shapes, which is essential as it is important to provide stable levels of drug within the patient, thereby providing a more consistent therapeutic effect. Furthermore, most of the polymers used for ODF formulation are hydrophilic in nature and sensitive to moisture; thus, an appropriate packaging is needed for moisture protection for a long term preservation.

1.4 Strategies to enhance the loading capacity of ODFs

1.4.1 Solubilisation of poorly water soluble drug

Most of the drugs being developed in the pipeline today are poorly water soluble (Kakran *et al.*, 2012), which remains a challenging task for the design of oral dosage forms. Furthermore, poor solubility might also influence the drug distribution within the dosage from. To overcome the challenges, various solubilisation techniques have been proposed to enhance the solubility profile of the drug substances.

Solid dispersion is a main explored technology, which incorporates the poorly soluble drug into an inert polymeric carrier. Blending drug particles within the polymer matrix prevents the aggregation and, thus, the dissolution of the drug is improved by increasing the available surface for wettability or enhancing the amorphicity (Kakran *et al.*, 2012). Solid dispersions have been widely employed to enhance the solubility properties of different types of drugs, such as paracetamol, nifedipine and ritonavir (Akiladevi *et al.*, 2011, Lalitha and Lakshmi, 2011, Huang and Dai, 2014). In addition, the formulation of the poorly soluble drug, glipizide, as a solid dispersion using hydrophilic carriers such as polyethylene glycol and poloxamer, has been extensively reported in the literature (Choudhary *et al.*, 2009, Verma *et al.*, 2011, C Patel *et al.*, 2012).

Particle size reduction is another similar approach to enhance the dissolution rate, where the improvement of dissolution profile of the drug is achieved through increased surface area. Micronisation is, for example, a conventional common method, which has been successfully applied to enhance the bioavailability and clinical efficacy of fenofibrate or griseofulvin (Vogt *et al.*, 2008, Kawabata *et al.*, 2011). Also, the use of nanoparticles by dispersing preformed polymers to produce the colloidal particles in the range of 10-1000 nm, promotes the dissolution rate of poorly soluble drugs, enhances the drug loading capacity and reduces the toxicity level of the excipients (Rabinow, 2004). The incorporation of BCS class II drug nanoparticles, e.g naproxen, fenofibrate and griseofulvin, into the edible strips using hydroxypropyl methyl cellulose as a film former exhibited the enhancement of dissolution performance and bioavailability of those studied drugs (Sievens-Figueroa *et al.*, 2012).

Cyclodextrins (CDs) have been extensively used in drug development for improving the aqueous solubility and bioavailability of those problematic drugs via the formation of inclusion complexation. CDs and their derivatives were used primarily for their solubilising effect, whilst also serving as drug carriers (Szejtli, 1998). Numerous studies have demonstrated the enhancing property in the literature. Hydroxypropyl β-cyclodextrin (HPCD) was used to develop complexation with prednisolone by solvent method in order to improve the dissolution profile, which was subsequently loaded into ODFs (Patel and Patel, 2014). The hydrophobic drug etoricoxib incorporated with beta cyclodextrin also showed enhancement in the aqueous solubility of the drug, as well as achieving good uniform distribution of drug once they are loaded into films (Senthilkumar and Vijaya, 2015).

Surfactants are also alternative options for aiding drug dispersion of poorly soluble drug, since they can act as dispersing and solubilising agents by reducing the surface tension, and potentially may be used for enhancing the dose uniformity of these class II drugs, as well as maintaining formulation flexibility with improved stability (Billany, 2002, Mishra *et al.*, 2009). Tweens, a non-ionic solubilising agent, are the most commonly used surfactants for development of ODFs (Siddiqui *et al.*, 2011, Irfan *et al.*, 2015), whereas Span, a non-ionic surfactant belonging to the sorbitan esters group, has a safe history of use in food and pharmaceutical formulation (Croda, 2010).

1.4.2 Taste masking approaches

Most drugs have a bitter taste, making taste masking a crucial parameter in the development of ODFs, as well as other dosage forms. In particular, palatability of ODFs is vital, since the films remain in contact with the oral mucosa by the hydrolysis with saliva. As such, several approaches of taste masking are available for enhanced performance and acceptability. One of the simplest techniques used in taste masking is the addition of taste modifiers, such as sweeteners and flavouring agents for bitter APIs. However, the choice of taste modified agent must be compatible with other excipients, stability and tolerance for optimal use (Walsh *et al.*, 2014).

Furthermore, coating with polymer is also a suitable approach for improved palatability. This method is achieved by introducing a physical barrier on the API itself, or the dosage form, from the taste buds of the tongue. As a result, the coating material inhibits the release of APIs in the oral cavity and, therefore, only dissolve to release at the enteric pH (pH < 5). The advantage of using this technique, especially for ODF formulation, is to prevent the contact of drug particles merging on the oral mucosa and, hence, ensuring taste masking (Xu *et al.*, 2008).

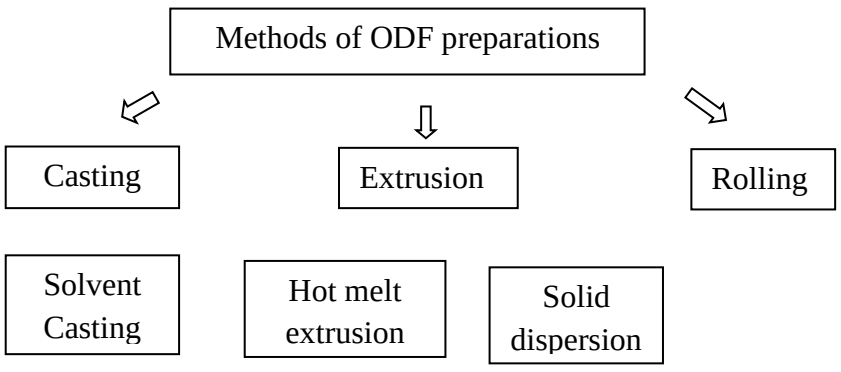
Cyclodextrin complexation has also been widely used for taste masking of bitter drugs. The process is carried out by the formation of inclusion of guest molecules into the CD cavity; the bioavailability, solubility and stability of these entrapped drugs is enhanced by the complexation. Most of all, they are capable of decreasing the taste perception of the drug. However, the extent of taste masking depends upon the size of cavity and the size of molecules (Davis and Brewster, 2004). For example, beta CDs are the most widely

used and are suitable for encapsulation of a wide range of drugs, especially for cyclic molecules. Alpha CDs tend to prove insufficient in drug inclusion, due to having the smallest cavity size, while gamma CDs are the largest but display a weaker complex ability than the other two CDs (Challa *et al.*, 2005). This method has been reported to be more suitable for taste masking for low dose drugs (Sohi *et al.*, 2004). In addition, CD complexation has improved taste masking of many unpleasant tasting drugs, but also achieved good uniform distribution of drug when incorporated into films (Mahesh *et al.*, 2010, Poluri *et al.*, 2013, Senthilkumar and Vijaya, 2015).

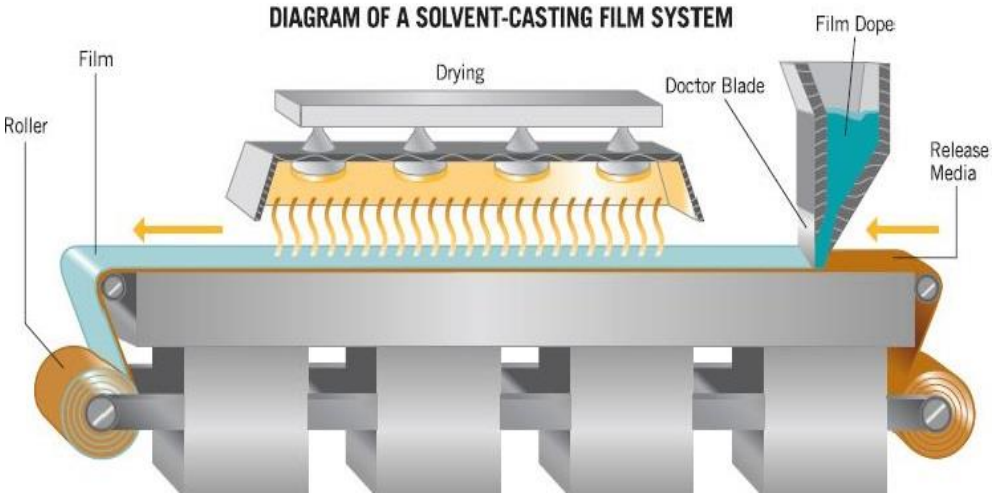
Although there are a selection of taste masking technologies available to assist the current issues of taste masking, a deep consideration of appropriate technique for the palatability of paediatric medicinal products is required.

1.5 Film preparation- new challenges and opportunities

Several manufacturing methods are employed for ODFs, including solvent casting, hot melt extrusion, solid dispersion, and rolling. They can be used as one process or combination techniques (Figure 1.4).



A)



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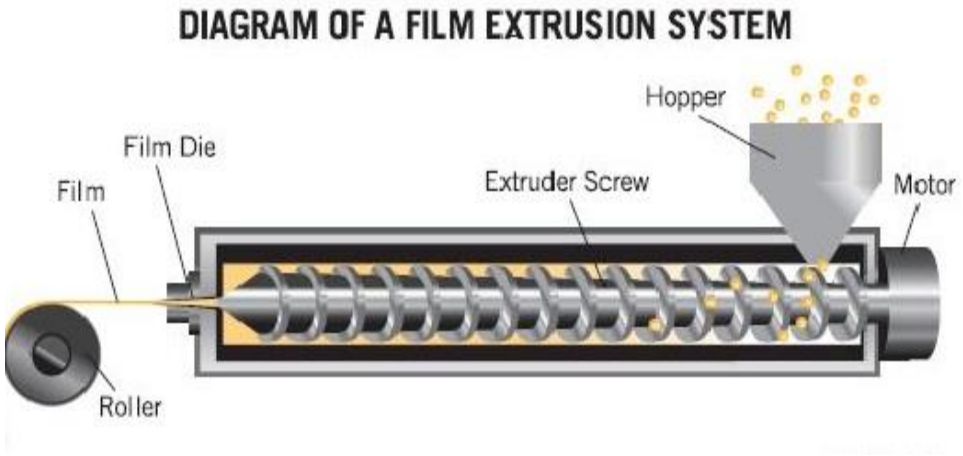


Figure 1.4- Schematic representation of ODF manufacturing as a commercial systems. Adapted from (Science, 2010).

1.5.1 Film manufacturing techniques

Two methods predominate for film preparation: solvent casting method and hot melt extrusion (Figure 1.4). The casting process can be defined as the simplest, oldest procedure in film manufacturing, which was first introduced at the end of the 19th century, driven by the needs of the photographic film industry (Collins, 1990). Lately, this technology is becoming a versatile tool for thin film production with high quality control. Solvent casting method involves the process where water–soluble polymers are firstly dissolved in water to form uniform clear viscous solutions, followed by addition of drug and other excipients, to form a homogenous solution. The solvent is subsequently cast on the substrate and evaporated during the drying period, resulting in a thin film that can be processed into desired properties (Siemann, 2005). Hence, solvent casting is widely considered as the method of choice, due to feasibility and low cost of manufacturing (Irfan *et al.*, 2015). In hot melt extrusion, the API and other excipients are mixed in a dry state, and undergo a heating process without the use of solvents. However, this method has not been a selected choice of film manufacturing as the higher temperature could be the cause of thermal degradation of film materials, and there are limited suitable film forming agents for heating (Crowley *et al.*, 2007, Dixit and Puthli, 2009, Mishra and Amin, 2011). Nevertheless, hot melt extrusion has been extensively used for sustained drug release for transdermal and transmucosal delivery systems (Repka and McGinity, 2000, Prodduturi *et al.*, 2005), tablet and granule manufacturing (Crowley *et al.*, 2007), and ODF formulation by exploring a multiple combination of polymers (Crowley *et al.*, 2004, Repka *et al.*, 2005).

Moreover, novel methods such as inkjet printing and three dimensional (3D) printing have become an interesting approach for manufacturing of ODFs, where the dosage solution is either sprayed accurately onto the film surface (Scoutaris *et al.*, 2011, Genina *et al.*, 2013, Janßen *et al.*, 2013), or the design configuration is created in 3D by deposition of thin layers of dosage form (Goyanes *et al.*, 2015). These promising technologies allow wide ranges of desired materials in good quantities (Birtchnell and Urry, 2013) and could be the platform for producing accurate dosage forms tailored to patients' needs (Goyanes *et al.*, 2015, Karki *et al.*, 2016). Recently, inkjet printing was used for preparation of personal dose oral films containing salbutamol sulphate made of potato starch for paediatric patients (Buanz *et al.*, 2011). With the advantages of printing technologies in terms of flexible dosing, the potential in drug dosing tailoring of ODF could bring benefits for patients, regardless of the age.

1.5.2 Factors affecting film manufacturing process

The physicochemical properties of APIs are the criteria factors that should be primarily considered. Drugs should be compatible with a suitable solvent and other film forming agents (Mishra and Amin, 2011). Another parameter is the moisture present in the solution; the moisture is observed to modify the mechanical properties of film, therefore a suitable humidity environment is a subject to be controlled during manufacturing (Rathi *et al.*, 2011).

1.5.3 Principal advantages of solvent casting methods

Solvent casting technology offers a unique drying process on the surface without the application of mechanical or thermal stress (Siemann, 2005). Additionally, both conventional and modern casting techniques deliver uniform thickness distribution, high optical purity and feasible processing of heat sensitive excipients (Siddiqui *et al.*, 2011). However, major drawbacks of conventional tools involve slow production speed and require further facilities for handling solvents (Siemann, 2005). Nowadays, the technology of solvent casting film promotes the production of wafer and tension-free film down to 5 µm in thickness. The use of polymers with high molecular weight can also be used for film development (Scheuermann, 2013).

1.6 Mechanism of Film Formulation

In order to understand the mechanical behaviour of polymers, numerous structural characteristics including chemical composition other physical factors should be reflected. Classifications of polymers are highly dependent on the types of polymerisation process as well as other schematically structures and properties (e.g thermos plastics, rubbers or thermosets).

1.6.1 Polymer classification

Polymers are defined as large molecular chains composed of a repetition of small molecules (monomers) (ASM International, 2003). They have unique chemical and physical properties and are categorised by their structure and properties. They may be either linear or branched molecules, whilst the chemical nature of the monomer and the flexibility of the polymer chain propose a significant impact on its mechanical performance.

ODFs are composed of one or more water soluble polymers (e.g. natural or synthetic), in order to dissolve quickly in the mouth. The selection of polymer is the key factor that contributes to successful film formulation; depending on the desired formulation design, the appropriate selection of type and concentration of polymer is a critical parameter for the ensuing mechanical properties, disintegration time, as well as the drug loading of the ODFs (Borges *et al.*, 2015). Given that ODFs target fast release and rapid onset of action, polymers therefore should be harmless and flexible, exhibit good wetting and spread ability, whilst also displaying adequate stability, sufficient peel, shear and tensile strengths (Leuner and Dressman, 2000, Dixit and Puthli, 2009). Hence, a variety of polymers have been extensively studied to modulate properties of films. Cellulose derivatives, especially hydroxypropyl methyl cellulose (HPMC) and hydroxylpropyl cellulose (HPC), available at different grades and the substitution groups, are widely used as film coating agents, as well as lubricants (Lopes *et al.*, 2006). For example, HPMC classified grades K and E are extensively used for film formulation; the use of type K is used as a delayed agent for sustained release formulations, whereas type E is more popular for being a film former (Karki *et al.*, 2016). Different grades of HPMC have also been

studied for the development of fast dissolving films of triclosan, indicating they were good film forming materials (Dinge and Nagarsenker, 2008). Similarly, certain drugs, such as famotidine or granisetron HCl, loaded into oral film using HPMC exhibited satisfactory mechanical properties (Sonawane *et al.*, 2012, Chaudhary *et al.*, 2013). Besides that, HPC used as a film former demonstrated good mechanical properties and carrying capacity of non-steroidal anti-inflammatory drug, ketorolac tromethamine, for buccal delivery (Alanazi *et al.*, 2007). Furthermore, other natural polymers, such as starch, sodium alginate as well as pectin are considerable choices for film development. Due to wide availability and low cost, starch is considered as the potential polymer for the film applications; nevertheless, film forming from starch was found to have a limitation of mechanical strength, brittle and crystal formation on films (Nagar *et al.*, 2011). Modified starch has been developed to improve the product performance and is available as maltodextrin or hydroxypropyl starch. Development of films with the modified starch, maltodextrin, showed a good quality with fast disintegration time (Shamekh *et al.*, 2002, Cilurzo *et al.*, 2010). Sodium alginate is another natural polymer that is widely used as a thickening, stabilising and emulsifier agent (Dixit and Puthli, 2009), but it has also been used to improve drug loading capacity of mucosal films using paracetamol and amoxicillin as model drugs (Boateng *et al.*, 2013). Pectin is the popular agent for sustained release of drug, therefore is not considered as a potential polymer candidate for orally dissolving films with the aims for fast release (Borges *et al.*, 2015, Karki *et al.*, 2016).

Synthetic polymers have also been used as polymer matrices for other drug delivery systems. Gelatin is well-known as the coating layer for the preparation of hard and soft capsules, but has also been utilised in implantable delivery systems, especially as sterile

film or ophthalmic film (Nagar *et al.*, 2011). Polyvinyl alcohol (PVA) has also been extensively employed as a film former (Nagaraju *et al.*, 2013). Kollicoat® IR and Kollicoat® protect (KP) are both new generation pharmaceutical excipients composed of polyvinyl alcohol – polyethylene glycol graft copolymer that are freely soluble in water. Films developed with these new excipients generate flexible films with great stability against moisture and fast disintegration profile (BASF, 2007).

Although the available choices of polymer for use in delivery systems is vast, a careful choice of polymer is necessary for producing good quality orally dissolving films with desired therapeutic dose for paediatric use.

1.6.2 Molecular mass and distribution

The distribution of chain length within polymers depends strongly on the intermolecular forces between neighbouring molecules, the molecular weight and chain figurations (Ward and Sweeney, 2004). Each polymer varies with the chain length and molecular weight. The correlation between mechanical behaviour and molecular weight distribution of polymer showed that molecular weight distribution of polymer might influence indirectly its mechanical behaviour, but directly alter its viscoelastic behaviour and brittle strength (Ward and Sweeney, 2004). Besides that, regarding to chain length of the polymer, longer polymer chains lead to reduction of the chain mobility and segmental motion of the molecules for supported film; whereas short chains have more free volume and less surface density, and hence lowering the glass transition temperature.

1.6.3 Interaction forces

The most recognised attractive forces between polymer chains include electrostatic interaction, hydrogen bonding and *Van der* Waals forces. The electrostatic interaction involves the interaction of charged groups, hydrogen bonding is considered as a strong physical interaction in self-assembly, whilst *Van der* Waals force is defined as the attraction of intermolecular forces between molecules. Polymers with strong polar groups and numerous hydrogen bonds (e.g. polyvinyl alcohol, polyacrylic acid) exhibit strong intermolecular forces, while other non-polar polymers held by weak Van der Waals interactions (Cao *et al.,* 2009). Therefore, a wide range of numerous factors should be investigated to achieve a better mechanical behaviour of polymers.

1.6.4 Stages of film formation

The processes of film formation are divided into two categories: dry and wet process (Guilbert *et al.*, 1997). There is no water and other solvents needed for the dry process of films. In this process, only heat is applied for drying the film forming materials. For the wet process, solvents are added to dissolve the polymers, subsequently drying to remove them and form a film product. Therefore, the selection of solvent is important.

The mechanism of film formation is highly dependent on whether the polymer is in a dissolved or dispersed state. Film formation from aqueous based polymeric solution is a process that spreads onto the surface of substrate, since the polymer is in the dissolved state. The stages of film formation undergo three mechanisms, which involve water

evaporation, particle packing (nucleation), particle deformation and particle coalescence (Fig. 1.5) (Felton, 2013).

Initially, the process starts with wetting and spreading polymeric solution onto the flat surface of the substrate. In this stage, the rate of solvent evaporation is the criteria parameter for film development. It has been proved that the solution is dried at a constant rate (Steward *et al.*, 2000). During drying, the polymer sphere starts packing tightly after application due to water evaporation. Subsequently, the polymer spheres deform into space filling, which happens at the same temperature for dry particles as well as particles casted in the wet film. With continuing drying film, the polymer particles anneal by migration of polymer chains to form the film (Figure 1.5).

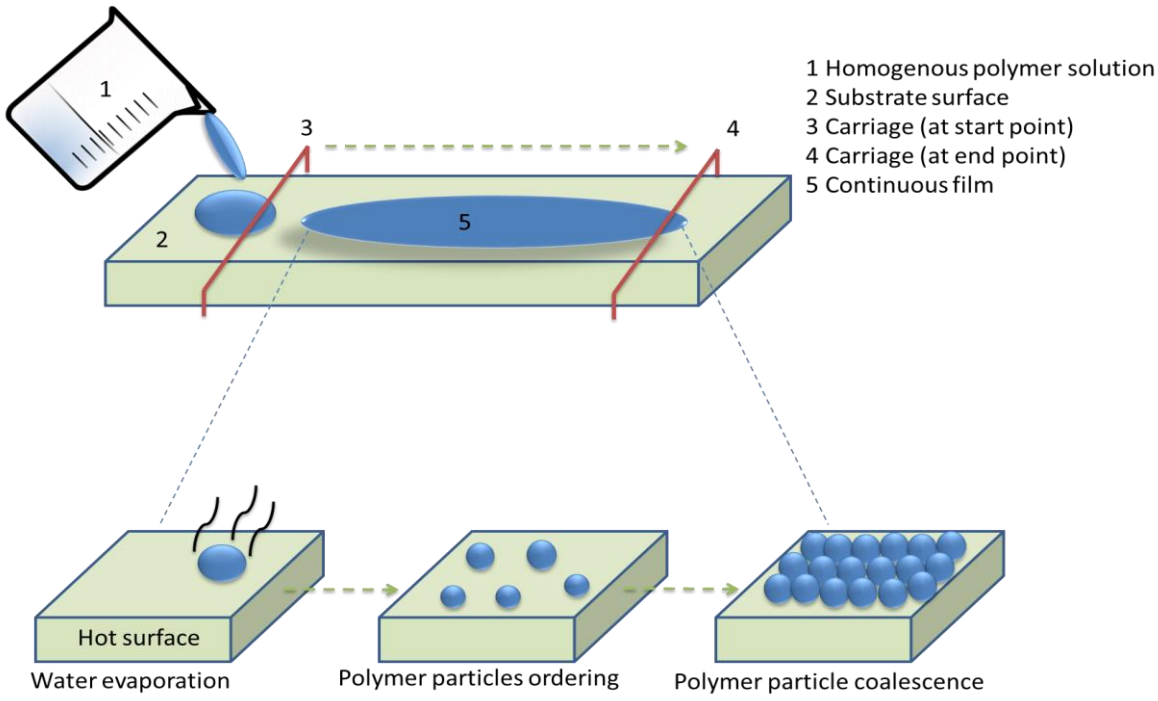


Figure 1.5- Schematic diagram of polymeric film formation.

1.6.5 Driving forces of film formation

Three existing theories have been discussed in literature reviews regarding the driving forces of film formation in dispersed state: polymer/ air surface tension (Dillon *et al.*, 1951), capillarity force or air/ water interfacial tension (Brown, 1956) and osmotic pressure (Sheetz, 1956). However, depending on the material's properties, polymer coalescence and film formation occurs as a consequence of any of those above mentioned driving forces.

The rate of drying has a strong impact on the film formation and is dependent on the relative humidity of the environment. Faster coalescence and increased polymer chain interdiffusion has been evidenced of high humidity conditions (Carlin *et al.*, 2008, Haley *et al.*, 2008).

1.7 Quality Control of Orally Dissolving Films and Their Challenges

Several challenges can be encountered when the formulation manufacture is transferred from laboratory scale to production scale. The final products should have adequate flexibility and good physico-chemical stability. Quality control of films is a pre- requisite to ensure the performance of films.

1.7.1 Thickness

Film thickness is directly proportional to the amount of loaded drug. The thickness of the ODF can be determined at different locations by digital micrometer gauge to ascertain uniformity in thickness. Films should have an ideal thickness of 50 to 1000 µm (Nair *et al.,* 2013).

1.7.2 Mechanical properties

Mechanical properties of ODFs play an important role for physical integrity for ODF production, but also for handling matters. Several encountered factors, such as types of film forming agents, quantity of plasticiser, film thickness, manufacturing process, storage conditions, as well as the use of APIs impact on the mechanical properties of films (Preis *et al.*, 2014). Literature revealed different methods to determine the mechanical strength, however there are no specifications available for ODF in terms of tensile strength and practical applicability (Morales and McConville, 2011). For instance, the Texture Analyser is the most common choice for determination of tensile property of ODTs, but films need to be cut into the standard template, which is difficult when taking into account comparisons due to a variety in sizes and shapes (Boateng *et al.*, 2009). Radebaugh *et al* (1988) designed the promising puncture testing system for polymeric films using different polymers. The use of texture analyser (TA.TX2® model, Stable Micro Systems) was firstly reported to analyse the mucoadhesion strength of controlled release Eudragit buccal films (Wong *et al.*, 1999). Hence, several modifications have been developed for adaptable evaluation of this parameter for ODFs (Eouani *et al.,* 2001, Prodduturi *et al.*, 2005, Perumal *et al.*, 2008, Garsuch and Breitkreutz, 2009, Preis *et al.*,

2014). The most relevant studies can be defined in terms of tensile strength, percentage elongation and Young's modulus, as follows (Dixit and Puthli, 2009, Preis *et al.*, 2014):

a) Tensile strength

Tensile strength is the maximum force applied to the film until the film specimen breaks. It is calculated by the applied forces of break divided by the cross- sectional area of the film. It can be computed with the Eq. (1)

$$\text{Tensile strength} = \frac{\text{Maximum force was applied}}{\text{Cross sectional area of film}} \quad (1)$$

b) Percentage Elongation

Elongation is defined as the deformation of film divided by the original length of the specimen and is calculated by the Eq. (2). The more plasticiser is added, the stronger the elongation of film is.

$$\%\ \text{Elongation} = \frac{\text{Length after force was applied}}{\text{Original length}} \text{ x } 100 \quad (2)$$

c) Young's Modulus

Young's modulus or elastic modulus is the measurement for the stiffness of film in the elastic region. It is obtained from the ratio of stress over strain using Eq. (3) as follow:

$$\text{Young's modulus} = \frac{\text{slope of stress}-\text{strain}}{\text{cross sectional area x corresponding strain}} \quad (3)$$

Depending on the designed formulations and chosen manufacturing methods, different behaviours of mechanical properties obtained from stress –strain curves are illustrated in Figure 1.6.

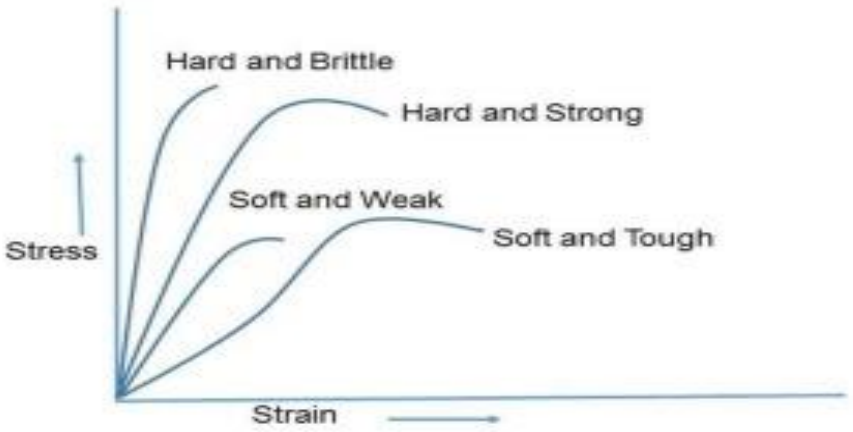


Figure 1.6- Behaviours of ODFs with varying mechanical properties, obtained from stress-strain curves. Apdated from Felton (Felton *et al.*, 2008).

Previous reports described that soft and weak polymers have low tensile strength, low percentage elongation and Young's modulus, whilst soft and hard polymers exhibit hard and stronger in the mentioned properties (Heng *et al.*, 2003, Morales and McConville, 2011). In fact, a wide variation of types of polymers and the choice of manufacturing creates the difficulty in establishing the values for these parameters. Hence, there are no standardised descriptions for appropriate values for mechanical strength and other studied ranges (Preis *et al.*, 2014, Borges *et al.*, 2015). Therefore, dependent on the formulation choice and delivery purposes, these crucial features are taken into account for controlling the physical strength of ODFs.

1.7.3 Disintegration time

As this is a new innovative technology, a set of associated limitations and challenges arise in the quality control of ODF formulations. ODFs are designed for quick release purposes when applied onto the tongue. Following the European Pharmacopeia, orodispersible tablets (ODTs) should disintegrate in less than 180 seconds (European Pharmacopoeia Commision, 2013) and less than 30 seconds by FDA guidance (FDA, 2008); whereas ODFs, recently subordinates to the "oromucosal preparations" monographs, only stated as "dissolve rapidly" without a defined time limit (European Pharmacopoeia Commision, 2013), although the disintegration time limit of 30 seconds or less is a recommended value for ODFs (Barnhart *et al.*, 2008). However, no standard pharmacopoeia disintegration test method for ODFs exists. Literature described that several disintegration test methods have been developing to verify the suitable system for disintegration time of ODFs (Table 1.4) (Hoffmann *et al.*, 2011). Reports showed that the standard pharmacopoeia disintegration system, without modifications, for tablets and capsules was applied to ODFs (Shimoda *et al.*, 2009, Cilurzo *et al.*, 2011, Liew *et al.*, 2014). The above method faces challenges with the end point determination due to film floating in a large volume of media (Low *et al.*, 2015). Alternatively, two simple methods for determining the time using a small volume of media, such as Petri dish and slide frame, has been studied in previous reports (Garsuch and Breitkreutz, 2010, Londhe and Umalkar, 2012, Preis *et al.*, 2012, Poluri *et al.*, 2013). However, these methods could not provide mechanical agitation of water droplets to create a hole or having enough water droplet to cover the surface of ODFs (Low *et al.*, 2015). Disintegration time can also be recorded by dipping films in a beaker containing 25 mL volume of media with a gentle stirring (Arya *et al.*, 2010, Tomar *et al.*, 2012, Bala *et al.*, 2013). Other literature reveals the exposition of

ODF on a stainless steel wire mesh, where the media is added to the surface until the films breaks, which is noted as disintegration time (Mishra and Amin, 2009, Joshi *et al.*, 2012, Bala *et al.*, 2014). In some cases, the measurement of swelling behaviour of films was applied to predict the disintegration time (Peh and Wong, 1999, Hoffmann *et al.*, 2011). On the other hand, those previous methods lack a defined and clear end point for disintegration. A novel disintegration test system was designed, with electronic end- point detection (Preis *et al.*, 2014). Films were clamped vertically into two clips equipped with a brass plate acting as a weight. When the film starts to disintegrate, it causes the dropping down of the clip weights, with the end point recorded visually by weight drop with time display. Low et al. (2015) set up a modified disintegration test system for ODFs, which attached six holder units where films were held in a horizontal position for test, by emerging them up and down at the modified speed and stroke. The breaking of ODF from the holder is defined as the end point disintegration (Low *et al.*, 2015). Dave et al. (2014) introduced the texture analyser as a disintegration testing system; the films are placed on the platform where the attached probe, mimicking the oral cavity pressure, moves with the trigger force until it touches the film. A small amount of media is applied and time for complete disintegration is recorded. The method developed using TA-CT3 texture analyser with probe provides clear end-point determination, whilst using small media volume aims to be biorelevant to the oral cavity volume (Dave *et al.*, 2014). Hence, an appropriate set up disintegration test system that is biorelevant to physiological conditions of the oral cavity with the consistency of measured disintegration time is needed for quality control settings.

Table 1.4- A review summary of disintegration test methods.

Media volume	Test system	Description of method
Small volume	Petri dish method	ODF is emerged into 2 mL of distilled water over the Petri dish until film is completely dissolved as noted as disintegration time.
	Slide frame method	Films are clamped into slide frame. A drop of water is added to the ODF. Time taken by the films to create a hole is recorded.
	Wire mesh method	ODF is placed on a stainless steel wire mess where 10 mL of media is added to the surface until the films break
	Beaker method	ODF is dipped into a beaker containing 25 mL water with a gently shaking until disintegration is observed.

Swelling studies	Contact angle measurement	The angle determination is carried out by a digital camera between a droplet of water and a planner surface of ODF.
	Modified disintegration apparatus	The degree of swelling is measured by the weight change of ODF before and after immersion into solution.
Large volume	USP disintegration system	ODFs are submerged into disintegration apparatus with certain speed until they are completely dissolved.
	Modified disintegration apparatus	ODFs are submerged into disintegration apparatus with certain speed until they are completely dissolved.

1.7.4 Stability studies

It is important to carry out stability studies for the prepared formulation to gain the information on how a new product is influenced by various exposed environmental factors, including temperature, humidity and light, and to identify any degradation in the formulation. Recommended storage conditions should be followed by according to International Conference on Harmonisation (ICH) guidelines (Q1A (R2)) for use termed: long term, intermediate and accelerated (Table 1.5).

Table 1.5- Recommended storage conditions for stability study detailed by the ICH guidelines.

Study	Environmental condition	Time period
Long term	25°C ± 2°C/60% RH ± 5% RH or 30°C ± 2°C/65% RH ± 5% RH	12 months
Intermediate	30°C ± 2°C/65% RH ± 5% RH	6 months
Accelerated	40°C ± 2°C/75% RH ± 5% RH	6 months

A new product, such as oral dissolving films, should be stored under controlled environment by ICH guidelines at the regular time point to establish the full stability profile of the ODF. The chemical instability of the polymer matrix may occur or the change of active ingredient may interfere with the matrix (Nagaraju *et al.*, 2013). Different parameters of films, such as physical appearance of the film, mechanical properties, and drug content, are required to be evaluated during its development.

Literature study showed that films were kept in aluminium foil under recommended conditions (eg. Long term and accelerated) for three months period (Bala *et al.*, 2013).

1.8 Thesis Aims and Objectives

The development of dosage formulations for paediatrics is still a big challenge, due to lack of mainstream research and development. However, orally dissolving films, a new drug delivery system for the oral route, serves as an alternative oral solid dosage form for paediatrics. This new system provides patients the medicine without the need of swallowing and offers rapid dissolution in a small amount of saliva without the consumption of water. Therefore, the overall aim of this project is to develop the ODF formulation with suitable physico-chemical and clinical properties as a new potential dosage form for paediatric use.

Therefore, the objectives of the research areas were focused as following:

- Screening of polymer materials and manufacturing optimisation for formulations of ODFs.

- Explore the strategic approaches for taste masking of bitter drugs and drug loading improvement, particularly of poorly soluble drugs.

- Optimisation of a suitable disintegration testing system and some prototype packaging development.

Chapter 2
Materials and Methods

2.1 Materials

Pectin from apple, Sodium alginate, Hyproxylmethylcellulose (10,000), Kollicoat Protect, Kollicoat Immediate release (IR), Maltodextrin, Starch, Gelatin, Sodium carboxy methylcellulose (90,000), Polyvinyl pyrrolidone (K15, K30), Polyvinyl alcohol (31,000-50,000), Polyvinyl alcohol (13,000- 23,000), pullulan were purchased from Sigma-Aldrich, UK. Glycerol (≥ 99.5 %), polyethylene glycol (PEG) 400 and Tween 80 used as plasticiser were obtained from Sigma- Aldrich, UK. Different types of cyclodextrins such as alpha, beta, gamma, 2-hydroxypropyl- β- cyclodextrin were purchased from Discovery Fine Chemical Ltd (Dorset, UK). Polycaprolactone (average Mw ~14,000), pluronic® F-127, phosphate buffer saline were also obtained from Sigma Aldrich (UK).

Dexchlorpheniramine maleate was acquired from Sigma- Aldrich, UK. Metformin hydrochloride and glipizide was obtained from Discovery Fine Chemicals Ltd. All other materials were of analytical grade.

2.2 Methods

2.2.1 Screening of film former for ODFs pre-formulations

Different types of water soluble polymers, such as pectin from apple, sodium alginate, hydroxypropylmethylcellulose (HPMC) (10,000), kollicoat protect (KP), kollicoat IR, maltodextrin, starch, gelatin, sodium carboxy methylcellulose (90,000), polyvinyl pyrrolidone (K15, K30), methylcellulose, polyvinyl alcohol (31,000- 50,000), polyvinyl alcohol (13,000- 23,000) and pullulan were selected in order to choose the suitable polymer for the formulation development of ODFs. Each polymer (10 % w/v) was

accurately weighed and prepared by dissolving in 10 mL distilled water to form uniform clear viscous solution. The solutions were then mixed and stirred to form a homogenous viscous solution, which was subsequently degassed under vacuum to remove the air bubbles. The solution is finally cast onto a baking tray and dried in the oven at 60 ºC for at least 2 hours to form a thin film. The films were carefully removed from the mould and cut into desired size and shape (3x2 cm^2 per strip) (Alam *et al.*, 2015, Senthilkumar and Vijaya, 2015). Casting films were examined for their physical and mechanical properties.

Figure 2.1- Images of ODFs developed by the baking tray method.

2.2.2 Screening of plasticiser and plasticiser concentration

The selected polymers from the initial screening were studied with plasticiser for their compatibility. Glycerol, polyethylene glycol (PEG) 400 and Tween 80 were added in varying amounts (5, 10 or 20 µL of plasticiser), to a clear polymer solution (10 mL). Both the solutions are then mixed and stirred to form a homogenous viscous solution, which was subsequently degassed under vacuum to remove the air bubbles and allowed to dry in the oven at 60 ºC for at least 2 hours to form films. Plasticiser that is compatible with film forming polymer was further selected for characterisation, including its effect on tensile properties and disintegration time as well as the capability of drug loading.

2.3 Preparation of drug loaded ODFs

2.3.1 Metformin HCl

Polymer solution prepared as described above was used for the loading capacity of drug into ODFs. A quantity amount of Metformin HCl at a dose loading of 10 mg, 20 mg and 30 mg per 3x2 cm^2 of ODF was added to the polymer solution. The entire solution was sonicated to remove entrapped air and to enhance the drug dispersion. The above method was replicated for producing drug loaded films (see 2.2.1). The prepared films were stored and studied for physicochemical stability (see chapter 3).

2.3.2 Preparation of glipizide- cyclodextrin complexation

Glipizide and different types of CDs (alpha, beta, gamma, and HPCD) were accurately weighed in the molar ratio of 1:1 (drug: carrier). Glipizide-CD complexes were produced by the kneading method (Aly *et al.*, 2003, Choudhary *et al.*, 2009), where F1= αCD/ glipizide complex, F2= βCD/ glipizide complex, F3= γCD/ glipizide complex, F4= HPCD/ glipizide complex. Glipizide was firstly solubilised in 2 mL of ethanol and then added to slurry of CD in ethanol to produce a paste. The paste was then kneaded by mortar and pestle for one hour to produce a homogenous mixture, followed by drying at 40 ^{0}C for 24 hours to remove all solvents.

2.3.3 Preparation of glipizide-CD complex loaded films

Films containing glipizide-CD complexes were prepared using Kollicoat Protect (KP) by the solvent casting method. The water soluble polymer (KP) was first dissolved in 10 mL distilled water to form a uniform clear solution. Glycerol as plasticiser (1 % w/v) was added to all formulations. After the polymer was completely dissolved, each type of glipizide-CD complex was added and stirred further in order to form a homogenous viscous solution. This solid dispersion was subsequently degassed under vacuum to remove the air bubbles. After degassing, the solution was finally casted on the aluminium flat surface of Elcometer 4340 Automatic film applicator (Elcometer Ltd., Manchester, UK) and dried at 50 ºC for 45 minutes. The films were carefully removed from the surface and cut into the desired size and shape (3 x 2 cm^2 per strip). The films were stored in a desiccator for further analysis (see chapter 4).

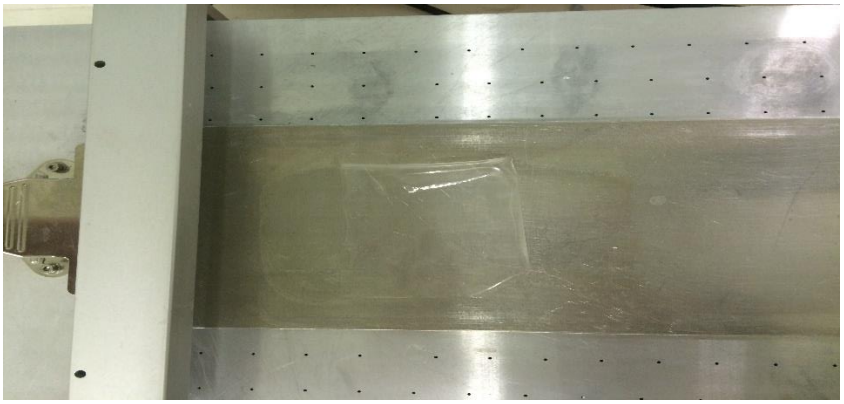


Figure 2.2- Films developed by Elcometer 4340 Film Applicator.

2.3.4 Preparation of ODF containing anti-histaminic drug

Films containing Dexchlorpheniramine maleate (DCM) at a dose of 1 mg and 2 mg were prepared using Kollicoat protect as a film former by solvent casting method using Elcometer 4330 film applicator (see 2.3.3) . DCM and glycerol was added to all formulations and stirred further to uniformly incorporate into the solution. The similar method was replicated for producing these formulations (see section 2.3.3). Dried films were carefully removed and stored in the desiccator till further use.

2.3.5 Preparation of drug loaded nanoparticles

Glipizide was chosen as a model BCS class II drug, due to its poor solubility and its clinical efficacy at low dose. The preparation of glipizide loaded nanoparticles was prepared by the solvent displacement method; briefly, an accurate amount of poly-ε-caprolactone (0.2- 0.5 % w/v) was dissolved in 10 mL of acetone, under continuous stirring with gentle heating for 30 minutes. Glipizide (10 mg drug) was added into the dissolved polymeric solution and mixed thoroughly to obtain a homogenous solution. The

organic solution was then added dropwise to 10 mL phosphate buffer saline solution containing Pluronic F- 127 (0.25 % w/v) and stirred well using a magnetic stirrer for 10 minutes at room temperature. The resultant nanospheres were formed by rapid solvent diffusion, whilst the residual solvent from the nanosphere suspension was removed under pressure by a rotary evaporator. The non-entrapped drug particles from the nanospheres were further separated by centrifuging them for 30 minutes at speed of 3200 revolutions per minutes using centrifugation Universal 32 (Hettich Zentrifugen, Germany). The retained supernatant containing nanoparticles was collected and redispersed in the same volume of water as before centrifugation.

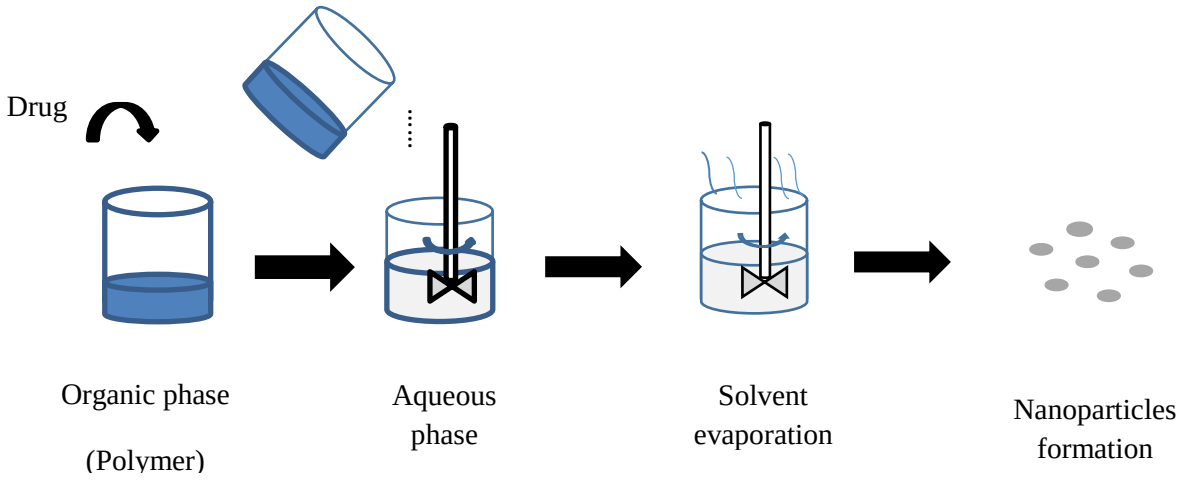


Figure 2.3- Schematic representation of producing nanoparticles by nanoprecipitation method.

2.3.6 Preparation of ODFs loaded with drug containing nanoparticles

Films containing glipizide nanoparticles were prepared using Kollicoat Protect (KP) as a film former by the solvent casting method. The water soluble polymer (10 % w/v) was first dissolved in 10 mL distilled water to form a uniform clear solution. Glycerol as plasticiser (1 % w/v) was added to the polymeric solution. Once the polymer was completely dissolved, the drug-loaded nanosphere suspension was further added to the polymer solution and stirred well to ensure the nanoparticles uniformly distributed. This solution was finally casted on the aluminium flat surface of Elcometer 4340 Automatic film applicator (Elcometer Ltd., Manchester, UK) and dried at 50ºC for 45 minutes. The films were carefully removed from the surface and cut into the desired size and shape (3 x 2 cm^2 per strip). The films were stored in a desiccator for further analysis.

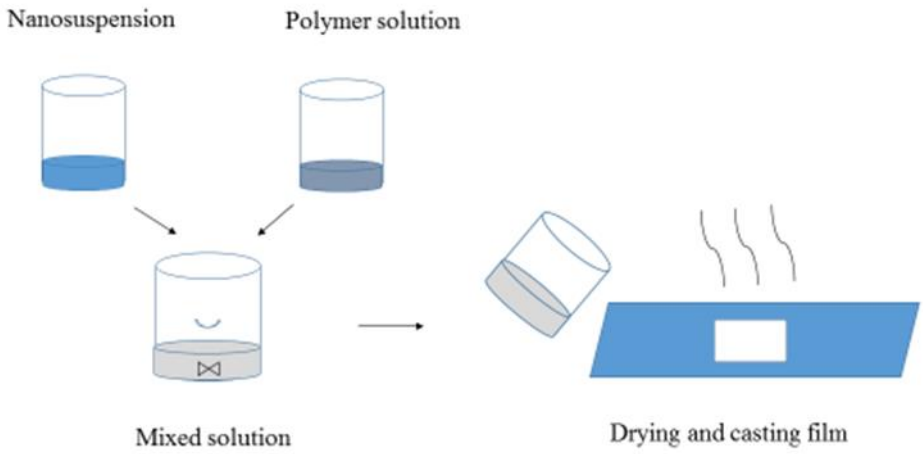


Figure 2.4- Process schematic for the casting film containing nanoparticles.

2.4 Characterisation of glipizide complexation

2.4.1 Phase solubility studies of glipizide

Phase solubility studies are carried out to study the affinity binding between CDs and glipizide. The technique was assessed by Higuchi and Connors methods (Higuchi and Connors, 1965). An excess amount of glipizide and different types of CD complexes were added to 10 mL of 70:30 methanol: water containing various concentrations of CDs (0-0.025 M). The mixture was shaken for 24 hours on the rotary flask shaker until equilibrium was reached. The solution was withdrawn and filtered through filter paper (Whatman, Grade 1), followed by drug assay by UV spectrophotometry at 276 nm. The experiments were conducted in triplicate. The binding constant (Kc) can be calculated using the equation below:

$$K_{1:1} = \text{slope} / S_0 (1\text{-slope})$$

Where S_0 is the intrinsic solubility of glipizide, and slope is obtained from the calibration curve of glipizide concentration against CDs.

2.4.2 Solubility studies of glipizide

Solubility studies of glipizide were determined using glipizide and complex equivalent to 10 mg of drug in distilled water and phosphate buffer pH 7.4 stirring for 24 hours, which was then assayed by UV spectrophotometry at 276 nm. The solubility studies were assessed in triplicate and data were the average values.

2.5 Characterisation of nanoparticles distribution

2.5.1 Particle sizes, polydispersity index determination

The average particle size and polydispersity index (PDI) of blank nanoparticles and drug loaded nanoparticles was determined by dynamic light scattering particle size analyser using NanoBrook 90Plus Zeta (Brookhaven, New York, USA). A cuvette containing 100 µL of the nanosuspension was diluted with the hydration phase up to 1 mL and all the measurement were carried out in triplicate at 25 ^{0}C at a 90 ^{0} angle.

2.5.2 Measurement of zeta potential

The zeta potential of these nanoparticles was measured using an Electrophoretic Light Scattering technique with NanoBrook 90Plus Zeta (Brookhaven, New York, USA) at 25 ^{0}C in distilled water. 100 µL of the sample was diluted in 1 mL distilled water. Three measurement of samples were carried out for the determination.

2.5.3 Scanning Electron Microscope (SEM)

To obtain the morphology of nanoparticles located inside ODFs, scanning electron microscopy technique was performed. The film sample was cut into small piece and placed on the double adhesive carbon tape over an aluminium tub. Samples were further coated with gold layer in an Emscope SC500 sputter coater at 20 mA for 1 minute (Quorum Technologies, Lewes, UK) before scanning of the samples. The images were captured by a field scanning electron microscope (Phillips XL 30, Eindhoven, Netherlands).

2.6 Preparation of simulated saliva solution

The simulated saliva solution in this study was prepared according to Koland et al (2011), with the formula given in table 2.1. First, 2.382 g of disodium hydrogen phosphate was dissolved in 1 litre of distilled water and the solution was stirred until completely dissolved. Potassium dihydrogen phosphate and sodium chloride were then added to form a homogenous saliva solution**.** The pH of saliva fluid was adjusted to 6.75 with phosphoric acid and it was used as test medium for the disintegration study.

Table 2.1- Composition of simulated saliva solution. The preparation of saliva fluid was adapted from (Koland et al., 2011).

Component	Quantity
Disodium hydrogen phosphate	2.382 g
Potassium dihydrogen phosphate	0.19 g
Sodium chloride	8.0 g
Distilled water	Up to 1 litre

2.7 Evaluations of ODFs

2.7.1 Visual inspection of films

Developed films were evaluated by visual inspection for their transparency and the capability to form a thin film, which should be removed easily from the casting surface. They are classified ranging from good to poor (Kulkarni *et al.*, 2010).

2.7.2 Thickness

The thickness of films was determined by a micrometer dial thickness gauge (Coventry, UK). The film was hung on the anvil and the reading on the dial was recorded. Every film was measured five times to calculate the average value.

2.7.3 Uniformity in weight

The uniformity of film weight was carried out by using 10 preparation units (BP, 2013). Each film (3 x 2 cm^2) was taken randomly and was weighed collectively. The values are the mean of 10 samples (n= 10) and the values are expressed as mean ± SD.

2.7.4 Mechanical properties of films

The mechanical properties of films containing water soluble drug (DCM) and poorly water soluble drug (glipizide) were evaluated using Hounsfield Tensometer, S Series testing machine (Tinius Olsen Ltd, Surrey, UK), with load cell 50N. Films with the size 3x2 cm^2 were attached on two clamps at the distance at 30 mm. These films were pulled

by two clamps at rate of 50 mm/min. The parameters of mechanical properties including tensile strength, elastic modulus and elongation were assessed. Three replicates were done by the following equations.

a) Tensile strength

$$\text{Tensile strength } (\text{N/mm}^2) = \frac{\text{Maximum force was applied}}{\text{Cross sectional area of film}}$$

b) Percentage Elongation

$$\% \text{ Elongation} = \frac{\text{Length after force was applied}}{\text{Original length}} \text{ x } 100$$

c) Young's Modulus

$$\text{Young's modulus } (\text{N/mm}^2) = \frac{\text{slope of stress-strain}}{\text{cross sectional area x corresponding strain}}$$

2.7.5 Determination of moisture uptake

Films were cut at the size of 3x2 cm^2 for evaluation. The moisture uptake was carried out by exposing them to an environment at 75 % relative humidity at room temperature (25 ± 2 ^{0}C) and in a desiccator for 1 week (Dinge and Nagarsenker, 2008)**.** Each film from

different formulations was measured in triplicate and calculated as percent increase in weight.

2.7.6 FTIR studies

To investigate the interaction between drug and excipients in the films, the FTIR spectra of each compound were tested in the regional wavelengths of 400 – 4000 cm^{-1} by Thermo Scientific Nicolet IS5 FTIR Spectrometer (Massachusetts, USA) implemented with an iD5 reflectance diamonds. A small sample of film and other powders (10 mg) was placed securely on the surface of diamond eyes followed by 16 scans to produce the resulting spectra.

2.7.7 DSC studies

Thermal properties of film formulations and other excipients were carried out by DSC Q200 V24.4 build 116, TA instrument (Delaware, USA). An empty pan was crimped and used as reference. The film sample (3 mg) was cut and then placed in aluminium T zero pans. Both the sample and reference pan were heated at the temperature ramp speed of 10 ^{0}C / min in the presence of nitrogen gas as effluent gas. The heat flow was set in a range of 20 ^{0}C to 250 ^{0}C. This was then followed by the graphs expressing the melting onset peaks and enthalpy fusion by the TA instrument universal analysis software.

2.7.8 HPLC method for glipizide

The reverse phase HPLC analytical method for glipizide was obtained from an Agilent 1200 Series (Waldbronn, Germany) with a multiple UV/ Vis detector and a C18 Phenomenex Luna column (150 x 4.6 mm, 5 µm). The mobile phase was prepared, consisting of acetonitrile: phosphate buffer solution (65: 35 % v/v). The mobile phase was filtered and sonicated before use. The elution was carried out at the constant flow rate of 1 mL/ min and the injection volume of 20 µL under the UV detection at 233 nm. The calibration curve was obtained using a range of concentrations from 0.1 to 0.5 mg/ mL of standard solution.

2.7.9 Drug loading

A) Glipizide

The drug loading of glipizide, glipizide film and glipizide complex film was determined. Three films (3 x 2 cm^2) from the same batch were dissolved in 10 mL of 70: 30 ethanol: water (see Appendix 1). The solutions were then filtered and diluted, which were then analysed at 276 nm by UV. The results were expressed as mean of three determinations.

B) DCM

Each film (3 x 2 cm^2) from the same batch containing 1 mg and 2 mg DCM were selected randomly. Films were completely dissolved in 100 mL of distilled water. After the dilution, the samples were analysed at 254 nm by UV. The results were expressed as mean of three determinations.

2.7.10 Drug content uniformity

The content uniformity of glipizide loaded films was determined by were dissolved in 10 mL of 70: 30 ethanol: water (see Appendix 1). The solutions were then filtered and diluted, which were then analysed at 276 nm by UV. The results were expressed as mean of three determinations. Each film's drug content was compared with the mean measured of drug content in the sample. Individual value of film sample should fall within 85- 115 % specification range of the drug monograph.

2.7.11 Drug entrapment efficiency

The drug entrapment efficiency of nanoparticles was analysed after the centrifugation of nanosuspension by measuring the concentration of non- entrapped drug. The supernatant was filtered through a 0.45 µm filter and then analysed by UV spectroscopy at 276 nm.

The drug entrapment efficiency of nanoparticles was calculated using this following formula:

$$\text{Drug entrapment effeciency (\%)} = \frac{\text{Total amount of added drug} - \text{amount of free drug}}{\text{Total amount}} \text{ x } 100$$

2.7.12 Drug release studies

A) Glipizide loaded nanoparticles

Drug release studies of glipizide loaded nanoparticles were carried out in 50 mL glass beaker containing 25 mL simulated saliva fluid (pH 6.8) and phosphate buffer solution

(pH 6.8) using the centrifugation. The temperature was set at 37 ± 0.5 ^{0}C and was stirred at 100 rpm. Aliquot of 5 mL was withdrawn from the dissolution medium at 1, 5, 10, 15, 20, 30, 45 and 60 minutes and same amount was replaced with the fresh media. The amount of drug released was analysed by UV spectrophotometer at the wavelength of 233 nm.

B) DCM

For drug release studies, ODFs loaded with DCM were performed in a 50 mL glass beaker containing 25 mL simulated saliva fluid (pH 6.8) and phosphate buffer solution (pH 6.8). The temperature was set at 37 ± 0.5 ^{0}C and each film was stirred at 50 rpm. During the experiment, the dissolution medium (3 mL) was withdrawn at 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 minutes and was replaced with the equal amount of fresh solution. The amount of drug released was analysed by UV spectrophotometer at the wavelength of 254 nm. Each sample was done in triplicate.

2.7.13 Stability studies

The stability studies followed the guidance of ICH for the ODFs as new pharmaceutical formulations. These studies were carried out by storing films at long term (25 ± 2 ^{0}C and 60 ± 5 % RH) and accelerated stability condition (40 ± 2 ^{0}C and 75 ± 5 % RH). All film samples were monitored at the initial day, 1 month and 3 months and evaluated for visual inspection for any change in appearance, mechanical properties, moisture content, disintegration time and drug content uniformity (see chapter 5).

2.8 Statistical Analysis

Statistical analyses were performed using GraphPad software version 6.0 (California, USA). Data analyses were compared using one way analysis of variance (ANOVA) and pair – wise multiple comparison by Tukey's test, with differences between means with $p < 0.05$ being considered significant for all the experiments. Each study represented the mean of triplicate results ($n=3$) ± standard deviation of this set of records, unless otherwise stated. Standard deviation was used as errors in the figures.

Chapter 3
Preliminary studies of film formulation

3.1 Introduction

Polymers, which may include both natural and synthetic materials, are widely used in pharmaceutical formulation, since they are generally well tolerated in the body and have a high capacity of drug loading. Thus, polymers have attracted interest for their application in dissolvable film formulations for fast and convenient drug delivery.

When used as the basis for oral dissolving films with rapid disintegration in the mouth cavity, the film-forming polymers should be harmless and flexible, exhibit good wetting and spread ability, whilst also displaying adequate stability, sufficient peel, shear and tensile strengths (Leuner and Dressman, 2000, Dixit and Puthli, 2009).

According to the classification of a polymer's mechanical properties, an ideal oral dissolving film (ODF) should have moderate tensile strength, high percentage elongation and low Young's modulus (Nalluri *et al.*, 2013). Indeed, a successful ODF formulation is dependent on the selection of polymer and polymer concentration, as they not only impact on the mechanical properties, but also influence the release of the active ingredient into the oral cavity through disintegration. Yet, there are no specific requirements or limitations that are defined to ensure the appropriate mechanical properties of films (Preis *et al.*, 2014).

Many different polymers for use in oral films are proposed in the patent literature, and various research groups have introduced different materials. As polymers govern the release profile of the drug, their choice is the main criterion for the selection of the intended release profile and site of action (i.e. local or systemic effect). Pullulan is the

first natural polymer, obtained from starch by the fungus *Aureobasidium pullulans*, employed for edible films (Leathers, 2003). The transparency, flexibility and low oxygen permeability profile of pullulan make it of great use in food associated applications; edible films made of pullulan have shown great potential in food coating materials, as they protect fats and vitamins in food from oxidation, therefore helping retain the flavours and quality (Cheng *et al*., 2011). Alternatively, pullulan can be used as a food additive. With regards pharmaceutical applications, the strong adhesive and anti- static property of pullulan can be exploited for use as a tablet binder as well as a coating agent for hard capsules (Prajapati *et al.*, 2013). The most common cellulose derived film forming polymers, such as hydroxypropyl methyl cellulose (HPMC) and hydroxylpropyl cellulose (HPC), are extensively used as film coating agents, as well as lubricants, because of their low cost and biodegradable properties. The cellulose based polymers are available in different grades depending on the degree of substitution and viscosity; the higher grade of polymers function as delayed agents for sustained release formulations (Lopes *et al.*, 2006), whereas the lower grade polymers are used for immediate release formulations (Kou *et al.*, 2011). For instance, HPMC has been shown to act as a protective coating agent for diclofenac sodium tablets at 25 mg, delivering a faster rate of drug release in the GIT environment (Roy *et al.*, 2009). Different grades of HPMC have also been studied for the development of fast dissolving films of triclosan, indicating they were good film forming materials (Dinge and Nagarsenker, 2008). Sodium alginate is a natural, water-soluble polysaccharide derived from brown seaweeds, which is widely used in the pharmaceutical industry because of its safety and availability. Due to its colloidal properties, sodium alginates have been used as thickening, stabilising and emulsifier agents, but also utilised for film formation (Nagar *et al.*, 2011). Films prepared from sodium alginate showed great potential for controlled drug release systems (Juliano *et al.*,

2008). Besides that, sodium alginate has been used to improve drug loading capacity of mucosal films using paracetamol and amoxicillin as model drugs (Boateng *et al.*, 2013). Pectin is also a natural polymer extracted from fruits and apples. It has a good capacity of loading drug, even at low pH function, and pectin is widely used for sustained release of drugs (Dixit and Puthli, 2009, Borges *et al.*, 2015). Kollicoat® IR and Kollicoat® protect (KP) are both new generation pharmaceutical excipients composed of polyvinyl alcohol – polyethylene glycol graft copolymer that are freely soluble in water. They have been successfully used as a film former for tablet coating for instant release formulations (Nagar *et al.*, 2011). Kollicoat® IR also works as a wetting binder and pore former in sustained released coatings (BASF, 2007), whilst films formed of Kollicoat protect showed both great protection against moisture vapour with taste masking effect with the addition of polyvinyl alcohol compared to Kollicoat® IR (Yadav, 2013). With special advantages including instant release profile, great flexibility, good film forming agent with taste masking and moisture barrier properties, this novel polymer, KP, has a sound rationale for its use as an alternative potential candidate to currently used polymers for ODFs.

Plasticisers have been considered as one of the significant factors in the formulation of ODFs, since it strongly affects the mechanical properties of films, such as the flexibility and tensile strength, by reducing the glass transition temperature (Tg) of the polymers (Rahman and Brazel, 2004, Roth and Dutcher, 2005, Cao *et al.*, 2009). The selection of plasticiser and its concentration depends on the polymers and solvents in the formulation, whilst it should also be compatible with the drug and the other components (Cao *et al.*, 2009, Vieira *et al.*, 2011).

3.1.1 Glycerol

Glycerol is a colourless, viscous compound that has versatile uses and applications in the food and pharmaceutical industry. It has three hydrophilic hydroxyl groups that result in high miscibility in water and a hygroscopic nature (Fundo *et al.*, 2011). Glycerol plays a major role in increasing the hydrophilic character of films and acts in a plasticising manner on the mechanical properties; several studies have reported the effect of glycerol on tensile strength, water permeability and thermal properties of films made from sodium alginate, starch and gelatine (Vanin *et al.*, 2005, Lukasik and Ludescher, 2006).

3.1.2 Polyethylene glycol

Polyethylene glycol (PEG) is one of the most popular excipients used in pharmaceutical formulations. It has been utilised as an ointment, tablet lubricant and plasticiser due to low toxicity (Topchiyeva, 1990). Depending on the molecular weight of PEGs, they are graded in different forms; the higher, solid grade PEGs (PEG > 900 Daltons) are usually used as lubricant for tablets, but they also improve the aqueous solubility and dissolution of poorly water soluble drugs (Joshi *et al.*, 2004, Paus *et al.*, 2015). The solid grade can be used either alone or in combination for tablet-coating polymers. The low liquid grade PEGs (200-600 Daltons) are used to enhance the water permeability and dissolution for formulations coating. PEGs have been widely employed as a plasticiser for prevention of film rupture and to improve the flexibility; e.g. PEG 400 was used as a plasticiser for diclofenac sodium tablet coatings (Roy *et al.*, 2009).

3.1.3 Tween 80

Tween 80 is one of the hydrophilic surfactants most commonly used as a non- ionic solubilising agent for poorly soluble drugs, as an emulsifier in foods and cosmetics, and as a wetting agent for oral suspension (Leuner and Dressman, 2000, van Zuylen *et al.*, 2001, Savjani *et al.*, 2012). It has been shown to reduce the surface tension of two phases and it can also perform as a plasticiser, whilst reports have also shown that Tween 80 is capable of improving the drug permeability for P-glycoprotein substrates (Zhang *et al.*, 2003). Furthermore, Tweens are the most commonly used surfactants for development of ODFs, since they may accommodate film dissolution, in addition to enhancing the immediate release of incorporated API (Kalyan and Bansal, 2012, Irfan *et al.*, 2015).

The aim of this investigation was to screen different film- forming materials used for the preparation of oral dissolving films, with assessment of mechanical properties and disintegration behaviour in order to optimise and propose suitable polymers and plasticisers for film formulations.

3.2 Results & Discussion

3.2.1 Visual inspection of blank films

The successful development of film formation is highly dependent on the properties of polymers; the film forming capacity of the polymer is defined as the ability to form a film that can be easily removed from the casting surface without damage or rupture, with classifications ranging from good to poor (Kulkarni *et al.*, 2010). Hydrophilic polymers are widely used in film formulation, since the hydrolysis of the polymer when in contact of saliva aids the rapid dissolution of APIs (Dixit and Puthli, 2009, Irfan *et al.*, 2015).

Initial studies were carried out to determine which type of polymers would be most suitable for film formulation; different hydrophilic polymers were chosen to develop preliminary studies of visual inspection, mechanical properties and other physicochemical characterisations. The homogenous polymer solution was casted to a non- stick metal mould. Based on the previously stated definition of film former capacity, polymers such as kollicoat IR, maltodextrin, starch, gelatin, sodium carboxy methylcellulose (90,000), polyvinyl pyrrolidone (K15, K30), methylcellulose, polyvinyl alcohol (31,000 - 50,000), polyvinyl alcohol (13,000 - 23,000) and pullulan showed that the blank films were sticky, which were difficult to peel, or resulted in no film forming (Figure 3.1 A C, D). Film forming from starch was found to have a limitation of mechanical strength and also crystal formation on the film (Nagar *et al.*, 2011) (Figure 3.1 B); starch is composed of two main natural constituents, amylose and amylosepectin, which are responsible for the ageing of starch films by the loss of water by evaporation and physical ageing during heating, which leads to re-crystallisation (Dureja *et al.*, 2011).

Indeed, films made from starch are heat-sensitive during the casting process due to poor water permeability (Van Soest and Knooren, 1997), whilst it has been reported that unplasticised starch films contained high levels of amylose, which causes a decrease in water vapour permeability and an increase in tensile strength (Nagar *et al.*, 2011, Pathare *et al.*, 2013). Polyvinyl pyrrolidone films are generally tacky in nature due to its hygroscopic property (Ali and Quadir, 2007). Films made of pullulan and Kollicoat® IR were difficult to peel due to their strong adhesive properties.

Four polymers – sodium alginate, pectin from apples, HPMC (average Mwt 10,000) and KP – showed a smooth, transparent appearance with good film forming capacity (Figure 3.1E, F, G and H). Therefore, these four hydrophillic polymers were selected for further studies, because they exhibited the capacity of being film formers, as well as being transparent and non- sticky (Figure 3.1E, F, G, and H). They are also low heat - sensitive polymers which are the suitable choice for fast release systems.

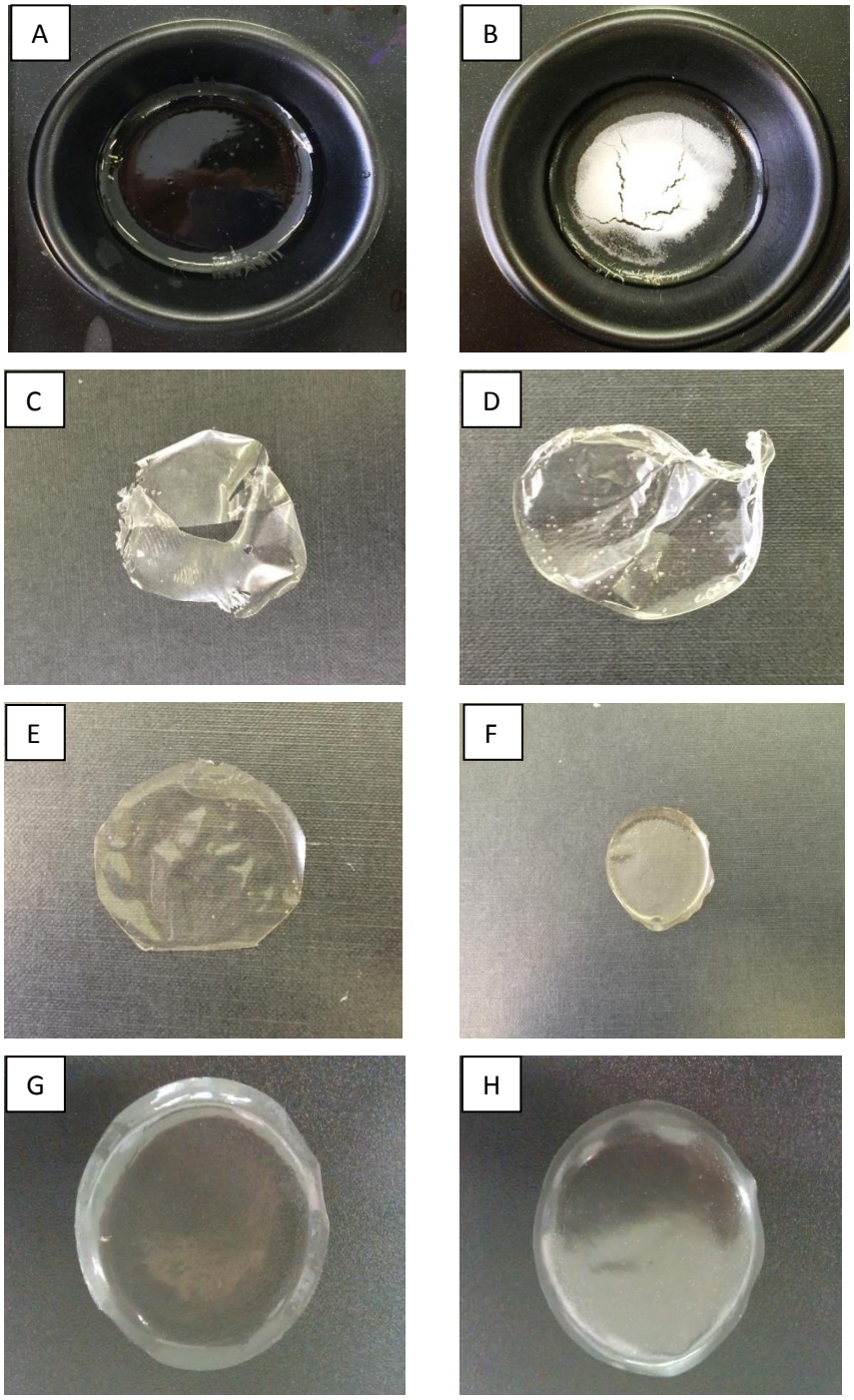


Figure 3.1-Visual image of polymer blank film A) PVP K 30 B) starch C) Kollicoat IR D) pullulan- too difficult to peel or no film formation whereas E) sodium alginate F) Pectin from apple G) Kollicoat protect H) HPMC (Mwt= 10,000)- achieved smooth texture with good film capacity after casting on the non- stick metal mould.

3.2.2 Mechanical properties of blank films

Following initial screening, four selected polymers were used to prepare films and further evaluate the resulting mechanical properties (Table 3.1). Mechanical properties of film formers are important with regards to film casting, handling and drug release. Results showed that unplasticised films exhibited adequate mechanical properties by exhibiting a low tensile strength but high percentage elongation, therefore producing soft and tough films. In addition, the strength of each film strip was directly dependent on the polymer type and concentration.

Table 3.1- Mechanical properties of blank films made from HPMC (10,000), SA, pectin and KP by initial screening. Films were cut at the size at 3x2 cm^2 (mean ± SD, n=3). Statistically significant differences are noted as follow: ns ($p > 0.05$) in tensile strength; **** ($p < 0.0001$) in % elongation and Young's modulus between polymer types, followed by two way ANOVA.

Polymer types	Film forming capacity	Thickness (*mm*)	Mechanical properties		
			Tensile strength (N/mm^2)	Elongation (%)	Young's modulus (N/mm^2)
HPMC (10,000)	Good	0.14 ± 0.01	6.64 ± 0.36	25.34 ± 1.82	23.08 ± 2.60
SA	Good	0.18 ± 0.01	3.82 ± 0.49	37.78 ± 1.27	11.09 ± 0.83
Pectin	Good	0.21 ± 0.01	4.76 ± 0.21	25.54 ± 0.40	17.10 ± 2.33
KP	Good	0.09 ± 0.01	4.72 ± 0.53	51.08 ± 2.93	10.01 ± 0.30

In this case, the thickness of blank films relates to the extent of barrier properties towards water vapour; films made of pectin have a higher thickness than the KP films, as pure pectin has a low ability to absorb water vapour and a high level of structural discontinuities caused by the drying conditions at high content of pectin in the films, which generates thicker films, in agreement with previous studies (Galus *et al.*, 2012, Meneguin *et al.*, 2014). SA and KP have a lower Young's modulus than the other polymers, which indicates films were flexible and softer to stretch due to their natural properties. Although starch and pullulan are both natural products, mechanical properties of films made from them are highly dependent on different chemical structure and functional attributes. Films made from starch exhibit poor mechanical properties due to its poor water permeability and the brittle nature of starch, thus affecting film forming ability (Krogars *et al.*, 2003). In contrast, pullulan possesses superior adhesive which makes it difficult to peel (Chaen, 2011).

3.2.3 Selection of plasticiser

Glycerol, Tween 80 and PEG 400 (1% v/v) were studied to optimise the formulation, since each may influence the physical properties of films. Films prepared using Tween 80 (Figure 3.2) and PEG 400 (Figure 3.3) as the plasticiser did not possess good appearance and also caused films to become harder and more brittle, which affects its appearance and handling. Therefore, Tween 80 and PEG 400 were considered to be incompatible with KP, SA, HPMC and pectin films, which is in agreement with reports elsewhere (Galgatte *et al.*, 2013). As they are polysaccharide - based films, these polysaccharides are generally resistant to fats and oil, which results in the formation of an oily layer on the surface of the film with the addition of Tween 80 and PEG 400, hence

affecting the film appearance and handling (Vieira *et al.*, 2011). On the other hand, it can be seen that films prepared using glycerol resulted in better appearance with good film forming compared to those prepared by other plasticisers at the same polymer concentration (Figure 3.4). As a result, glycerol was chosen as a suitable plasticiser in these formulations, as it has shown excellent plasticising ability due to stability and compatibility with the polymers used here, with previous results also confirming that glycerol has been an appropriate plasticiser for sodium alginate and CMC (Chillo *et al.*, 2008, Boateng *et al.*, 2009, Boateng *et al.*, 2013).

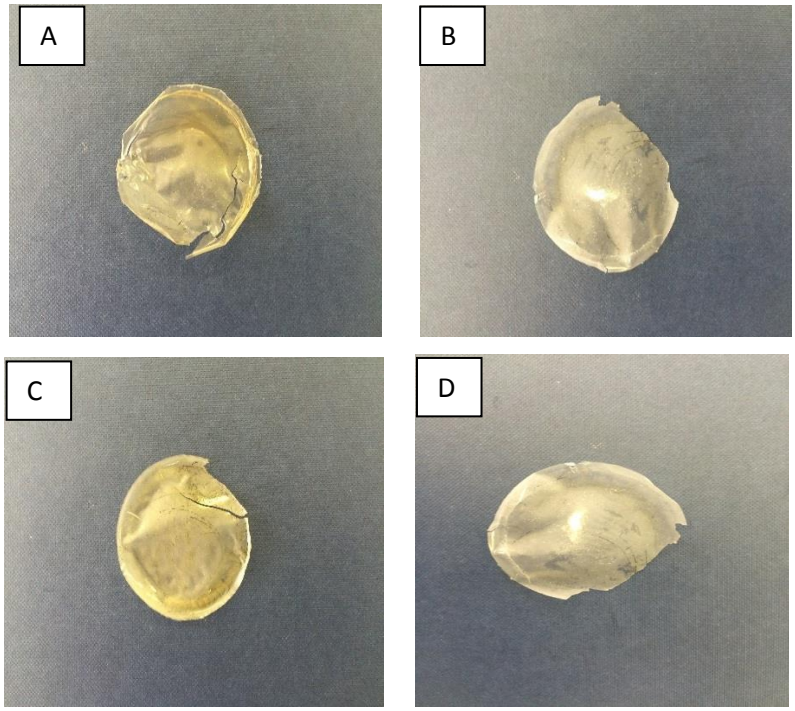


Figure 3.2- Visual image of polymeric film A) Pectin made from apple, B) Kollicoat protect, C) sodium alginate and D) HPMC (Mwt = 10,000) using Tween 80 as plasticiser- Films turned yellowish or cloudy and were too brittle after casting on the non- stick metal mould.

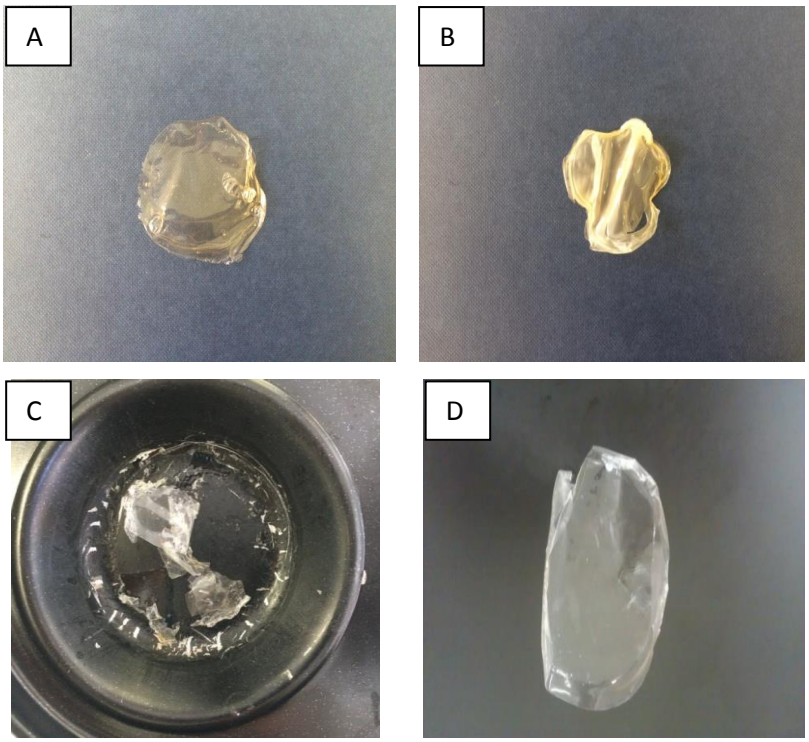


Figure 3.3- Images of the plasticised films made from A) Pectin, B) SA C) HPMC (10,000), D) Kollicoat protect using PEG 400 as plasticiser. Films did not exhibit good appearance or they were too brittle to form films.

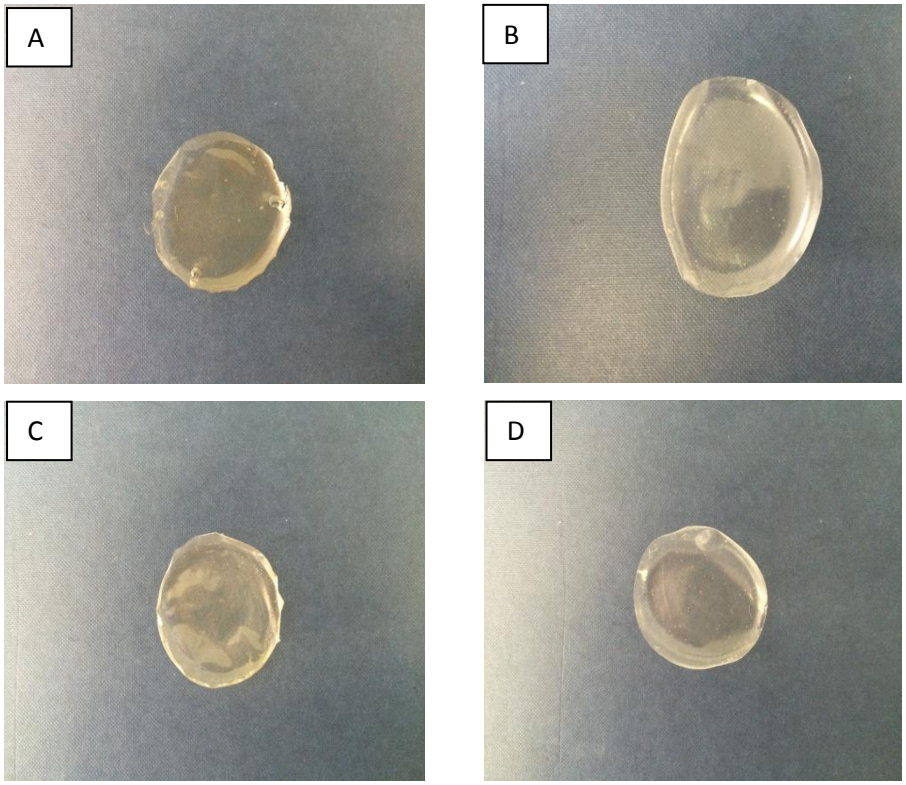


Figure 3.4- Images of the plasticised films made from A) Pectin, B) KP, C) SA D) HPMC (10,000) using glycerol as plasticiser. Films exhibited good appearance and smooth texture with good film capacity.

3.2.4 Effect of plasticiser concentration

Varying amounts of plasticiser was added to investigate the impact of plasticiser (in this case, glycerol) on the physicochemical properties of films (elastic behaviour, disintegration properties). As expected, the higher portions of plasticiser have a statistically significant impact on the mechanical properties of films ($p < 0.05$, ANOVA followed by Tukey's test) (Table 3.2). Tensile strength and Young's modulus properties of KP, SA and Pectin reduced significantly at the highest concentrations of plasticiser (20 µl), which indicated that KP and pectin films became even softer when the amount of plasticiser was increased, having a strong effect on the Young's modulus. Whilst for HPMC films, there was no significant difference in tensile strength and % elongation ($p > 0.05$, ANOVA followed by Tukey's test), but a significant difference in Young's modulus ($p < 0.05$, ANOVA followed by Tukey's test). This could be the antiplasticising effect of plasticiser within the polymer systems. The migration of glycerol from the film matrix enables the HPMC molecules to strongly interact with plasticiser when the plasticiser concentration is above its compatibility limits, hindering polymer mobility. Hence, no change in mechanical properties of HPMC was observed (Sanyang *et al.*, 2015).

It was observed that the thickness of each film formulation was increased with an increase in glycerol content; the films were produced with a high content of glycerol molecules in the film forming solution, leading to more glycerol molecules occupying the void of the polymer matrix and interacting with the polymer chains, thus creating an increased distance between the polymers within the matrix, which thereby lead to them being thicker (Fundo *et al.*, 2014). Similar results have also highlighted the effect of plasticiser

concentration on film thickness (Imran *et al.*, 2010, Jouki *et al.*, 2013, Razavi *et al.*, 2015, Sanyang *et al.*, 2016).

Table 3.2- Mechanical properties of HPMC, SA, pectin and KP films at different glycerol concentration added at 5 µl , 10 µl and 20 µl .Film size taken at 3x 2 cm^2 (mean ± SD, n=3). Elongation and elastic modulus are also important parameters for films, since they provide an indication of the deforming properties and flexibility of films; the more brittle the film, the lower the elongation value. Statistically significant differences are noted as follow: ns ($p > 0.05$) in tensile strength; **** ($p < 0.0001$) in % elongation and Young's modulus between polymer types at different plasticiser concentrations, followed by two way ANOVA.

Polymer	**Amount of plasticiser**	**Film forming capacity**	**Thickness (*mm*)**	**Mechanical properties**		
				Tensile strength (N/mm^2)	**Elongation (%)**	**Young's modulus (N/mm^2)**
HPMC (10,000)	5 µl	Good	0.10 ± 0.01	8.93 ± 0.55	34.37± 3.18	25.54 ± 0.80
	10 µl	Good	0.17 ± 0.01	10.33± 1.47	30.84 ± 0.49	33.51 ± 4.75
	20 µl	Good	0.34 ± 0.01	5.55 ± 0.20	31.24 ± 3.39	17.94 ± 2.34
Pectin	5 µl	Good	0.07 ± 0.01	9.74 ± 0.59	35.60 ± 3.24	27.40 ± 0.84
	10 µl	Good	0.16 ± 0.01	4.19 ± 0.63	29.39 ± 2.92	14.21 ± 0.83
	20 µl	Good	0.34 ± 0.01	0.69 ± 0.10	55.77 ± 4.29	1.24 ± 0.10
Sodium alginate	5 µl	Good	0.12 ± 0.01	3.73 ± 0.19	31.90 ± 1.31	11.71 ± 0.95
	10 µl	Good	0.22 ± 0.01	3.38 ± 0.69	48.93 ± 2.10	6.89 ± 1.13
	20 µl	Good	0.30 ± 0.01	0.40 ± 0.08	68.37 ± 8.70	0.58 ± 0.60
Kollicoat Protect	5 µl	Good	0.09 ± 0.01	5.26 ± 0.66	44.78 ± 1.68	11.77 ± 1.05
	10 µl	Good	0.16 ± 0.01	2.48 ± 0.20	58.53 ± 2.66	4.23 ± 0.21
	20 µl	Good	0.36 ± 0.01	0.36 ± 0.03	23.30 ± 0.89	1.54 ± 0.15

Higher concentrations of plasticiser in all formulations studied produced softer, more flexible films by reducing the tensile strength and elastic modulus, whereas the % elongation was increased. For example, the tensile strength of HPMC decreased from 10.33 to 5.55 N/m^2 and that of pectin reduced from 9.74 to 0.59 N/mm^2. This is because the more plasticiser added to the homogenous polymer solution, the greater number of plasticiser molecules interact with the polymer chains, which thereby weaken the intermolecular forces within the polymer molecules and possibly integrating H-bonding of glycerol to polymers. Thus, the films become more flexible and soft. Similar results have been reported for the effect of glycerol on films (Cheng *et al.*, 2006, Fundo *et al.*, 2011, Fundo *et al.*, 2014).

3.3 Disintegration time

Disintegration time is defined as the time needed for a substance to disintegrate completely in a given volume of solution (USP37, 2008).

The European Pharmacopeia only states that ODFs "dissolve rapidly" without a defined time limit (European Pharmacopoeia Commision, 2013), although the limit of disintegration time of 30 seconds or less is a recommended value for ODFs (Barnhart *et al.*, 2008), whilst FDA guidance for a related dosage form, orally disintegrating tablets (ODTs), suggests a disintegration time of less than 30 seconds but European Pharmacopeia state less than 180 seconds (European Pharmacopoeia Commision, 2013). The disintegrating time of films was within the range of 6 to 30 seconds (table 3.3).

Results showed that the effect of plasticiser on disintegration time was different for various polymers ($p > 0.05$, ANOVA).

Table 3.3- The disintegration time of HPMC, SA, pectin and KP films with glycerol amount added at 5 µl, 10 µl and 20 µl. Film at 3x2 cm^2 were dissolved in 25 mL distilled water at 37 ^{0}C (mean ± SD, n=3). No statistical difference in disintegration time of different films by amount of plasticiser was observed using one way ANOVA, ns ($p>0.05$).

Polymer	Amount of plasticiser	Disintegration time (s)
HPMC (10,000)	5 µl glycerol	26 ± 1.15 s
	10 µl glycerol	23 ± 0.58 s
	20 µl glycerol	22 ± 0.58 s
Pectin	5 µl glycerol	23 ± 1.15 s
	10 µl glycerol	17 ± 0.58 s
	20 µl glycerol	30 ± 0.58 s
Sodium alginate	5 µl glycerol	11± 0.58 s
	10 µl glycerol	11 ± 0.58 s
	20 µl glycerol	14 ± 0.58 s
Kollicoat Protect	5 µl glycerol	11 ± 0.58 s
	10 µl glycerol	7 ± 0.58 s
	20 µl glycerol	10 ± 0.58 s

Films with weaker tensile strength (section 3.2.4), due to ease in polymeric chain movements by the plasticising effect, show an increase in the film water affinity through the hydrophilic property of the plasticiser, since the three hydroxyl groups present in the glycerol molecules are available for water adsorption (Cheng *et al.*, 2006, Chillo *et al.*, 2008); all of which leads to the ODF requiring less swelling time, thereby producing fast disintegration. In contrast, KP is a synthetic copolymer composed of polyvinyl alcohol – polyethylene glycol, which allows itself to act as an internal plasticiser, thus, it dissolves faster than other film formulations (Nagar *et al.*, 2011). Given the results from this and the previous section, it was observed that 10 µl glycerol in the film formulation was the optimum amount to yield the desired mechanical properties and disintegration time in accordance with relevant guidance.

3.4 Effect of drug loading

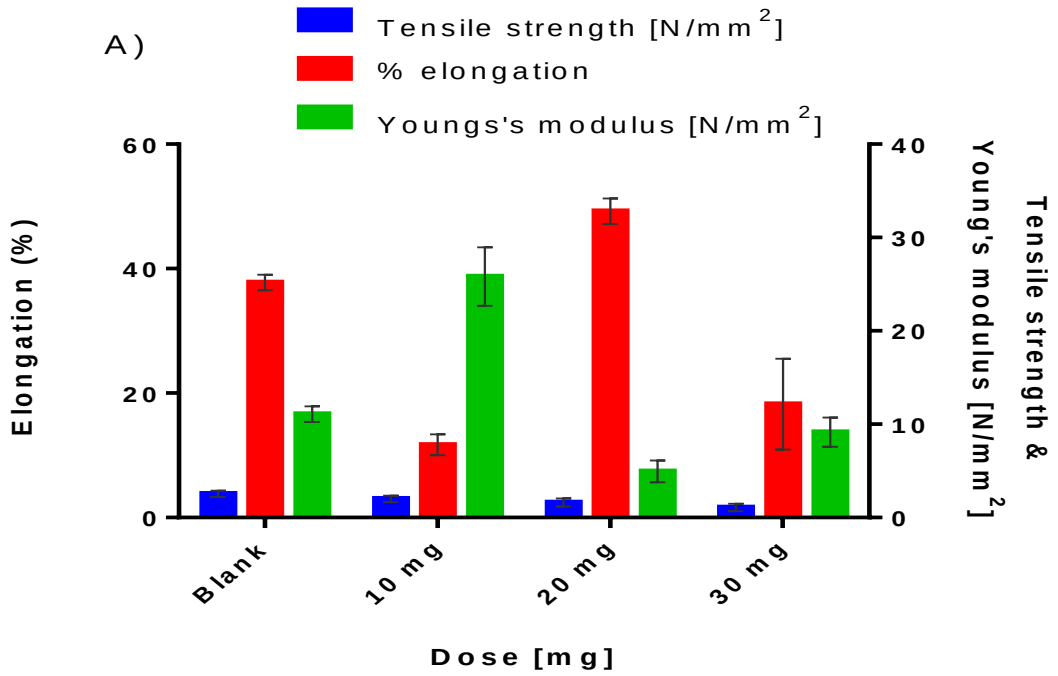
3.4.1 Mechanical properties of drug loaded films

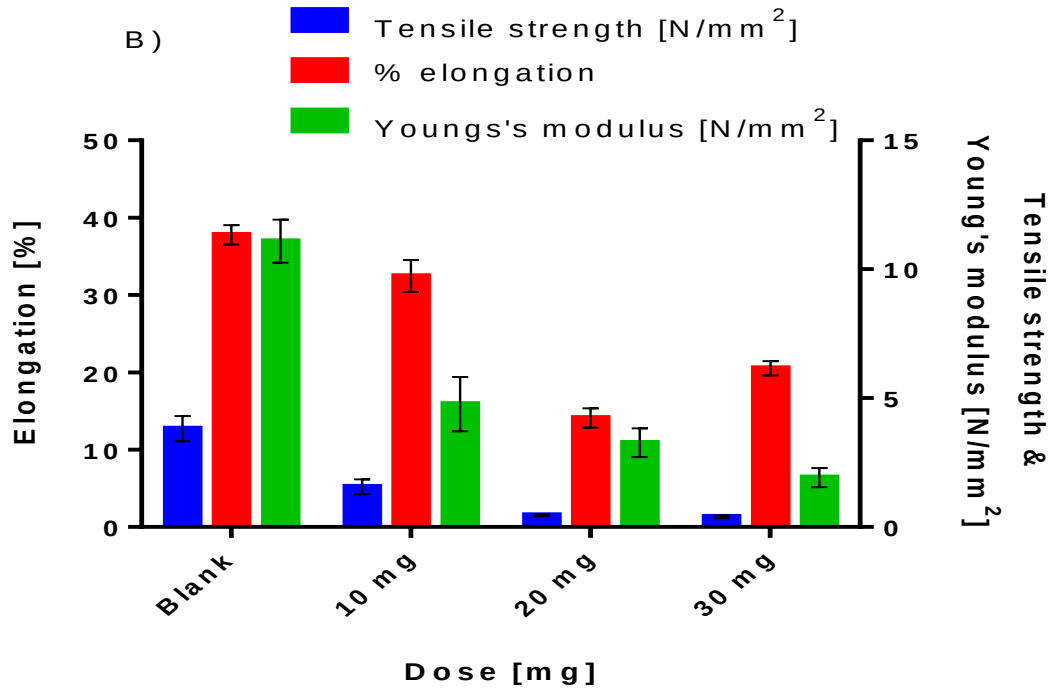
The formulations showing the ideal characteristics from the results above were further selected for drug loading. Metformin hydrochloride, an oral anti-diabetic drug, was chosen as a water soluble drug model for this study. HPMC was not selected further for drug loading due to being sticky or difficult to peel off from the non- stick mould and turning cloudy upon drug loading. HPMC films become cloudy due to the poor loading capacity of the produced ODF, and it has been reported that HPMC type E, as HPMC Mwt 10,000, is associated with films being brittle and non-peelable at low concentrations (Mahesh *et al.*, 2010).

Table 3.4- Film thickness of Metformin HCl- loaded SA, KP and Pectin polymeric films at dose loading at 10 mg, 20 mg and 30 mg. The thickness of films made from SA, KP and Pectin increases with a higher drug loading (mean ± SD, n=3). No statistical difference in thickness of different films by amount of dose was observed using one way ANOVA, ns ($p>0.05$).

	Film thickness (mm)		
Dose	**SA**	**KP**	**Pectin**
10 mg	0.13 ± 0.005	0.12 ± 0.005	0.18 ± 0.006
20 mg	0.15 ± 0.005	0.14 ± 0.006	0.20 ± 0.005
30 mg	0.16 ± 0.005	0.14 ± 0.006	0.21 ± 0.005

The average thickness of drug loaded films for SA, KP and pectin varied from 0.13 to 0.20 mm with an increase of drug loading from 10 mg to 30 mg (Table 3.4). It was observed that the presence of drug significantly influenced the mechanical properties (e.g. tensile strength, % elongation and Young's modulus) of the polymeric films in comparison to drug free film formulation ($p < 0.05$, ANOVA followed by Tukey's test), with similar observations recorded for all three types of polymers, as expected (Figure 3.5 A, B and C). As more hydrophilic drug molecules occupy the polymer matrix, greater interactions occur with the polymer chains, which thereby weaken the intermolecular forces, thus reducing the mechanical properties of all films (Mali *et al.*, 2008).


A)
Tensile strength [N/mm 2]
% elongation
Youngs's modulus [N/mm 2]
Elongation (%)
Young's modulus [N/mm 2]
Tensile strength &
60
40
20
0
40
30
20
10
0
Blank
10 mg
20 mg
30 mg
Dose [mg]


B)
Tensile strength [N/mm 2]
% elongation
Youngs's modulus [N/mm 2]
Elongation [%]
Young's modulus [N/mm 2]
Tensile strength &
50
40
30
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10
0
15
10
5
0
Blank
10 mg
20 mg
30 mg
Dose [mg]

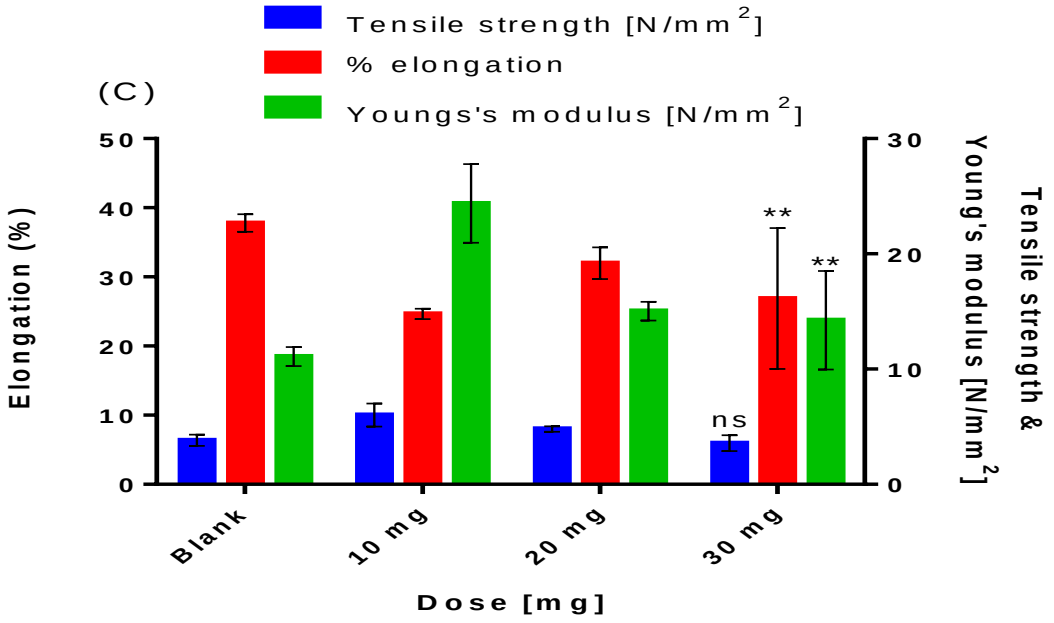


Figure 3.5- The influence of drug loading on mechanical properties of drug- loaded films - A) sodium alginate B) Kollicoat protect C) Pectin from apple at different dose strength: 10 mg, 20 mg and 30 mg compared to blank films (mean ± SD, n= 3). Statistically significant differences are noted as follow: ns ($p > 0.05$) in tensile strength; **** ($p < 0.0001$) in % elongation and Young's modulus of SA, KP and Pectin films at different amount of drug loading, followed by two way ANOVA.

An increase in drug loading also had an effect on all formulations, although this was dependent on polymer type (Figure 3.5 A, B and C). For KP films, increasing the drug loading from 10 mg to 30 mg showed no significant difference in tensile strength ($p > 0.05$, ANOVA followed by Tukey's test), yet significant differences were seen in % elongation and Young's modulus ($p < 0.05$, ANOVA followed by Tukey's test). Whilst for pectin films, there was no significant difference in tensile strength and % elongation ($p > 0.05$, ANOVA followed by Tukey's test), but a significant difference in Young's modulus ($p < 0.05$, ANOVA followed by Tukey's test), which indicated that KP and pectin films became even softer when the amount of drug was increased, having a strong effect on the Young's modulus. This indicates that the presence of water- soluble drug in a higher concentration promotes softening of the polymer, suggesting that the drug could

have an additional plasticising effect on the films produced. Moreover, it is seen that drug loading affects the mechanical properties of the polymeric film, due to the differences in chemical structure of the polymer. KP film is a highly flexible polymer due to the presence of polyvinyl alcohol that acts as an internal plasticiser (BASF, 2007). Pectin generated thicker films with an increase in drug loading, whilst extra force was required for breaking the films, which indicates a higher tensile property of pectin compared to the other two polymers. The higher Young's modulus value of pectin corresponds to the stiffness of films due to its colloidal thickening properties (Galus and Lenart, 2013). SA films have the lowest mechanical properties compared to KP and Pectin, showing that films become ductile and more flexible as more drug molecules are incorporated into the material, promoting a higher mobility between polymer chains. Also, Metformin HCl is a hygroscopic drug, and therefore responsible for moisture uptake during handling (Barot *et al.*, 2010).

Results illustrated that drug loading has a significant effect on the mechanical properties within the boundaries investigated.

3.4.2 Disintegration time of drug loaded films

The disintegration time of drug loaded films is generally faster than that of blank films. All drug loaded films were completely dissolved in less than 40 seconds, fulfilling the criteria of fast dissolving films (Table 3.5). KP films showed the shortest disintegration time – within 5 seconds for all levels of drug loading – due to the hydrophilic nature of the polymer and the plasticising effect of the drug, which is readily soluble in water, whereas pectin based films required more than 20 seconds to fully dissolve, but still less

than one minute; due to its highly colloidal properties, pectin tends to dissolve slowly as it is a high molecular weight compound composed of a complex of heteropolysaccharides, whose carboxylic acid groups are present in ester form, which are responsible for the extent of solubility of pectin (Nagar et al., 2011, Galus et al., 2012). SA is a hydrophilic colloidal polymer, which consists of the sodium salt of alginic acid, that makes films dissolve quickly in water (within 10 seconds).

Table 3.5- Disintegration time of drug-loaded films made from sodium alginate, Kollicoat protect and pectin. Film at 3x2 cm^2 were dissolved in 25 mL distilled water at 37 ^{0}C (mean ± SD, n=3). No statistical difference in disintegration time between SA and KP films, but recorded significantly difference in Pectin films by amount of dose was observed.

Film forming polymer	Amount of drug loading	Disintegration time of films (s)
Sodium alginate	No drug	12 ± 1.15 s
	10 mg	8 ± 0.58 s
	20 mg	9 ± 0.58 s
	30 mg	10 ± 1.53 s
Kollicoat Protect	No drug	7 ± 0.15 s
	10 mg	5 ± 0.58 s
	20 mg	5 ± 0.58 s
	30 mg	4 ± 0.58 s
Pectin	No drug	45 ± 1.58 s
	10 mg	42 ± 2.65 s
	20 mg	40 ± 2.08 s
	30 mg	25 ± 2.08 s

Incorporating higher quantities of API can be seen to influence the disintegration profile of films; films with weaker tensile properties and high flexibility (section 3.4.5) require less time for rupture of the films, which is evident with faster disintegration times for all formulations upon high levels of drug loading (Sungthongjeen et al., 2004). Pectin films disintegrated faster as the facilitate action of hydrophilic drug, especially at higher doses.

3.4.3 FTIR studies

To investigate the interaction between metformin HCl and polymers in the films, the FTIR spectrum of the polymer, drug, physical mixture and drug-loaded films were tested between wavelengths 4000 – 500 cm^{-1} by Thermo Scientific Nicolet IS5 Spectrometer.

KP demonstrates a broad absorption band at 3302 cm^{-1}, corresponding to the hydroxyl group, sharp bands at 1727 to 1709 cm^{-1} and 1041 cm^{-1} of the stretching vibration of (-C=O) group, with the C-H stretch vibration occurring in the region of 2361 to 2155 cm^{-1} (Figure 3.6). Sodium alginate displays a broad absorption band at 3274 cm^{-1} of the hydroxyl group, sharp bands recorded at 1593 and 1081 cm^{-1} of the asymmetric stretching vibration of carboxyl (-COO) group and characteristic bands at 2361 to 2155 cm^{-1} assigned to C-H stretch vibration (Figure 3.7) (Daemi and Barikani, 2012). Pectin exhibited a broad absorption band at 3375 cm^{-1} of O-H stretch, sharp band at 1733 cm^{-1} of the stretching vibration of (-C=O) group and strong bands at 1014cm^{-1} of C-O stretching band (Figure 3.8) (Seslija *et al.*, 2016)

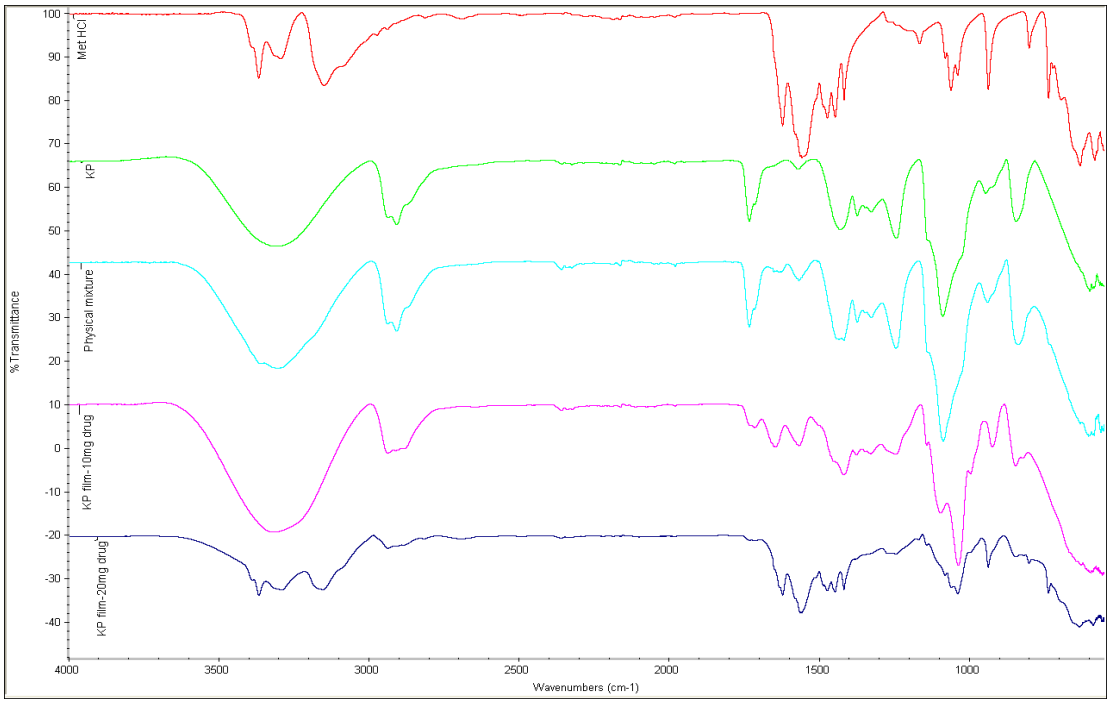


Figure 3.6- FTIR of Metformin HCl, KP, physical mixture and drug loaded KP films

The incorporation of drug at 10 mg dose into the film caused a significant reduction in the intensity and band shift from 1727 to 1715 cm^{-1} for the -CO group observed in all spectra. A new band formation at 1087 cm^{-1} in the drug-loaded film (10 mg) indicated the possible strong arrangement of C-O group due to hydrogen bonding (Figure 3.6, 3.7 and 3.8). However, the intensive absorption bands of drug were observed in the films with 20 mg drug loading. The N-H stretching of C=N-H group has strong absorption peak at the range of 3387 - 3156 cm^{-1}. A weak intensity band at 1035 cm^{-1} corresponded to C-N stretching was observed and this could overlap with the C-O band from the polymer. The drug interaction with polymer at higher doses can explain further the influence of amount of drug loading on the mechanical properties of these film formulations by showing a strong peak intensity of drug in the spectrum of all film loaded drugs (20 mg). The

apparent differences between the drug loaded films and the physical mixtures might be masked by the film peaks, particularly as they were used in less quantity than the polymer.

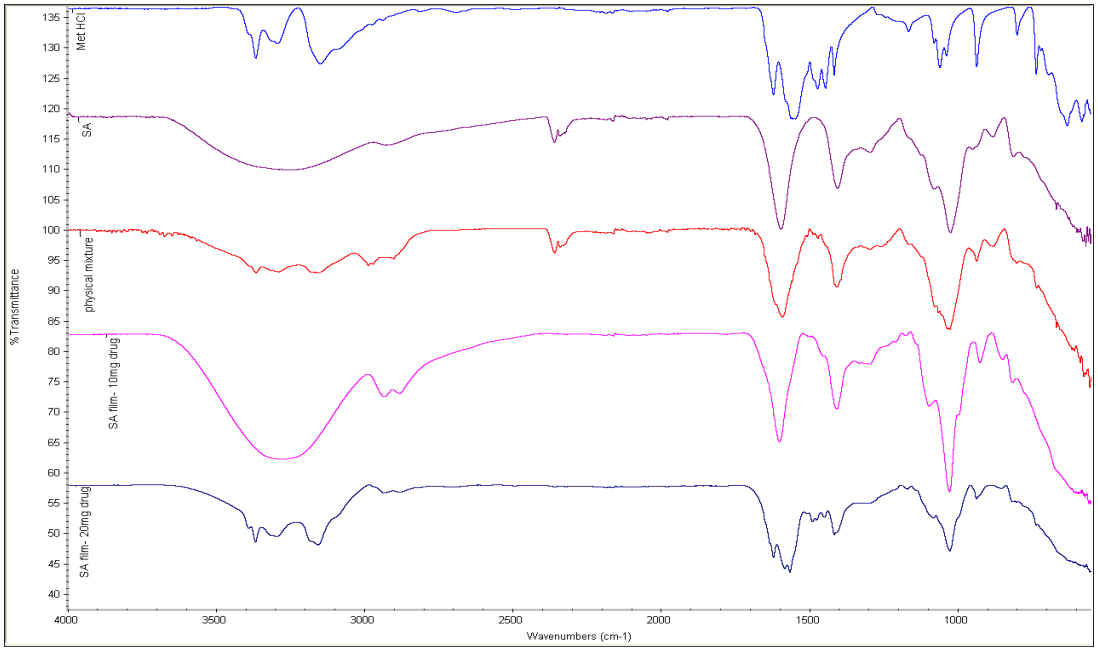


Figure 3.7- FTIR of Metformin HCl, SA, physical mixture and drug loaded SA films.

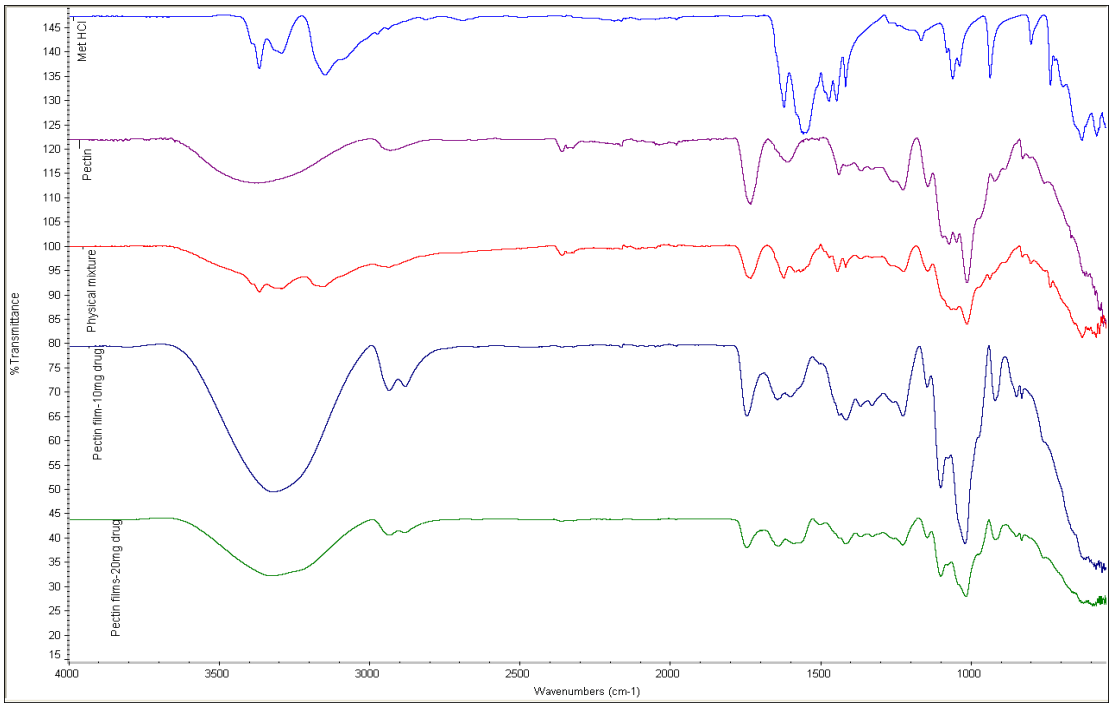


Figure 3.8- FTIR of Metformin HCl, pectin, physical mixture and drug loaded Pectin films.

3.4.4 Disintegration time of commercial products

Several commercialised ODFs, such as Listerine and Nicotine strips, were studied for the disintegration times for comparison to the developed ODF formulations. The reported data showed that Listerine displayed a rapid disintegration (7 seconds) (Dave *et al.*, 2014), whereas the disintegration time of NiQuitin strip containing 2.5 mg API was 120 seconds. In contrast, Gas- X® thin strips, containing 62.5 mg API, disintegrates within 60 seconds (Table 3.6). The disintegration time reflects on the amount of drug loading and the type of polymer used based on the release target. Listerine Oral Care strips, using pullulan as a film former with loading of inactive ingredients, showed fast release as pullulan is a hydrophilic polymer (Nagar *et al.*, 2011). Nicotine strip was formulated using Eudragit L100 polymer, which is a combination of methacrylic acid and ethylacrylate, for controlled time release of active ingredient via pH- dependent swelling (Sonje and Chandra, 2013). Modified starch was chosen for developing Gas- X® thin strips as it is widely used for coating of immediate release dosage forms with a low production cost (Dixit and Puthli, 2009, Nagar *et al.*, 2011). Due to its high solubility profile, film formulations made of KP polymer displayed the shortest disintegration time, comparable to commercial ODF products. Results confirmed that the optimised film formulations have satisfactory physicochemical properties compared to market products based on the type of polymer chosen for fast delivery system.

Table 3.6- Comparison of disintegration time of drug- loaded film formulation and commercial products.

Formulation	Drug loaded film formulation			Commercial products		
	SA	Pectin	KP	Listerine ®	Gas- X thin strips	Niquitin strips
Dose	10 mg	10 mg	10 mg	N/A	62.5 mg	2.5 mg
Time (s)	8 ± 0.58	42 ± 2.65	5 ± 0.58	7 ± 1.1	58 ± 2.7	120 ±0.58

3.5 Uniformity of dosage form

It is important for new dosage forms to achieve the consistency of dosage units. Each batch unit should comply within the acceptable limit of the label claim, 85- 115 %, according to British Pharmacopeia (BP, 2013). The quality control is carried out by two methods: weight variation or uniformity of content. Both SA and KP film formulations were assessed according to the standard of British Pharmacopeia, followed by the relevant testing for solid dosage forms (BP, 2013). Pectin was not selected further for this test due to films being so brittle with high amount of drug loaded when peeling off.

3.5.1 Uniformity of weight

The deviation of individual weight from the drug-loaded SA films at 10 mg and 20 mg dose loading was 2.54 ± 0.22 mg (% RSD of 8.55) and 2.92 ± 0.28 mg (% RSD of 9.56)), respectively. The weight uniformity of drug- loaded KP films at 10 mg and 20 mg dose loading was 2.88 ± 0.33 mg (% RSD of 9.97) and 2.94 ± 0.25 mg (% RSD of

8.44), respectively. Each batch of formulation showed RSD of less than 10%. Thus, the preparation met the criteria of BP (2013) weight uniformity (Table 3.7).

Table 3.7- Summary of weight uniformity of SA and KP film formulation at 10 mg and 20 mg drug dose (mean ± SD, n= 20).

Formulation	Weight uniformity (mean ± SD), n= 20	% RSD
SA film-10mg Met HCl	2.54 ± 0.22	8.55
SA film-20mg Met HCl	2.92 ± 0.28	9.56
KP film- 10mg Met HCl	2.88 ± 0.33	9.97
KP film- 20mg Met HCl	2.94 ± 0.25	8.44

3.5.2 Uniformity of content

Although the general acceptable limit of the label claim of each unit should be 85- 115 %, depending on the dosage form, drug type and doses, the criteria are stated differently in the monograph. The criteria of BP content uniformity for Metformin HCl should be within the range of 98.5-101 % (BP, 2015). However, the content percentage of metformin HCl in the films was found to be 114.44 ± 4.67 % and 113.42 ± 6.59 % for SA and KP films, respectively (Table 3.7). Thus, these preparations failed to meet the criteria of content uniformity, which indicated that metformin HCl distributed unevenly in the films. Since the portion of film used for analysis was taken from the centre of the dried films, drugs were accumulated in the middle of the baking mould after drying, which may

explain the reason of higher percentage content uniformity. Therefore, using a baking mould for solvent casting may not be the most appropriate method for film preparation.

3.6 Method Development of Film Manufacturing

As mentioned in section 3.5.2, the previous method is not the appropriate method for film development due to the failure to achieve the required content uniformity. Hence, an alternative system, employing the Elcometer 4340 film applicator, was investigated to accomplish the criteria and produce reproducible uniform films under mini industrial scale.

3.6.1 Elcometer 430 Film Applicator

The Elcometer Film Applicator is a robust, reliable, easy handling equipment for applying uniform and reproducible film products, based on the principal application of solvent casting technology. Solvent casting method involves the process which water - soluble polymers are firstly dissolved in water to form uniform clear viscous solutions with the following addition of drug and other excipients, to form a homogenous solution, subsequently cast on the substrate and evaporated during the drying period (Siemann, 2005). The applicator is operated by numerous controls mounted on the front panel of the instrument (Figure 3.9).

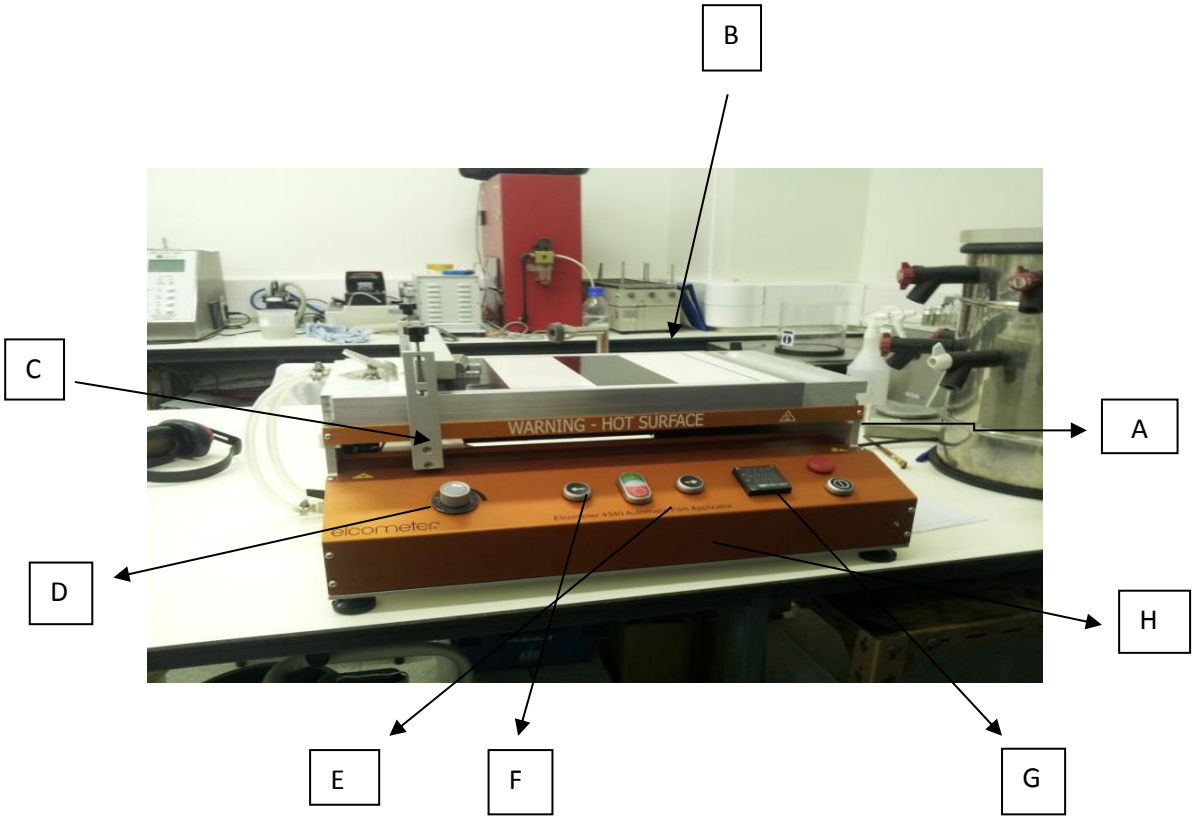


Figure 3 9- Schematic parts of Elcometer 4340 Motorised Film Applicator

A-Table, B- Substrate, C- Carriage, D- Carriage speed, E- Carriage return, F- Carriage start, G- Control system, H- Temperature control/ display.

3.6.2 Uniformity of Content

Following on from the optimisation process above for the content uniformity of films, metformin HCl loaded KP and SA films were carried out for further investigation using the Elcometer. Results showed that all films achieved the desired content uniformity when uniformity in thickness is maintained, as expected (see table 3.8), compared to the baking tray method. This indicates that metformin HCl distributed evenly in the films and, hence, the Elcometer Film Applicator was ideally chosen for further study of film formulation and development.

Table 3.8- Comparison of content uniformity of Metformin HCl loaded films using baking tray and Elcometer method.

Parameters	Baking Tray		Elcometer method	
	SA film	KP film	SA films	KP films
Thickness (mm)	0.13 ± 0.005	0.12 ± 0.005	0.14 ± 0.01	0.14 ± 0.01
Content uniformity	114.44 ± 4.67 %	113.42 ± 6.59 %	99.15 ± 4.45 %	101.35 ± 4.25 %

3.7 Conclusion

In order to optimise the formulation of ODFs, it is first critical to screen the components used in the formulation, as they can greatly influence the physicochemical and mechanical properties of the films produced. The choice of polymers to be used as film formers are important, as they are the major component in the formulation and can influence not only the tensile properties of the films, but also impact on drug release. After initial screening of a range of polymers, SA, pectin, and KP were selected as film forming polymers, as they exhibited good texture with excellent film forming capacity; other polymers were excluded from further studies, as they were difficult to peel or no film developed. These selected polymers were studied with plasticisers (glycerol, PEG 400 and Tween 80), with only glycerol proving to be suitable, as films obtained adequate mechanical properties to handle, possibly due to glycerol being a molecule with smaller molecular weight and size, thus possibly achieving greater interaction by H- bonding with the film forming polymers. Different amounts of plasticiser used also significantly influences the mechanical properties as well as the disintegration time of films. The tensile properties of all film formulations were decreased with an increase in the plasticiser amount (5 µl, 10 µl and 20 µl per 3 x 2 cm^2 strip), indicating that the

plasticising effect has taken place. Amount of plasticiser (10 µl glycerol) achieved the optimum mechanical properties in the film formulation. Results indicated that the film forming was governed by the amount of plasticiser and the amount of polymer could influence on the structural rearrangement of films. The reason is that glycerol on the polymer chains reduce the spaces of polymer molecules, which are available for the hydroxyl groups for water adsorption (Cheng et al., 2006).

Drug loading of the selected film formulations resulted in further reductions in tensile properties (e.g. tensile strength and elastic modulus of films were reduced, but the percentage elongation was increased) at different dose strengths. The results showed that the loading of drug into films produced more flexible films, as further plasticising effects have occurred, due to the hydrophilic nature of the drug investigated. The disintegration time of drug-loaded films was faster than that of blank films, which was expected, since films with weaker tensile strength require less swelling, thereby producing fast disintegration. The study of drug- polymer interaction using FTIR suggested the interaction of polymer and drug via the formation of hydrogen bonding. All films showed good uniformity in weight. However, the content uniformity of films failed to meet the pharmacopeia criteria due to uneven distribution of drugs in the films. These results showed that the solvent casting method by baking mould was not suitable for oral dissolving film preparation. A new alternative method has been approached for further ideal film development in order to achieve content uniformity.

Overall, the optimisation of formulations for oral dissolving films was achieved through the selection of parameters such as preparation method of casting films, polymer selection and properties of drug that produced films with desired properties.

Chapter 4
Investigation into the effect of cyclodextrin complexation on drug loading, content uniformity and solubility of poorly water soluble drug loaded ODFs

4.1 Introduction

The rate and extent of drug absorption is governed by two key factors; the solubility and permeability of a drug (Miller *et al.*, 2012). According to the Biopharmaceutical Classification System (BCS), drug candidates are divided into four classes based on their permeability and solubility profile (Amdion *et al.*, 1995, Yu *et al.*, 2002) (Table 4.1). A highly soluble drug is a substance that, at the highest dose strength, is soluble in 250 mL or less of aqueous media over the pH of 1- 7.5 at 37 ^{0}C, while a highly permeable drug achieves the extent of absorption of greater than 90 % of the total administrated dose (FDA, 2015). Class I drugs dissolve rapidly across the gastrointestinal membrane when administrated, with the rate limiting step of this class of drug being the gastric emptying rate when the dissolution is fast. In contrast, class II comprises relatively poorly water-soluble drugs, with aqueous solubility of < 0.1 mg/ mL, yet with a high absorption profile; thus, *in-vivo* dissolution becomes the rate limiting step in this case. Class III drugs encompasses water soluble substances that have permeability as the rate of limiting step for absorption; hence, it is vital to design this drug class to be released rapidly for maximising its residence time in the gastrointestinal tract in order to improve permeability. Conversely, class IV drugs are mainly water insoluble drugs, which also suffer from poor permeability and, as a result, exhibit poor oral bioavailability. Currently, it its estimated that a large number of new chemical entities (up to 70 %) undergoing development are generally high molecular weight, poorly soluble and lipophilic compounds, which affects the rate and extent of oral drug absorption across the gastrointestinal mucosa (Kakran *et al.*, 2012, Ku and Dulin, 2012, Kurkov and Loftsson, 2013). Despite their high permeability, the associated low aqueous solubility and slow release of drugs in the gastrointestinal tract results in low oral bioavailability, leading to

consequences such as ineffective treatment, frequent dose escalations to achieve therapeutic effects, and increased levels of toxicity (Kawabata *et al.*, 2011).

Solubility is a fundamental parameter that plays an important role for oral dosage forms to reach desired therapeutic drug concentrations following absorption via oral administration (Savjani *et al.*, 2012). Most of active pharmaceutical ingredients (APIs) used during the formulation design and development stages are poorly-water soluble. They are eliminated from the gastro-intestinal tract (GIT) before getting absorbed in the systemic circulation, which causes limited bioavailability. Whereas, the permeability is the diffusion of drug across the GIT membrane (Martinez and Amidon, 2002), which is also a crucial step in the determination of bioavailability, is determined by the physicochemical factors of drug, including molecular size, polar-/non-polar surface area, lipophilic or hydrophilic property, as well as other physiological properties of GIT (e.g. GIT pH, blood flow, gastric emptying and absorption mechanism) (Martinez and Amidon, 2002, Song *et al.*, 2004, Dahan *et al.*, 2009).

Moreover, in addition to the issues pertaining to solubility and permeability, most therapeutic drugs have a bitter taste and it becomes a serious problem affecting patient compliance and acceptability, particularly to paediatric patients. Hence, several techniques of taste masking have been employed to provide a palatable and pleasant taste, including using sweetening agents (Sohi *et al.*, 2004), coating with polymers (Douroumis, 2007), ion- exchange resin (Puttewar *et al.*, 2010) or forming complexation (Arima *et al.*, 2012).

Furthermore, development of new formulations with high drug loading becomes a challenge as a result of poor powder flowability and sticky tendency, which affects the content uniformity of the final dosage form (Shanmugam, 2015). Regarding to ODF formulations, a high amount of drug loading is an even greater problematic issue for film development, since the size of dosage form is the limiting factor (Dixit and Puthli, 2009). Besides that, a poor solubility profile of a drug, especially those belonging to BCS class II, could influence the disintegration/ dissolution of the film in the oral cavity as a consequence of the limited amount of saliva solution in the oral cavity (Shanmugam, 2016), thus negating the intention of fast disintegration without the need of water.

Table 4.1- Biopharmaceutical Classification System of Drug Substances adapted from Wu and Benet (Wu and Benet, 2005) .

	High solubility	Low solubility
High permeability	**Class I**	**Class II**
	Acetaminophen	Diclofenac
	Chlorpheniramine	Glipizide
	Midazolam	Ibuprofen
	Nifedipine	Loratadine
Low permeability	**Class III**	**Class IV**
	Captopril	Amphotericin B
	Cetirizine	Ciprofloxacin
	Metformin	Furosemide
	Ranitidine	Neomycin

As solubility and permeability are the important factors that determine the bioavailability of APIs, solubility can be modified by different strategies to enhance the solubility profile, such as salt formation for both acidic and basic drugs (Berge *et al.*, 1977, Elder *et al.*, 2013), pH adjustment (Vemula *et al.*, 2010), particle size reduction (Rabinow, 2004), solid dispersion (Vo *et al.*, 2013) and complexation (Loftsson and Duchene, 2007). Particle size reduction can be achieved by milling and creating nanosupensions, which produce smaller particle sizes with great surface area. However, milling and other techniques can lead to aggregation of particles and, consequently, reduction in flow capacity and wetting properties due to restricted surface area; as well as an extreme amount of mechanical forces applied to drug substances, which induce drug degradation, resulting in insignificant or no dissolution improvement. Alternatively, the complexation of poorly soluble drugs with cyclodextrins has been used to improve the aqueous solubility, palatability and stability of drugs due to their unique structures and their ability to modify the physicochemical properties of guest molecules (Miranda *et al.*, 2011).

4.1.1 Complexation

Discovered in 1891 by Villiers (Villiers, 1891), cyclodextrins (CDs) are natural molecules comprising a family of cyclic oligosaccharides derived from starch through fermentation in a cyclic manner to produce six, seven and eight glucopyranose units known as α, β, γ CDs, respectively (Figure 4.1) (Vyas *et al.*, 2008). CDs possess the shapes of truncated cone made up from 1,4 glycosidic bonds with a hydrophilic exterior, whereas the internal cavity creates the lipophilic character consisting of a specific volume (Loftsson and Duchene, 2007).

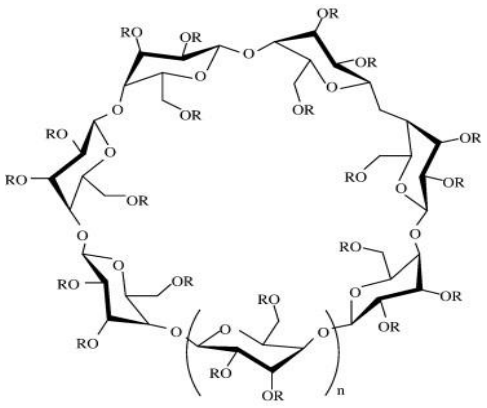


Figure 4.1- General chemical structure of beta cyclodextrin comprising of seven glucopyroranose units, where n=1. Correspondence of "n" to 0 or 2 represents the structure of alpha and gamma CD, respectively.

The cavity size is the major parameter to define their binding affinity of each sub- group natural CD for complexation (Challa *et al.*, 2005, Concha-Santos *et al.*, 2013). For example, beta CDs are the most widely used and are suitable for encapsulation of a wide range of drugs, especially for cyclic molecules. Alpha CDs tend to prove insufficient in drug inclusion due to having the smallest cavity size, while gamma CDs are the largest but display a weaker complex ability than the other two CDs (Challa *et al.*, 2005).

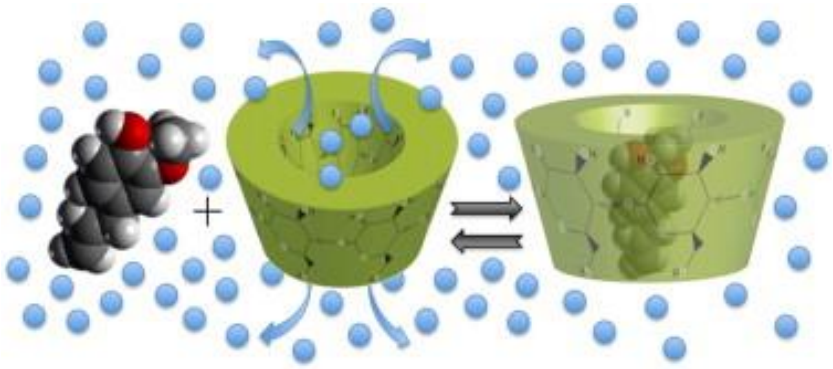


Figure 4.2- Schematic diagram of the formation of inclusion complexation of drug and cyclodextrins. Adapted from Mura (Mura, 2014).

These molecules were used primarily for their solubilising effect, whilst also serving as drug carriers through the formation of inclusion complexes to accommodate many drugs (Fig. 4.2), including polar, non-polar, aliphatic and aromatic molecules (Szejtli, 1998). Yet, the aqueous solubility of natural CD molecules is still limited due to strong intermolecular hydrogen bonding, which affects the complexation. Various CD derivatives were synthesised to extend the physicochemical properties and inclusion capacity for enhancing the aqueous profile. They exhibit a higher degree of substitution of hydrogen bond- forming hydroxyl group, and even by lipophilic methoxy functions, results in dramatic improvement in their aqueous solubility compared to the traditional CDs (Loftsson and Duchene, 2007). For example, synthetic derivatives of β-CD, such as methyl-β-CD and hydroxypropyl β-CD (HPCD), are also accessible for formulation of poorly soluble drugs, as they offer better solubility, but their use is limited by the level of toxicity and cost (Miranda *et al.*, 2011). Depending on the properties of the different types, CDs have found extensive use in pharmaceutical formulation; hydroxypropyl β-CD and sulfobutyl ether-βCD, serving as the hydrophilic CDs, are widely used for improvements in dissolution and absorption of poorly water-soluble drugs (Hirayama and Uekama, 1999), whereas the hydrophobic CDs, such as ethylated and acylated CDs, are useful as carriers for sustained release of water-soluble drugs (Loftsson and Brewster, 1996). Chemical modifications of CDs were investigated for enhancement of the transdermal and rectal absorption of drugs for local and systemic use (Matsuda and Arima, 1999), as well as to improve the chemical stability of peptide and protein drugs (Bilati *et al.*, 2005, Vyas *et al.*, 2008). The introduction of hydrophilic polymer on drug – cyclodextrin complexation have shown an enhancement in the solubilising effect of CDs for irbesartan, a poorly water- soluble drug, by studying a range of polymer concentrations (Loftsson and Brewster, 1997, Mura *et al.*, 2001, Hirlekar *et al.*, 2009) .

Complexation of CD also improves the palatability of many bitter drugs as they inhibit the contact of drug particles to taste buds, thereby providing taste masking effect (Sohi *et al.*, 2004).

4.1.2 Glipizide, poorly water- soluble model drug

Glipizide (Figure 4.3) has the molecular formula $C_{21}H_{27}N_5O_4S$ with the molecular weight of 445.535 and a water solubility of 37.2 mg/ L (DrugBank, 2015).

Figure 4.3- The chemical structure of glipizide

Glipizide is used as an oral and short acting anti- diabetic drug. It belongs to the second generation of sulfonylurea, which is more potent in the suppression of the blood glucose level in patients with type 2 diabetes. It works as a potassium blocker by binding to the potassium channel receptor on the cell surface of the pancreas, resulting in depolarisation of the channel. Consequently, this depolarisation triggers the opening of voltage - gated calcium channels, which induce the release of insulin. Glipizide was introduced into the market under brand names of Glucotrol, Glucotrol XL, GlipiZIDE XL, Minidias, Glibetin. They are prescribed in tablet forms available at 2.5 mg, 5 mg and 10 mg.

This study aims to formulate ODFs for paediatric patients, incorporating the poorly soluble drug, glipizide, an oral anti- diabetic drug for the treatment of type II diabetes. The objective was to study the effect different types of CDs on the improvement of aqueous solubility of glipizide by forming inclusion complexes and enhancing the perception of bitter taste of drug, as well as potentially increasing the drug loading and content uniformity within the ODF.

4.2 Results & Discussion

4.2.1 Phase solubility studies of glipizide

The phase solubility diagrams of glipizide with different types of CDs within the concentration range studied displayed a typical A_L type, where the aqueous solubility of the drug increases linearly as the function of CD concentration (Figure 4.4), thus proving a certain degree of its inclusion complexation in aqueous solution. The linear correction with slope less than 1 indicating the formation of a 1:1 complexation is achieved. The binding constant showed an increase of 34 M^{-1}, 83 M^{-1}, 200 M^{-1} and 222 M^{-1}, for α CD, γ CD, β CD and HPCD, respectively. The lower stability constant given by the α - and γ- CD complexes suggests that the weaker interactions are more labile, thus leading to the premature release of drug (Szejtli, 1984). On the other hand, the higher stability constant of β CD and HPCD could show the better compatibility of the guest molecule and the strength of the interaction by partial fit into the CD cavity (Szejtli, 1984, Becket G S, 1999).

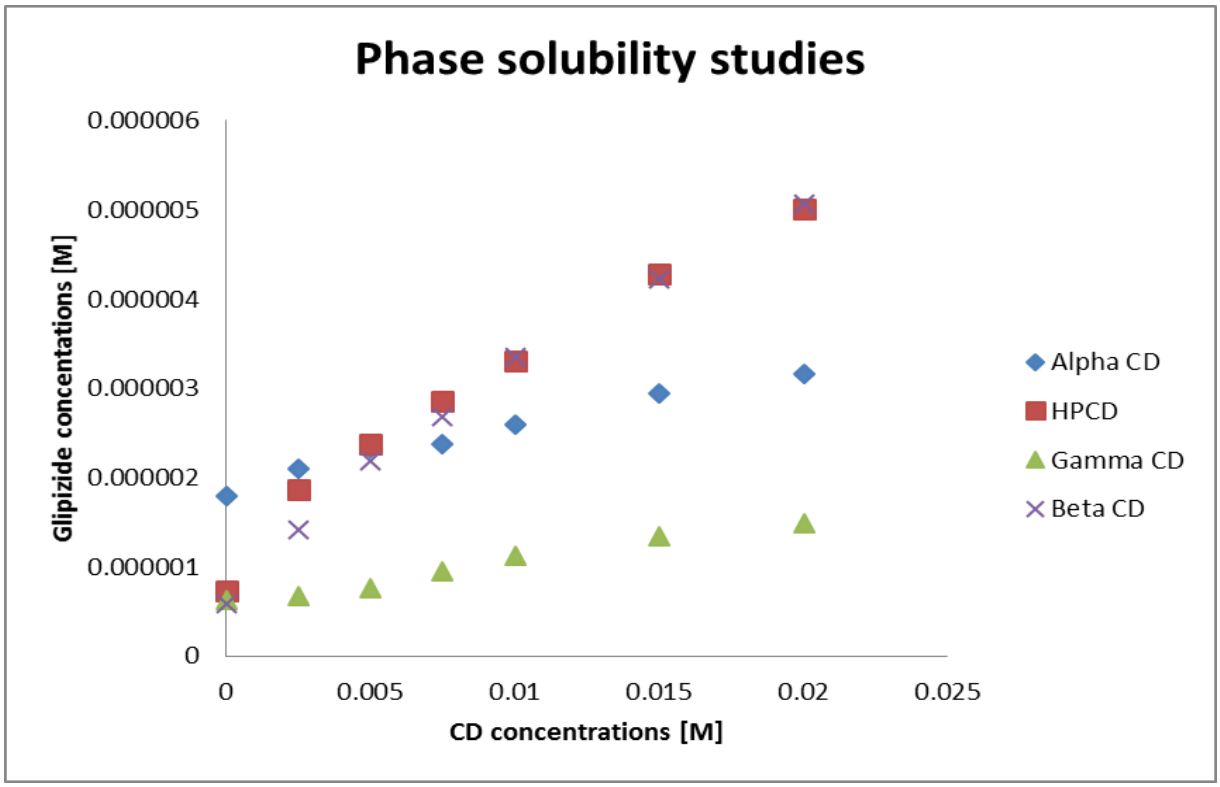


Figure 4.4- Phase solubility diagram of glipizide- alpha CD (◊), glipizide-beta CD (☒), glipizide-gamma CD (Δ) and glipizide- HPCD (□) systems in water at 25 ± 0.5 ^{0}C (mean ± SD, n=3). A typical A_L type was observed, where the aqueous solubility of the drug increases linearly as the function of CD concentration.

4.2.2 Solubility studies

The solubility profiles of glipizide and the CD complexes are shown in table 4.2. The solubility of glipizide in water was found to be 0.036 mg/mL, which is in agreement with the literature value (Dehghan *et al.*, 2010), whereas the use of CDs significantly enhanced the solubility of glipizide in both water and PBS ($p < 0.0001$, ANOVA followed by Tukey's test). Also, there were significant improvements in solubility of the poorly water soluble drug, glipizide, by individual CD complexes in comparison to each other ($p < 0.05$, Tukey's test) at the two media. Although there was an increase in solubility for glipizide in water, alpha CD complexation showed less efficiency in increasing the

solubility profile in water than the other CDs, as alpha CD itself has a limited aqueous solubility due to the strong intermolecular hydrogen bonding, thus restricting some hydroxyl groups coming into contact with water (Sapkal, 2013). In contrast, F4 formulation (HPCD complexation) showed great enhancement in the aqueous profile of this drug, as HPCD is a chemical modification of beta CD having a higher solubilising effect in water, compared to the pure drug ($p < 0.001$ Tukey's test). On the other hand, the aqueous profile of the poorly soluble drug, glipizide, gained improvement by using the beta or gamma complexation, despite the fact that these natural CDs molecules still retained their limited aqueous solubility due to strong intermolecular hydrogen bonding (Loftsson and Duchene, 2007).

Table 4.2- Comparison of solubility profile of glipizide from various complexes in distilled water and phosphate buffer solution pH 7.4 (n=3, mean ± SD). All the complex formulation showed statistically significant difference compared to the pure glipizide either in distilled water or PBS solution with $p < 0.0001$, two way ANOVA, Tukey's test). The aqueous profile of glipizide enhanced by the effect of individual CD complex, $p < 0.05$.

Formulation	Solubility in distilled water (mg/ml)	Solubility in phosphate buffer pH 7.4 (mg/ml)
Pure Glipizide	0.036 ± 0.001	0.199 ± 0.003
Alpha CD/ Gli complex	0.22 ± 0.002	0.33 ± 0.002
Beta CD/ Gli complex	0.34 ± 0.001	0.57 ± 0.005
Gamma CD/ Gli complex	0.37 ± 0.002	0.38 ± 0.002
HPCD/ Gli complex	0.41 ± 0.004	0.46 ± 0.016

However, it was observed that the CD complexation enhanced the solubility of glipizide more effectively in the phosphate buffer solution. As expected, the solubility of glipizide increased in higher pH since glipizide is a weak acidic drug with pKa of 5.9, thus glipizide becomes more ionised in solution – the weakly acidic sulfonylurea groups of glipizide become protonated at higher pH, due to the delocalisation of the nitrogen electron pair from the sulfonyl group (Jamzad and Fassihi, 2006). Nevertheless, the complexation revealed no great effect for F3 and F4 formulation either in water or PBS solution. This is possibly as a consequence of the drug and CD complexes forming water-soluble aggregation via hydrogen bonding, promoting solubilisation of the drug through non-complexation formation (Maragos *et al.*, 2009).

4.2.3 FTIR studies

FTIR analysis was performed on glipizide, KP, CD and drug- loaded films at 5 mg dose (Figure 4.5, 4.6, 4.7 and 4.8). The IR spectrum of pure CD (i.e. alpha, beta, gamma and HPCD) was characterised by a broad peak at 3329 cm^{-1} due to strong hydrogen bonding, and small sharp peaks at 2931 cm^{-1}, 1633 cm^{-1}, 1409 cm^{-1}, 1023 cm^{-1} and 996 cm^{-1}, corresponding to stretching associated with the absorbance of O-H, C-H, C-O functional group, respectively (Figure 4.5, 4.6, 4.7 and 4.8) (Gil *et al.*, 2004, Dua *et al.*, 2011). Glipizide showed the major double peaks at 3329- 3241 cm^{-1} due to NH-CO stretch, as well as other prominent peaks at 1651 cm^{-1} (CONH stretching), 1598 cm^{-1} (C=N stretch), 1370 cm^{-1} (SO_2NH Stretching), 1142 cm^{-1} (cyclohexyl stretching), 1651 cm^{-1} (C=O, Urea) (Behera *et al.*, 2008, Jain and Saraf, 2009). KP demonstrates a broad absorption band at 3200 cm^{-1} of hydroxyl group, sharp bands at 1727- 1709 cm^{-1} and 1041 cm^{-1}

associated with C=O stretching. The position of all absorption bands remained the same as in the spectrum of each component in the spectrum of the physical mixture. However, no peak of drug and complex was observed in the KP film, which suggests that the NH group of Glipizide emerged with the broad band of alpha CD in the same region of hydroxyl group. Furthermore, the drug and the complex could potentially form hydrogen bonding due to their broad band observed at the hydroxyl regions. Yet, these may well be masked by the film peaks, particularly as they were used in less quantity than the KP. Hence, other analyses are needed to determine its presence in the film formulation.

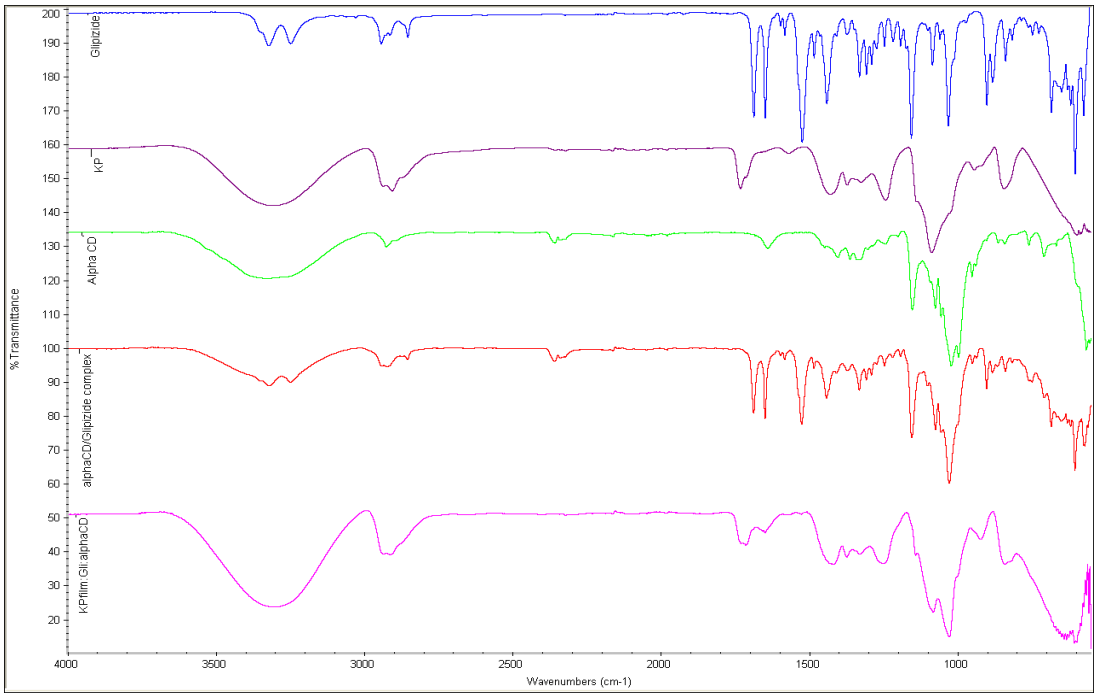


Figure 4.5- FTIR of glipizide, alpha CD, KP and glipizide - CD complex and drug-loaded KP film.

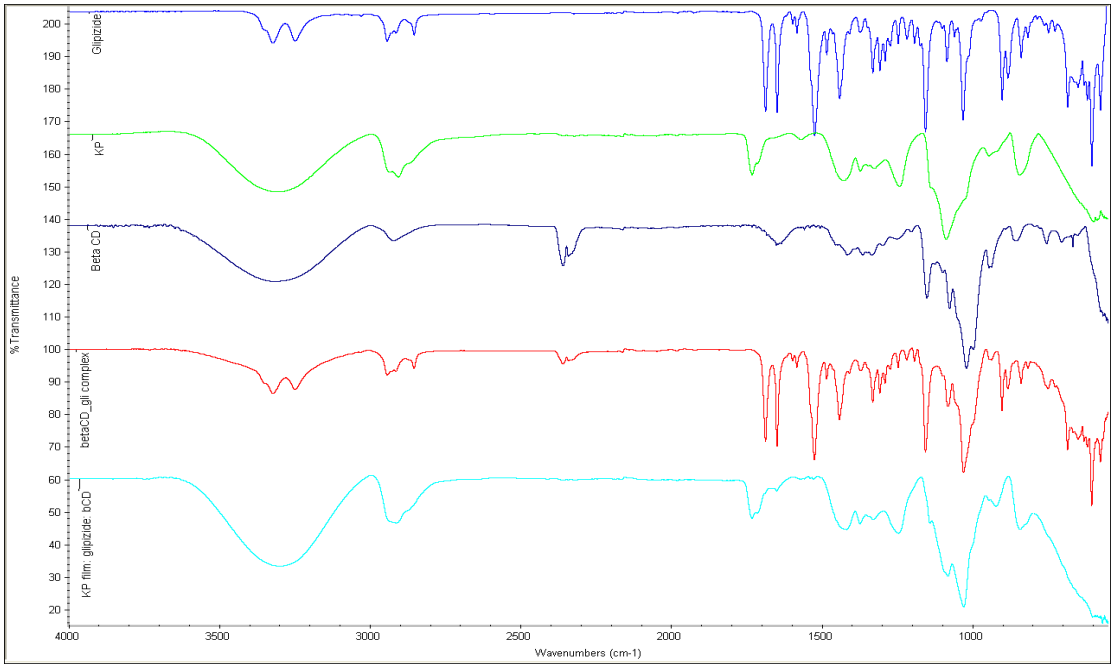


Figure 4.6- FTIR of glipizide, beta CD, KP and glipizide - CD complex and drug-loaded KP film.

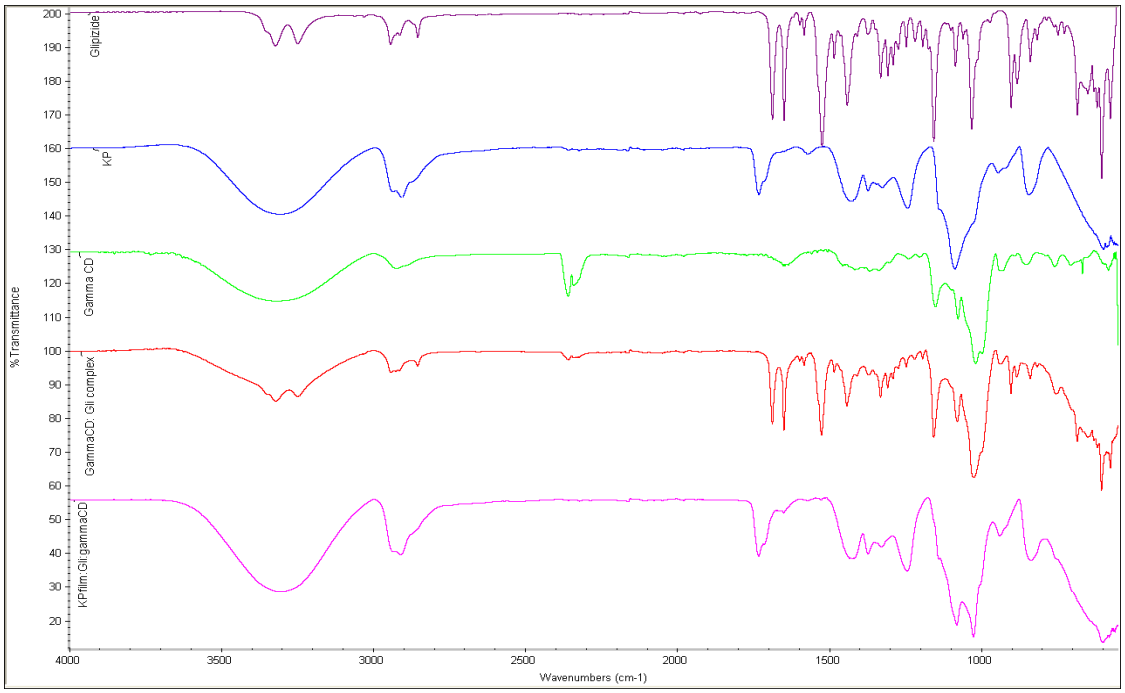


Figure 4.7- FTIR of glipizide, gamma CD, KP and glipizide - CD complex and drug-loaded KP film.

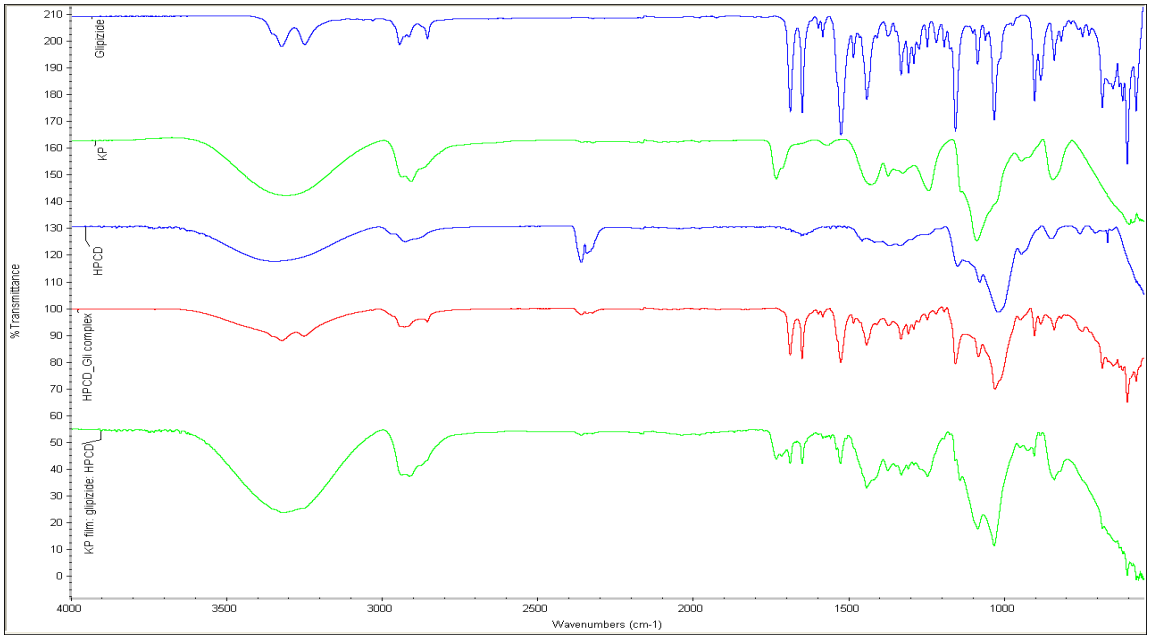


Figure 4.8- FTIR of glipizide, 2- HPCD, KP and glipizide - CD complex and drug-loaded KP film.

4.2.4 DSC studies

The thermogram of alpha CD exhibits three exothermic peaks at 80 ^{0}C, 106 ^{0}C and 120 ^{0}C, which correlates to previous reports in the literature (Figure 4.9) (J Szejtili, 1988). In case of the DSC curve for beta CD, the wide endothermic peak was recorded from 86.22 ^{0}C- 118.66 ^{0}C corresponding to the dehydration of water molecules within the CD cavity (Figure 4.10) (Dua *et al.*, 2011). Pure gamma CD exhibited the broad endothermic peak in a range of temperature of 50 ^{0}C – 150 ^{0}C, which is corresponding to the evaporation of water (Figure 4.11) (Gil *et al.*, 2004). Because of its amorphous form, HPCD showed a broad endothermic peak at about 96 ^{0}C and this peak is related to dehydration of water molecules within the CD cavity (Figure 4.12) (Kohata *et al.*, 1993). Pure powdered glipizide displayed a melting endothermic peak at 213.30 ^{0}C with the fusion enthalpy of 302.6 J/g, which suggests that the drug is in the anhydrous crystal form. The thermograms

of the glipizide- CD complexes express the presence of each component at a similar temperature to those thermograms of the raw materials due to their physical mixing. Blank KP films showed mainly the dehydration of water from 52- 91 ^{0}C, since KP is a polyvinyl alcohol-polyethylene glycol graft copolymer, where the polymer chains are composed of large amount of hydroxyl group (-OH group). The change of the drug melting peak position from 213.30 ^{0}C to 156 ^{0}C and intensity in glipizide- CD complex loaded film formulations could be attributed to the inclusion of glipizide in the CD cavity, which can sterically hinder the melting formation of the drug or a possible reduction in crystallinity upon complexation, where the changes were not recorded with the glipizide-CD complex scans. The appearance of low peak density of drug corresponds to the low amounts compared to the polymer that might masked the CD complex and possibly the reason that some of these thermal events cannot be detected well (Figure 4.9, 4.10, 4.11 and 4.12). Overall, DSC data did not show Tg of KP, and the effect of drug on Tg, perhaps, polymer and the complex remain its amorphous form.

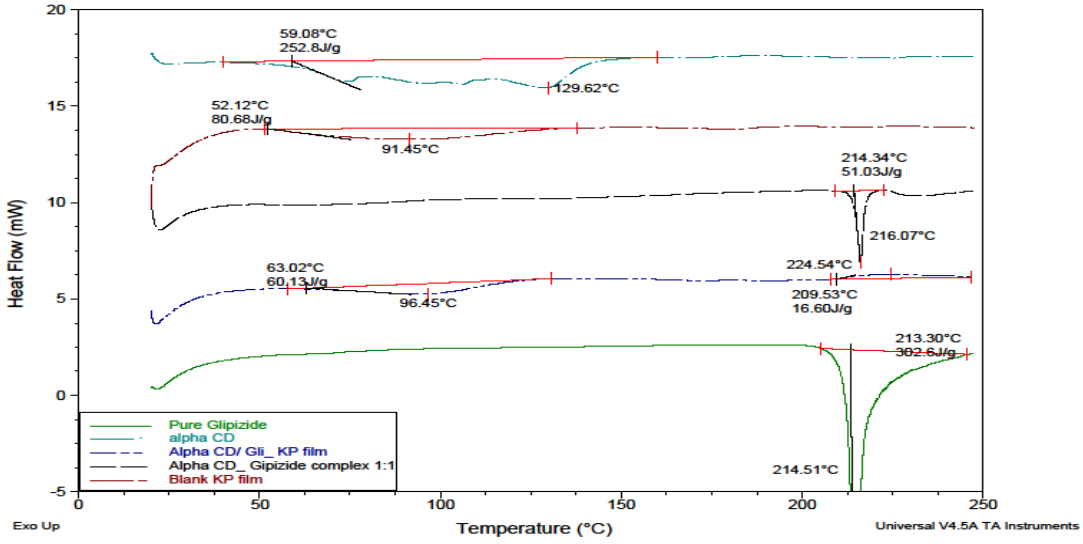


Figure 4.9- DSC of glipizide, alpha CD, KP and glipizide - CD complex and drug-loaded KP film.

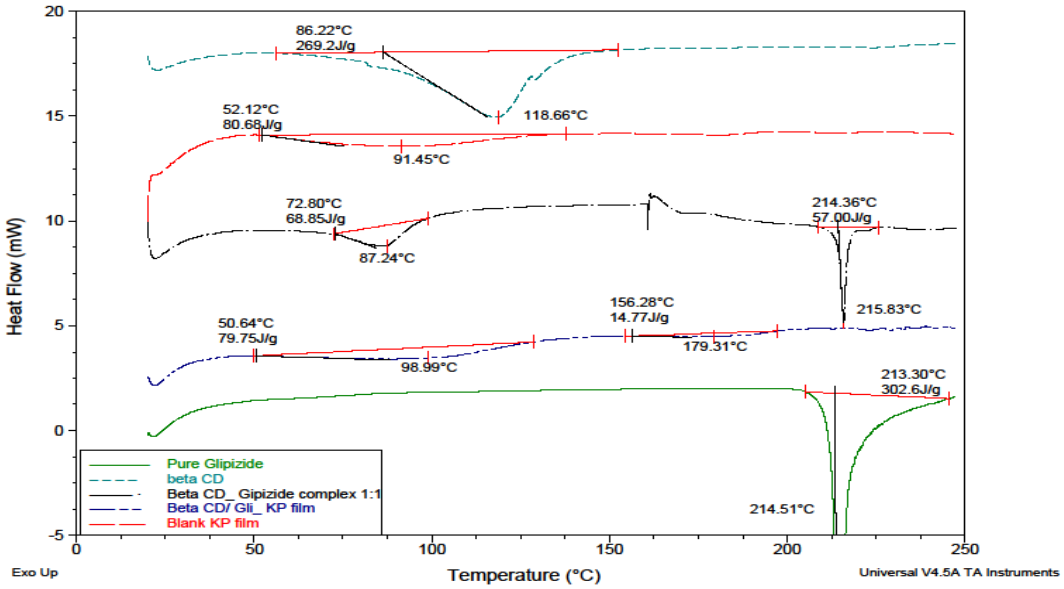


Figure 4.10- DSC of glipizide, beta CD, KP and glipizide - CD complex and drug-loaded KP film.

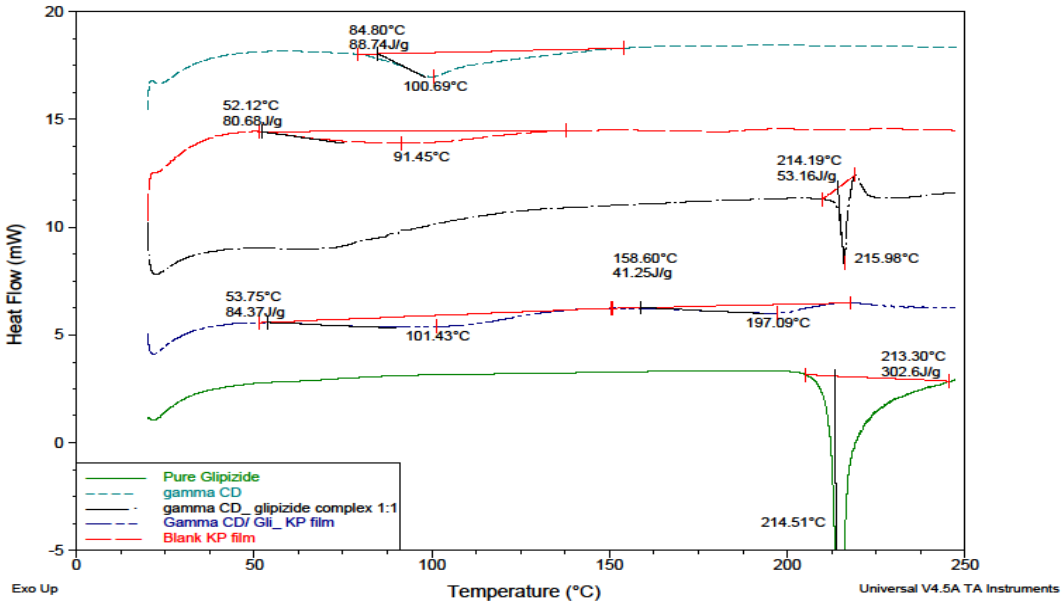


Figure 4.11- DSC of glipizide, gamma CD, KP and glipizide - CD complex and drug-loaded KP film.

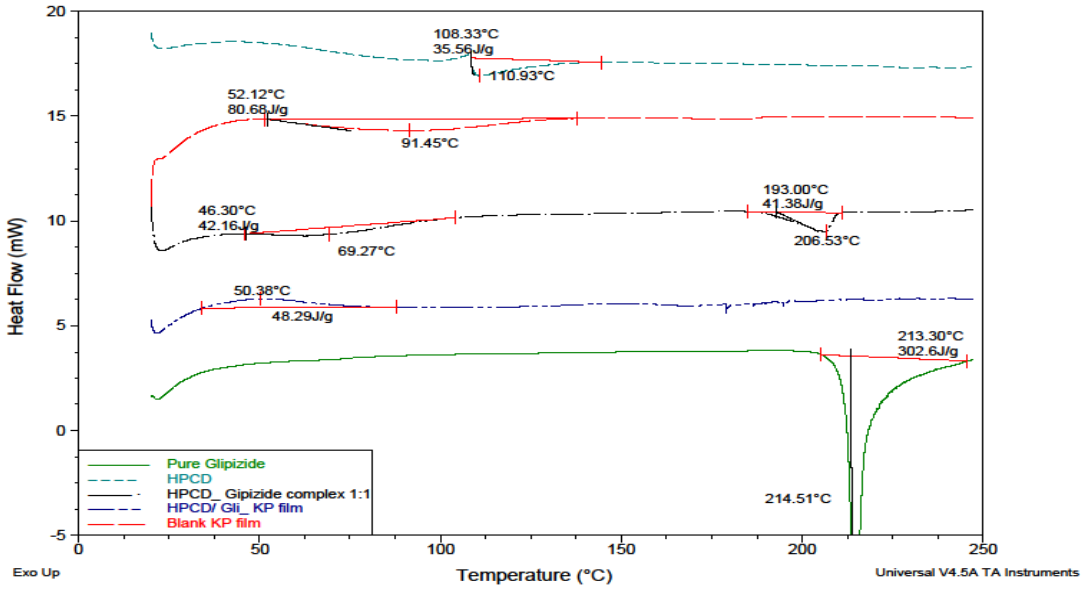


Figure 4.12- DSC of glipizide, HP CD, KP and glipizide - CD complex and drug-loaded KP film.

4.2.5 Moisture content

According to the moisture content allowance relating to Kollicoat protect (up to 5 %) (BASF, 2007), blank KP films remained stable as they achieved the acceptable boundary for moisture content (BASF, 2007). This is attributed to the strong barrier properties of KP against water vapour (Yadav, 2013). Percentage moisture uptake varied within the range of 0.45 ± 0.17 % to 1.02 ± 0.13 % at room temperature and of 0.52 ± 0.28 % to 1.53 ± 0.21 % in desiccators at room temperature (Figure 4.13). Nevertheless, these two storage conditions showed no significantly changes in weight for all films studied between day 0 to day 7 ($p > 0.05$, ANOVA followed by Tukey's test), further confirming the barrier properties of KP against water. It was also seen that beta CD/ glipizide complex loaded films have the lowest moisture content in both conditions ($p < 0.01$, ANOVA followed by Tukey's test), since beta CDs possess a high number of

intramolecular hydrogen bonding between the matrix that causes a decrease in the chain mobility, thus restricting the permeability of water vapour (Loftsson and Duchene, 2007). Conversely, alpha and gamma CD complexes possess weaker interactions within the cavity, which are more labile to exposure to water vapour in room temperature. However, the moisture content of HPCD complex loaded KP films is higher than that of the other natural CDs ($p < 0.05$, ANOVA followed by Tukey's test); since HPCD is a chemical derivative of natural beta CD, where an increase in substitution level is optimal for complexing capacity, this could distort the CD ring structure by weakening the intramolecular hydrogen bonding, which possibly allows water molecules to be more readily absorbed into the matrix (Miranda *et al.*, 2011). The incorporation of a complexation with less hydrophilic character inside the hydrophilic polymer could reduce the water absorption of films in comparison with the control blank film, as there may be cross- linking between the polymer and the complex, and hence minimising the migration of CD molecules and the drug molecules towards the polymer surface, causing the reduction of water absorption (López-de-Dicastillo *et al.*, 2011) .

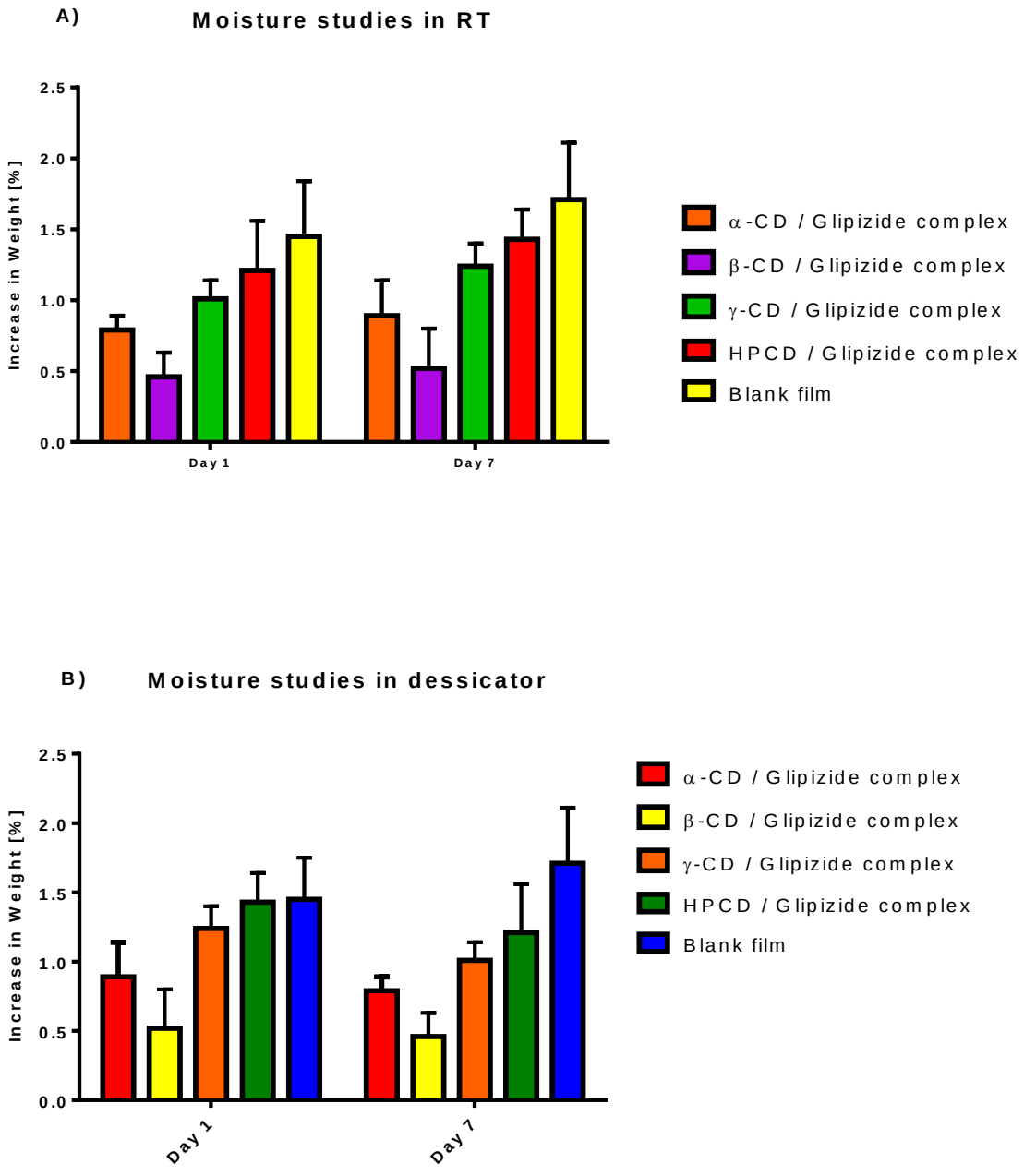


Figure 4.13- Moisture studies for blank KP films and films containing glipizide-CD complexes at A) room temperature (25 ± 2^0C) and B) in the desiccator at day 1 and day 7. The percentage moisture uptake is measured based on the final weight of the film in comparison to the initial weight of the film when freshly prepared at initial day**.** No significantly difference in the weight of films stored at room temperature and in desiccator from the initial day to day 7 ($p< 0.05$, Tukey's test).

4.2.6 Mechanical properties of films

4.2.6.1 The effect of CD complexation

The mechanical properties of ODFs were evaluated through their strength and elasticity. A soft, adequately strong film should retain moderate tensile strength, high % elongation, and low elastic modulus (Mashru *et al.*, 2005). All film achieved uniformity in thickness (0.14 µm) regardless to different types of complexation. Films loaded with different types of glipizide-cyclodextrin complex at 10 mg dose were flexible, transparent and non-sticky. Results showed that all films exhibited moderate tensile strength and recorded an increase in % elongation for the different CD formulations, which indicates films become more flexible in the order F1< F2< F3< F4 (Figure 4.14).

However, mechanical properties of all film formulation were insignificantly reduced by the incorporation of inclusion complex by different sub- classes of CDs**.** A slight reduction was apparent in tensile strength and Young's modulus of glipizide/ complex loaded films, but there was no major changes in these parameters compared to the controls ($p > 0.05$, ANOVA followed by Tukey's test), possibly as a consequence of the addition of CD complexes to the films causing aggregation and break down of the polymer matrix, which enhances the molecular mobility and free volume of polymer network (Fundo *et al.*, 2014). In contrast, there were no significant changes in mechanical properties of films by individual CD complexes in comparison to each other ($p > 0.05$, ANOVA followed by Tukey's test) (Figure 4.14). Since the drug has been incorporated in the CD cavity, there was no interaction with the polymer and the CD cavity, as consequently, no significant impact on the tensile properties of films for each example of CD were

observed. Based on the results, ODFs containing glipizide-CD complexes are soft and tough, exhibiting a low tensile strength but high percentage elongation (Felton *et al.*, 2008) with or without the addition of complexation.

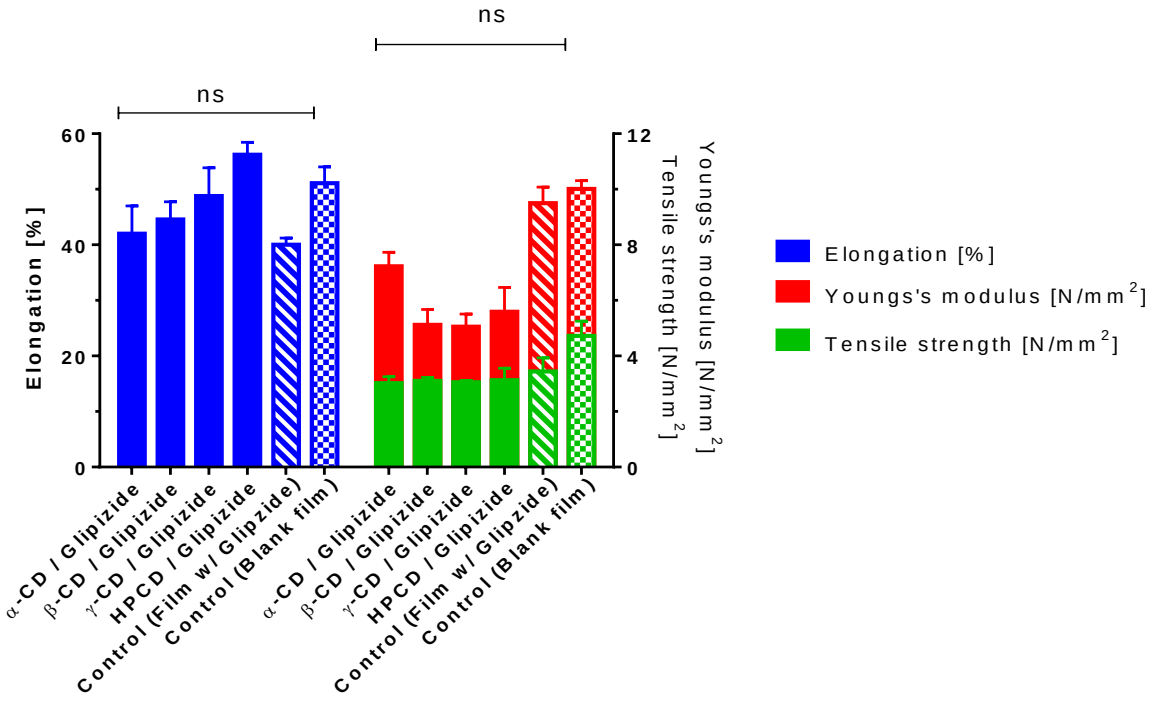


Figure 4.14- Mechanical properties of KP film containing glipizide-CD complex at 10mg dose where F1= αCD/ glipizide complex, F2= βCD/ glipizide complex, F3= γCD/ glipizide complex, F4= HPCD/ glipizide complex (n=3, mean ± SD). No statistical difference was observed using one way ANOVA followed by Tukey's test , between different types of CD complex loaded films and the controls, ns ($p > 0.05$)

4.2.7 Drug loading – dose escalation study

The drug loading capacity of pure drug into the KP films achieved 90.0 ± 0.30 %, 80 ± 0.35 %, 60 ± 0.24 %, 50.26 ± 0.45 % and 42.83 ± 0.28 % at a dose of 2.5 mg, 5 mg, 10 mg, 15 mg and 20 mg, respectively (Figure 4.15).The use of CD complexation showed a significant enhancement in the drug loading of glipizide into KP films compared to pure drug itself ($p < 0.01$, ANOVA, Dunnett's test). In the case of films incorporating glipizide-CD complexes, the drug loadings were achieved up to 93.0 ± 0.28 %, 94.30 ± 0.25 %, 96.50 ± 0.44 % and 97.20 ± 0.43 % at 2.5 mg dose for F1 to F4, respectively. The high achievement in drug loading is the possible use of a small dose (2.5 mg) and a better inclusion of drug inside the CD cavity with an increase in volume size of the different sub classes of CD. However, at a dose of 5 mg, the drug loading of F1 and F3 were 62.5 ± 0.23 % and 88.0 ± 0.20 %, respectively, but were higher for F2 and F4, up to 91.30 ± 0.24 % and 92 ± 0.12 %, respectively. Indeed, the drug loadings were significantly higher with the beta, gamma and HPCD complexes at 5 mg dose compared to without the use of CDs ($p < 0.001$, ANOVA followed by Dunnett's test), suggesting efficient inclusion of the drug within the hydrophobic cavity. However, a considerably lower drug loading was evident for the alpha CD complex, compared to the control ($p < 0.001$, ANOVA followed by Dunnett's test), possibly as a consequence of the smaller cavity size associated with alpha CDs, which leads to insufficient drug inclusion (Challa *et al.*, 2005). In contrast, there was a significant decrease in the percent drug loading efficiency with an increase in drug loading in the formulations ($p < 0.001$, ANOVA followed by Dunnett's test). KP films incorporating glipizide-CD complexes at a dose of 10 mg reached 43.35 ± 0.36 %, 49.30. ± 0.08 % , 53.65 ± 0.83 % and 68.8 ± 0.54 % of drug loading for alpha CD, gamma CD, beta CD and HPCD, respectively. Again, a

significant reduction in loading efficiency was observed for films incorporating glipizide-CD complexes a dose of 20 mg, to 13.69 ± 0.69 %, 13.83 ± 0.44 %, 17.50 ± 0.53 % and 35.89 ± 0.92 % for alpha CD, gamma CD, beta CD and HPCD, respectively ($p < 0.0001$, ANOVA followed by Dunnett's test). This suggests that there may be a lack of space available within the ODFs for a specific volume size of CD and/or drug, since a similar downward trend was also apparent for drug loading without CDs. In addition, for the lower loading associated with alpha CD - drug complex and gamma CD - complexes may be due to weaker drug-CD interactions, whereas other complexes show greater drug loading, indicating stronger drug-CD interactions, which is supported by the evidence of the stability constant values (see part 4.2.1). Amongst the different CDs investigated, HPCD complexes achieved the most efficiency in drug loading, as a consequence of the broader cavity volume as well having a better compatibility with the guest molecule and the strength of the interaction with the CD cavity, as evidenced from previous results (see section 4.2.1 and 4.2.2).

Furthermore, results indicate the limitation for drug loaded into film, since the maximum amount of drug able to be efficiently loaded into the ODFs, both with and without complexation, appeared to be around 12 mg, after which point no further drug could be loaded, regardless of initial dose added. Nevertheless, although the complexes provided no benefit with regards to drug loading at the higher doses, and in some instances were disadvantageous in this regard, there would still be the added benefit of taste-masking and enhanced stability compared to drug alone.

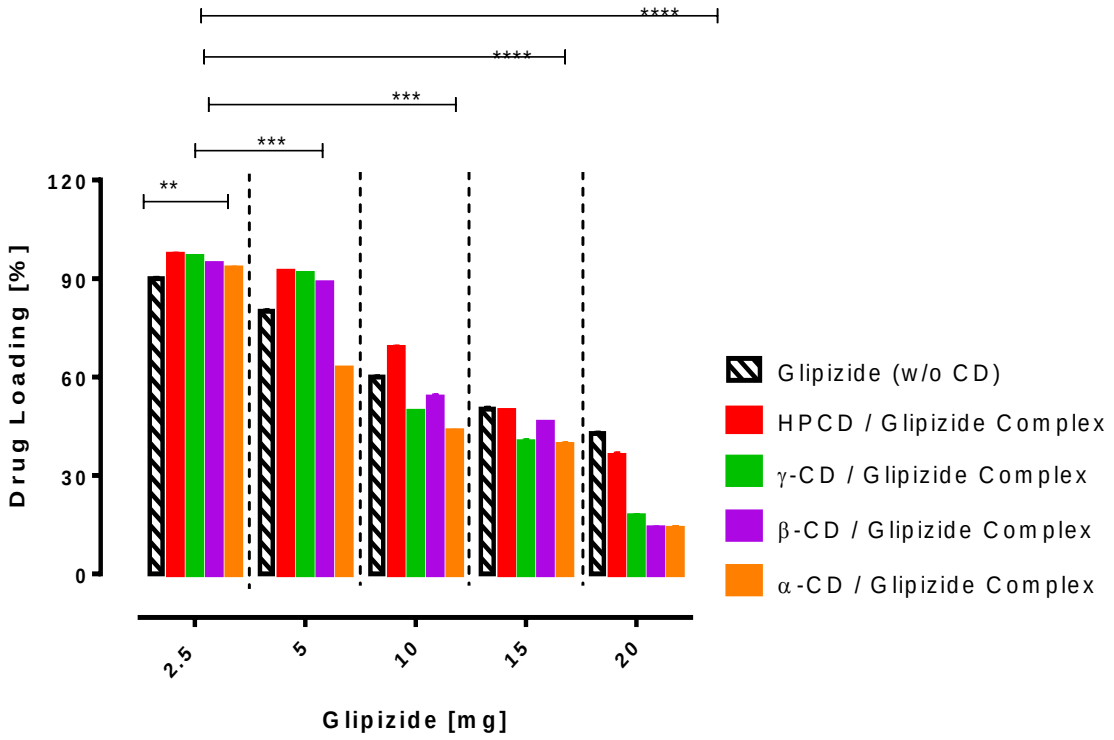


Figure 4.15- Dose escalation study of glipizide loaded CD complexation- Results showed the reduction in drug loading up to 20 mg of glipizide due to the availability of the cavity volume of each CD (mean ± SD, n=3). Statistically significant differences are noted as follow: ns ($p > 0.05$); * ($p < 0.05$); ** ($p < 0.01$); *** ($p < 0.001$); **** ($p < 0.0001$), two way ANOVA).

4.2.8 Drug content uniformity determination

Since glipizide is poorly soluble in water, achievement of drug content uniformity, which is vital for patient safety and efficacy since a uniform dose will provide consistent dosing and, therefore, more predictable and stable levels of drug within the patient, thereby providing a more consistent therapeutic effect, becomes a challenging parameter for formulation of dosage forms, especially for ODFs with limitation of drug loading.

The drug content of glipizide without the addition of CD complexation is on the limits of the acceptable boundaries for drug content uniformity (85 -115 %, BP 2013), suggesting poor distribution within films, whereas the content of uniformity of glipizide from ODF formulations loaded at 5 mg with the various CD complexes not only showed within the criteria standard, but also improved significantly in dose uniformity compared to the control ($p < 0.0001$, ANOVA followed by Dunnett's test) (Figure 4.16). Furthermore, the complexes improved drug loading at low doses, but it has proved that the content uniformity of poorly soluble drug is enhanced by the use of CD complexation. In addition, regarding to the clinical aspects, the use CD complexation could provide the additional benefit to the taste-masking and enhanced stability of bitter drugs.

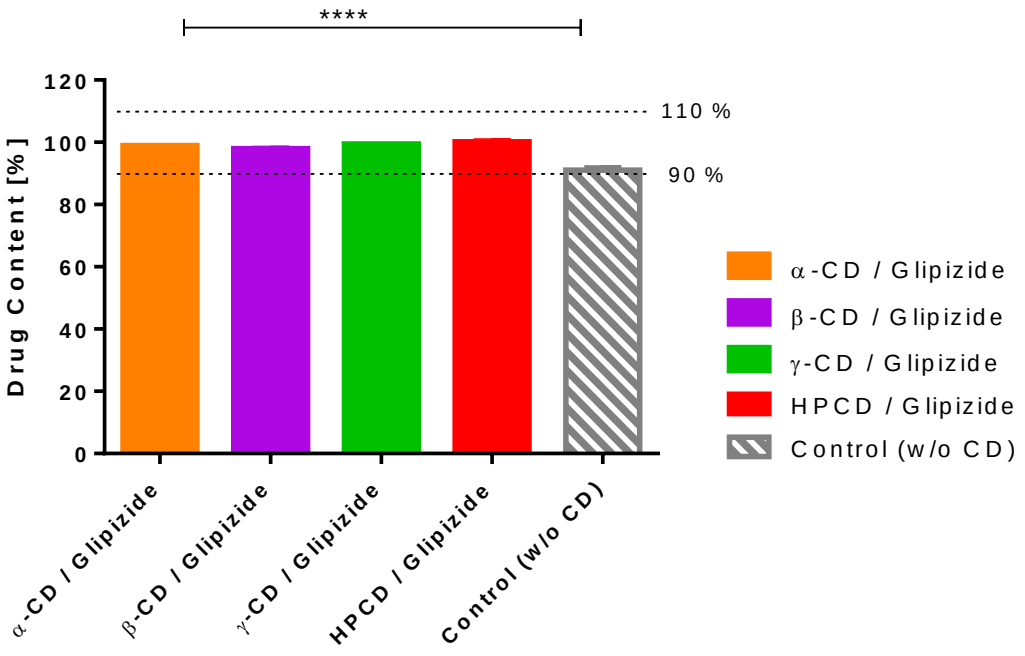


Figure 4.16- Drug content uniformity of 5 mg glipizide loaded ODF with and without CD. Results reported in 3 replications (mean ± SD, n=3). Dash line indicates the criteria for content uniformity of ODFs. Statistically significant differences are analysed using one way ANOVA with the variance as followed; **** ($p < 0.0001$).

4.2.9 Disintegration time

The disintegration test was carried out in 25 mL distilled water (Fig 4.17). ODFs are desired to dissolve quickly for fast release within a minute (Kalyan and Bansal, 2012). Disintegration profiles of orally dissolving films of glipizide demonstrated acceptable design, with all film formulations dissolving completely within 15 seconds, as expected due to the presence of the hydrophilic film former.

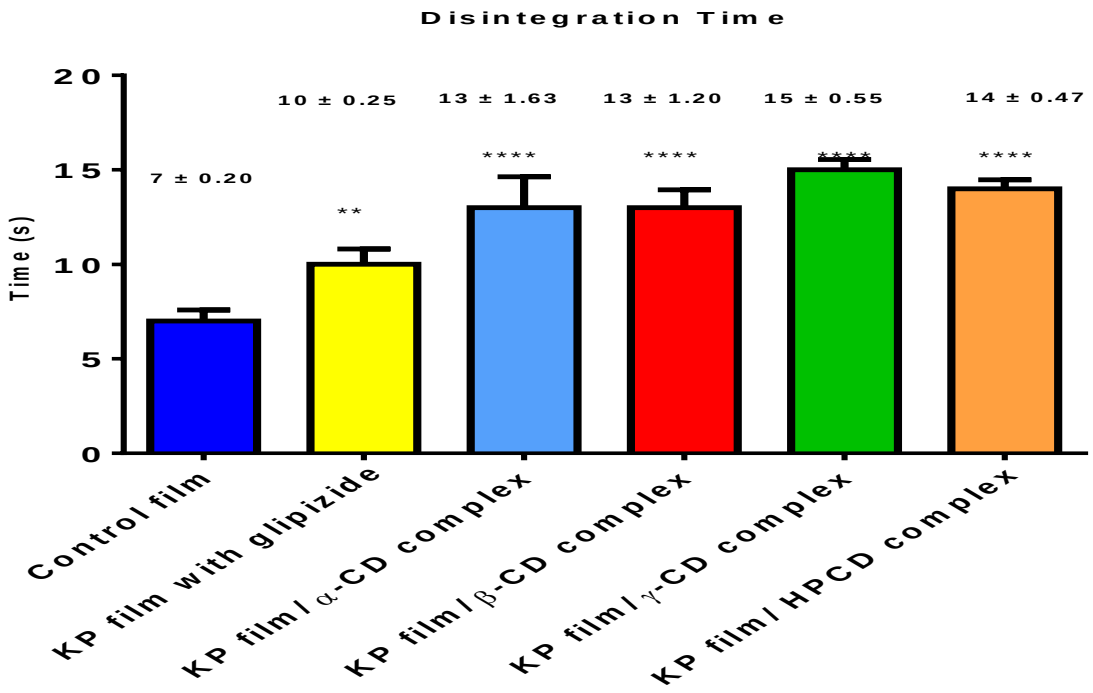


Figure 4.17- Disintegration time of blank films, KP film loaded glipizide only and KP films containing drug -CD complexes in 25 ml distilled water at 37 ^{0}C (mean ± SD, n=3). Statistical different was observed using one way ANOVA followed by Dunnett's post- test, between different types of CD complex loaded films and the controls, with the variance as follow: ** ($p < 0.01$); **** ($p < 0.0001$).

However, there is a significant increase in disintegration time when the films were loaded with glipizide- CD complexes compared to the blank film ($p < 0.0001$, ANOVA followed by Dunnett's test) and KP film loaded with glipizide without complexation ($p < 0.05$, ANOVA followed by Dunnett's test). The time for films to dissolve between control and glipizide without complexation was also found to be significantly different ($p < 0.01$, ANOVA followed by Dunnett's test). The time taken for blank films to disintegrate was only 7 seconds, as it is readily soluble in water, whilst it took longer time for CD complex loaded KP films to dissolve completely, as more time is required to uptake more water molecules for breaking the complexation and polymer matrix. Besides that, low moisture content recorded for complex loaded films, respectively (see 4.2.5- moisture studies), which probably implement on a longer disintegration time of films based on the strong barrier of KP against water, and thereby, it takes more time for water being absorbed into the polymer matrix. In contrast, there is no significant difference for each sub-class CD complexation in breaking up the films, as they have a similar chemical structure ($p > 0.05$, ANOVA, Dunnett's test) (Challa *et al.*, 2005).

4.3 Conclusion

Glipizide formed a stable inclusion complex with CDs with a typical A_L type. The stability constant showed a linear increase of 34 M^{-1}, 83 M^{-1}, 200 M^{-1} and 222 M^{-1} for α - CD, γ- CD, β - CD and HPCD, respectively, with a range of cavity size available for each type of CD. The stability constant of complexation is strongly dependent on the strength of the interactions of the guest molecule and the CD cavity. The solubility profile of glipizide was enhanced by incorporating within the complexes in both water and PBS

solution, which is to be expected, with a higher improvement of solubility of glipizide in PBS due to the high degree of ionisation from the weak acidic group. All film formulations were flexible, transparent and non- sticky and achieved uniformity in thickness (0.14 μm) and drug content (85-115%) at lower drug loadings. Glipizide-CD complex loading of the selected film formulations resulted in further reductions in tensile properties (e.g. tensile strength and elastic modulus of films were reduced, but the percentage elongation was increased) as observed in the order of α-CD β-CD, γ-CD, and HPCD complexation. The results showed that not only plasticiser but also complexation can potentially act as further plasticising agent, as the complex could aggregate and break down the polymer matrix, which enhances the molecular mobility and free volume of polymer network. There was no change in moisture content of film formulations in room temperature and oppositely reduced in the desiccator ($p > 0.05$). However, the interaction bonding within the cavity is responsible for moisture content determination as the evidence of beta CD/ glipizide complex loaded films have the lowest moisture content in both conditions, compared to other complexation due to alpha and gamma CD complexes possessing the weaker interactions within the cavity, which are more labile to expose to water vapour in room temperature. The disintegration time of films and glipizide-CD complex-loaded films was less than 20 seconds in 25 mL distilled water, due to the presence of hydrophilic film former. FTIR suggested that the NH group of Glipizide emerged with the broad band of CD in the same region of hydroxyl group and be masked by the film peaks, particularly as they were used in less quantity than the polymer. DSC data did not show Tg of KP, and the effect of drug on Tg, perhaps, polymer and the complex remain its amorphous form. Drug loading efficiency reduced significantly for all film formulation with an increase in amount of doses of pure drug and drug complexes into films (up to 20 mg), due to poor loading capacity of cavity volume of CDs ($p < 0.05$)

whilst they could impart taste masking and enhance stability. Overall, CD complexation showed the improvement solubility profile of poorly soluble drug- Glipizide. CD complexation improved uniformity of drug loading available at low dose based on the limited cavity sizes.

Chapter 5
Evaluation and physicochemical characterisation of dexchlorpheniramine maleate loaded orally dissolving films

5.1 Introduction

Allergy rhinitis is a common, chronic condition that results in inflammation by pollen or dust, with classic symptoms of nasal congestion, itching and sneezing, whilst severe conditions could lead to the impairment of quality of life and sleep disturbance (Rosenwasser, 2002, Kemp, 2009). It is estimated that between 10 to 20 % of the population of the population are affected by the disorder, with the prevalence increasing to upwards of 40 % within the paediatric community (Small and Kim, 2011). In most cases, the frequency of allergy increases with age depending upon on the geography, genetic and living environment (Mandhane *et al.*, 2011), with rates of exposure to allergens increased in older children aged from 13 to 14 compared to younger age group (Skoner, 2001). Besides the avoidance of allergens, several pharmacotherapeutical clinical treatments of allergy have been implemented, such as the use of corticosteroids, antileukotrienes and antihistamines, with the latter being the most traditional choice. The use of first generation H1 antihistamine is widely available and has shown great potential as first-line medication for both intermittent (seasonal) and persistent (perennial) allergic rhinitis (Simons, 2003). Moreover, antihistamine is a common API for treatment of cough and cold, runny nose and sneezing, with many different dosage forms available, such as tablets, capsules, oral syrups and spray solutions. Indeed, one of the commercially available dosage forms is based on the fast drug delivery systems as orally disintegrating tablets (ODTs) containing loratadine at 10 mg dose strength for relieving seasonal allergies; however, this is not licensed for children under 6 years old and, since ODTs are still a solid monolithic oral dosage form, there remains concerns over choking. Using orally dissolving films, as alternative new system to traditional oral dosages, bring

potential benefits for rapid onset of action and ease of administration especially for quick relief of allergy.

Dexchlorpheniramine maleate (DCM), an active isomer of chlorpheniramine, is a H1 antagonist from the first generation of anti-histamines (Fig. 5.1). DCM is a white, odourless, crystalline powder that is freely soluble in water and soluble in alcohol. It has the empirical formula $C_{16}H_{19}ClN_2 \bullet C_4H_4O_4$, with the molecular weight of 396 (DrugBank).

Figure 5.1- The chemical structure of DCM

DCM belongs to the alkylamine derivatives and its sedative activity is twice as potent as chlorpheniramine. DCM is used for systemic relief of allergic conditions, such as urticaria and angioedema or even for the treatment of common coughs and colds. DCM is mainly given orally and sometimes delivered topically.

DCM is available at 2 mg oral dose for every 4 to 6 hours. According to the BNF, DCM is available at 500 micrograms for children aged from 2 to 6 years and 1 mg for those

aged 6 to 12 years every 4 to 6 hours in the treatment of allergic rhinitis (BNF, 2010). DCM is the choice of drug for paediatric patients, due to its high solubility and acceptable bioavailability.

Aim & objectives

This chapter aims to investigate the formulation of ODFs containing antihistamine for fast onset of action for allergy relief at the suitable clinical doses for children.

The objectives were to investigate:

- The physico-chemical and mechanical properties of ODFs following incorporation of the anti-histamine DCM.

- The development of a more suitable testing system for quantification of the disintegration time of ODFs, by studying the impact of test media and media volume on the disintegration behaviour;

- The development of prototype packaging and their ability to maintain ODF stability at different storage conditions.

5.2 Result & Discussion

5.2.1 Mechanical properties

The mechanical properties of films containing DCM was assessed based on the setting method in chapter 2 (see 2.7.4). The thickness of the films before and after DCM loading was uniform (Table 5.1), whilst the texture of films was flexible, transparent and non-sticky, regardless of drug loading. Results showed that the mechanical properties of drug loaded films reduced slightly compared to control films, possibly due to the plasticising effect of the drug (Table 5.1).

Table 5.1- Mechanical properties of blank film and DCM loaded KP film at dose 1 mg and 2 mg at size 3 x2 cm^2. Drug loading has no effect on the mechanical properties of film due to small amount of drug present ($p > 0.05$, one way ANOVA, Dunnett's test).

Dose	**Thickness (*mm*) (n=5)**	**Mechanical properties**		
		Tensile strength (*N/mm²*)	**Elongation (%)**	**Young's modulus (*N/mm²*)**
Control	0.14 ± 0.001	4.72 ± 0.53	51.08 ± 2.93	10.01 ± 0.03
1 mg	0.14 ± 0.001	3.16 ± 0.10	52.58 ± 3.57	6.03 ± 0.58
2 mg	0.14 ± 0.001	3.02 ± 0.11	49.01 ± 4.52	6.27± 0.80

Even though in a low amount, the hydrophilic drug molecules are able to occupy the polymer matrix, thereby interacting with the polymer chains, which possibly leads to a reduction in the mechanical properties of films (Repka *et al.*, 1999). However, this difference was not deemed to be significant ($p > 0.05$, one way ANOVA, Dunnett's test).

Indeed, an increase in dose loading seems not to have any effect on mechanical properties of films, as a low amount of drug was used ($p > 0.05$, one way ANOVA, Dunnett's test) (Table 5.1).

5.2.2 FTIR Studies

DCM showed the major double peaks at 3329- 3241 cm^{-1} due to N-H stretch vibration. Other prominent peaks at 1697 cm^{-1} (C=O stretching), 1598 cm^{-1} (C=N stretch), carboxylate 1353.31 cm^{-1} and Maleate 860.78 cm^{-1}. KP demonstrates a broad absorption band at 3200 cm^{-1} of hydroxyl group, sharp bands at 1727- 1709 cm^{-1} and 1041 cm^{-1} associated with C=O stretching (Figure 5.2). Physical mixture demonstrated the superposition of pure drug and polymer. Although no major peak of drug was observed in the KP film, due to emerging of bonding in the same regions, new bond formation was recorded at 1093 and 1038 cm^{-1}, which suggests that the maleate group of DCM interacted with the polymer, which may explain the reduction in mechanical properties of films as a consequence of the potential plasticising effect of drug. In addition, these may well be masked by the film peaks, particularly as they were used in less quantity than the KP.

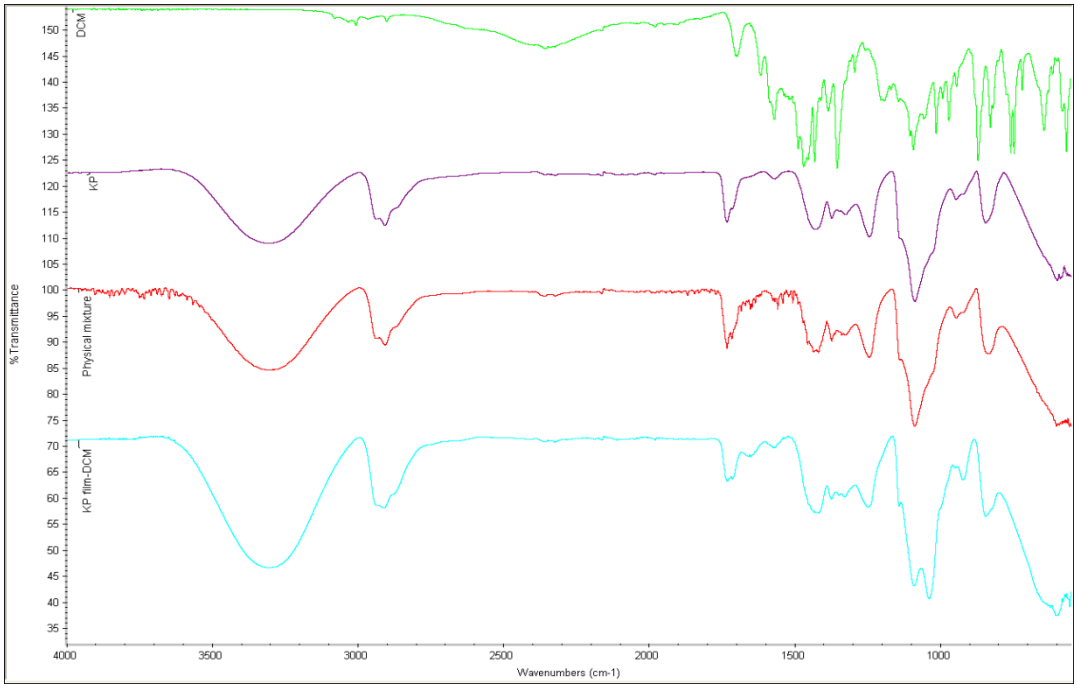


Figure 5.2- FTIR of DCM, KP, physical mixture and drug-loaded KP film.

5.2.3 DSC studies

The DSC curves obtained for pure drug and optimised formulation are shown in Figure 5.3. Pure powdered DCM displayed a single sharp endothermic peak at 112.96 ^{0}C with the fusion enthalpy of 88.31 J/g, which suggests the drug was in the crystalline form (Figure 5.3). Blank KP film recorded the distinctive melting peak at 206.33 ^{0}C and a broad peak at 76 ^{0}C responsible for the dehydration of water, since KP is a polyvinyl alcohol-polyethylene glycol graft copolymer. The thermal curve of the physical mixture showed the corresponded melting to the superposition of polymer and pure drug. A change in thermal behaviour of polymer and drug might be an indication of interaction between drug and polymer as seen in FTIR (see 5.2.2).In contrast, no peak of drug was observed in the DSC for the drug loaded film. However there is drug present, as confirmed by other analyses (e.g. drug loading, FTIR), but the nature of incorporation into the film

may help to preserve the amorphous nature of the drug, which could possibly the polymer inhibits the recrystalline state of the drug (Konno and Taylor, 2008, de Oliveira *et al.*, 2016).

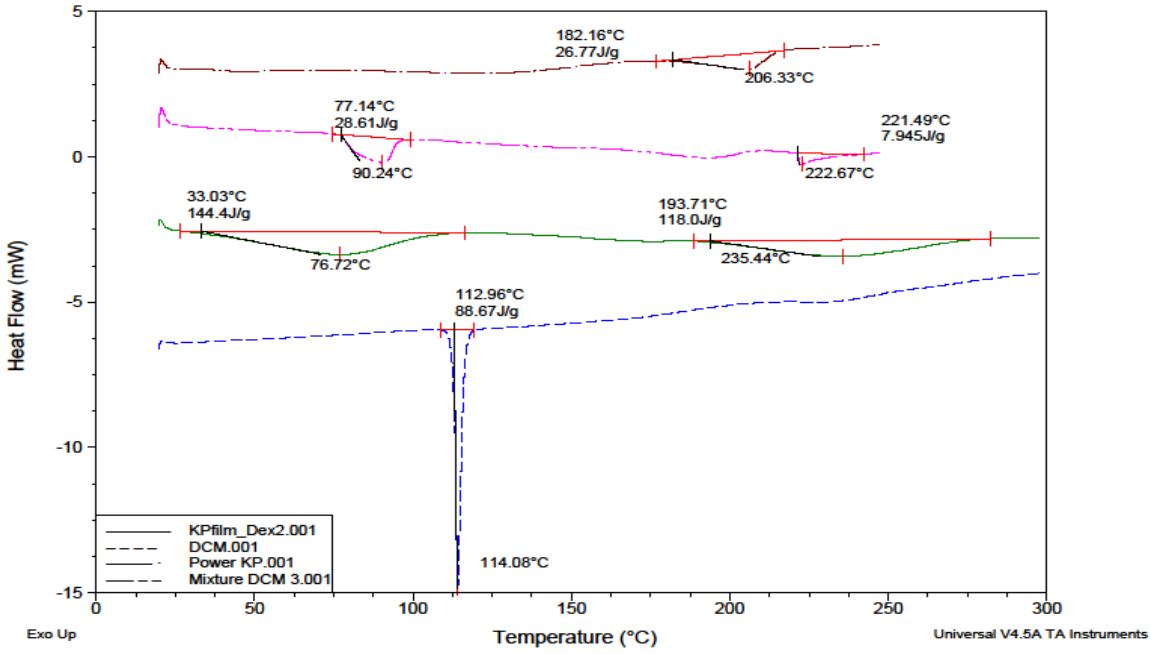


Figure 5.3- DSC thermograms of DCM (---), KP (— ·), physical mixture (— --) and drug-loaded KP film (—).

5.2.4 Disintegration time

5.2.4.1 *Method suitability and development*

Orally dissolving films are novel potential dosage forms, designed to disintegrate in the mouth upon hydrolysis with saliva. Since this is a relatively new pharmaceutical formulation, there is no standard pharmacopoeial disintegration test procedure specified for ODFs. Furthermore, the current conventional disintegrating tests (for tablets) that are described in the pharmacopoeia have been utilised to characterise ODFs (Donauer and Löbenberg, 2007). In this case, it is difficult to assess the disintegration profile due to its rapid disintegration with media. In addition, the conventional testing system is also challenging for visual inspection for end point determination, especially to film- forming polymers that swell immediately and float on the surface. Besides that, using 900 mL volume of test solution is not applicable to the condition of the buccal cavity in terms of media and volume, and, thus, is considered as not suitable for characterisation of ODFs (Bi *et al.*, 1996, Harada *et al.*, 2006, Kakutani *et al.*, 2010, Preis *et al.*, 2014). On the other hand, various alternative methods have been proposed in the literature to facilitate the assessment of the disintegration behaviour of ODFs. The petri dish and slide frame methods are considered as the simplest processes, where the film is placed in the stationary surface, with water dropping from a syringe until it tears apart, which is taken as the disintegration time (Figure 5.4) (Irfan *et al.*, 2015). Although the small volumes of droplet solution might be an appropriate saliva volume, these two methods show no mechanical agitation, which does not reflect on the human oral cavity (Preis *et al.*, 2013, Irfan *et al.*, 2015). The use of wire mesh is also an alternative method for measuring the disintegration time of ODFs, but the same problem is taken into account as films are immobile during the procedure (Joshi *et al.*, 2012). Each of the previously stated methods

suffer from a lack of a clearly defined end point, which may inevitably lead to an overestimate for disintegration time, as well as no mechanical force supplied, thereby considered insufficient for imitation of physiological conditions. Indeed, the disintegration testing system should be presented in a dynamic condition, where the movement is the influential factor on the performance of ODFs. An alternative analytical approach, that implements the dynamic situation, is one where the films are dipped into a glass beaker filled with 25 mL of water with gentle stirring (Arya *et al.*, 2010, Preis *et al.*, 2013). Moreover, a method employing an attached cylindrical probe with exerted force that mimics as oral cavity pressure, is contemplated as a realistic, dynamic reflection of the physiological environment of human mouth (Abdelbary *et al.*, 2005, Szakonyi and Zelkó, 2013). As such, the work described in the subsequent sections of this chapter describes a developmental approach to a more relevant and suitable testing method for the disintegration time of ODFs.

A) B)

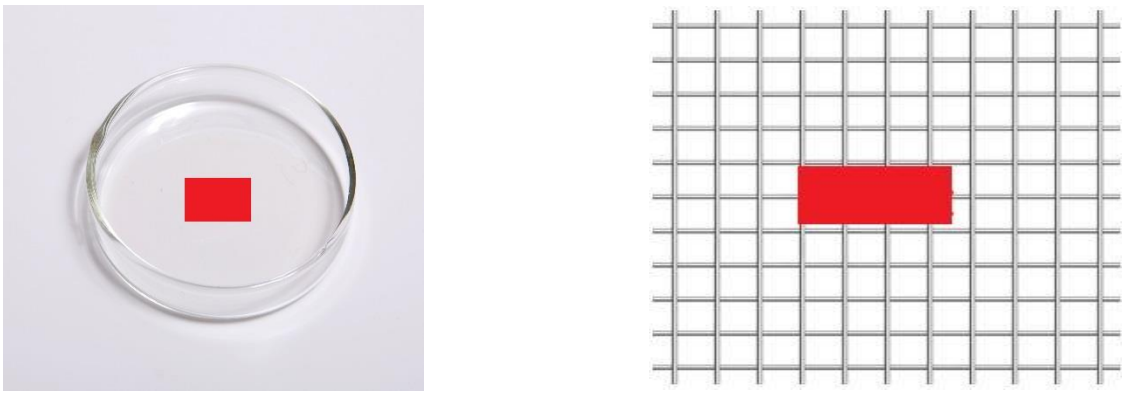


Figure 5.4- Schematic presentation of the disintegration system A) Petri dish and B) slide frame

5.2.4.2 *Texture Analyser- A promising approach*

Several studied methods, including the pharmacopoeia test currently used for the quantification of the disintegration time of ODFs, might be ineffective when used for prediction as a quality control parameter. The texture analyser instrument has previously been considered as a practical operating approach to evaluate the disintegration time of orally disintegrating tablets, which is proposed by many researchers (Dor and Fix, 2000, El-Arini and Clas, 2002, Brniak *et al.*, 2015). In this test system, a moving probe is employed, with a constant force applied to an object (e.g. tablet or film). The object of interest (e.g. tablet or film) is placed onto the flat surface and then submerged in a defined volume of disintegrating medium, with subsequent application of the exerted force, which imitates the action of a tongue. The end point of disintegration time is determined once the object is completely broken down, as determined by the contact of the probe with the underlying flat surface upon which the object is initially placed (Szakonyi and Zelkó, 2013, Scheuerle *et al.*, 2015). This apparatus system has previously been reported as a precise and convenient operating structure that more closely reflects the conditions of the human mouth, in addition to providing a clear visual assessment for disintegration (Szakonyi and Zelkó, 2013). Furthermore, using the texture analyser approach provides a better correlation with the *in vivo* disintegration time, as it demonstrates a simulation of the dynamic condition (Dor and Fix, 2000, Abdelbary *et al.*, 2005, Szakonyi and Zelkó, 2013). Based on the factors outlined above, the beaker method and texture analyser method show a good reflection of the dynamical condition that is applicable to the determination of the disintegration profile of ODFs; therefore, they were investigated for evaluation of the disintegration activities for films.

The disintegration time of the ODFs were determined by two systems for comparison. It was carried out by using TA-CT3 texture analyser with probe (Figure 5.5) or using the commonly employed method of simply stirring in a beaker. The disintegration test system conducted by CT3 texture analyser (TEXTUREPRO CT, Brookfield) requires films to be placed on the platform, where it mimics a lower oral cavity. The probe was set at pre - speed of 1.0 mm/ second with 5 g trigger load force. The probe, which acts to simulate oral cavity pressure, moves until a trigger force is sensed when it touches the film. As soon as the probe touches the film, 1 mL of media is added. The other system was using the commonly employed method of simply stirring in a beaker. The disintegration time was recorded when films completely break down.

Figure 5.5- Schematic presentation of the Text analyser TA-CT3 model (TEXTUREPRO CT, Brookfield) with probe attached.

5.2.5 Media suitability

In addition to the limitations of current analytical methods to determine the disintegration time of ODFs, the media employed in such methods is also questionable. Currently, water is the most commonly used media, which lacks many of the components of saliva, resulting in a potential impact on disintegration time of ODFs. With this in mind, to obtain the effect of media composition on the disintegration profile, the use of distilled water and simulated saliva fluid at pH 6.8 were used for investigation at 25 ^{0}C and 37 ^{0}C.

5.2.5.1 *Impact of media volume*

Along with the media itself, media volume is also an important controlling factor that could influence the disintegration behaviour of the dosage form, especially to ODFs, as they are quickly hydrolysed upon contact with the saliva. As a consequence of the lack of pharmacopoeial methodology for the testing of ODFs, the disintegration testing system employed for tablets or capsules, using large media volume, is often applied to ODFs (Abdelbary *et al.*, 2005, Harada *et al.*, 2006). For instance, using 900 mL as media volume to films could provide inconsistent results for determination of the end point for disintegration time, as a loss of visual detection can be problematic due to film floating or even hydrating immediately upon the contact with the media (Narazaki *et al.*, 2004, Mishra and Amin, 2009). Furthermore, this condition does not reflect on the condition of the human mouth. Moreover, a method for quantification of disintegration time using a small media volume is more suitable and biorelevant to saliva volume, in order to better replicate the disintegration process in the oral cavity, which is applicable to this novel dosage form, ODFs.

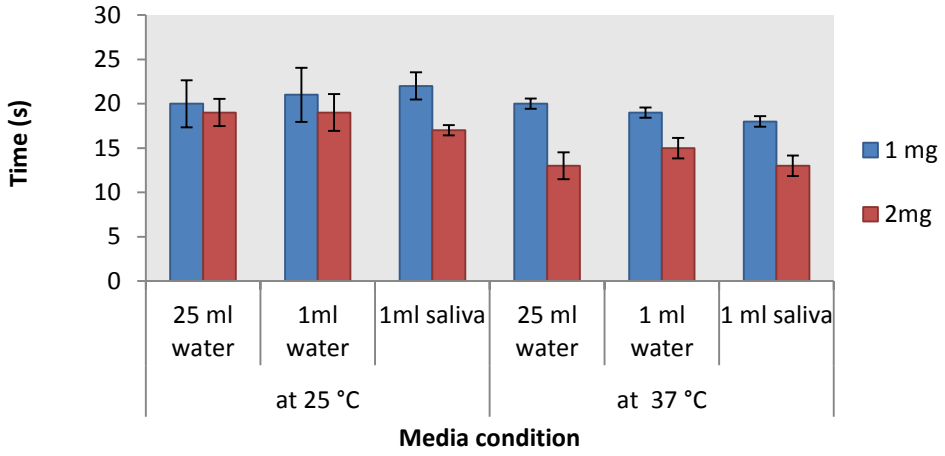
As such, the impact of media volume on the disintegration time of ODFs was additionally investigated. These films (3 x2 cm^2) were tested in a 50 mL beaker containing 1 mL or 25 mL of either distilled water or simulated saliva solution at 25 ^{0}C and 37 ^{0}C, with gentle stirring. 1 mL of the same two media were also analysed for probe methodology.

5.2.5.2 *Disintegration time of ODFs*

All films were dissolved in less than 25 seconds. Results showed that the use of distilled water and saliva solution as test medium resulted in almost similar disintegration time of the studied films (Figure 5.6.A). There was no significant difference in the disintegration time for dissolving films incorporated with 1 mg and 2 mg dose of DCM at 25 ^{0}C and 37 ^{0}C ($p > 0.05$, ANOVA followed by Tukey's test). The time recorded for ODF incorporating 1 mg to completely dissolve were 20 ± 2.65 s, 21 ± 2.00 s and 22 ± 1.15 s in 25 mL distilled water, 1 mL distilled water and 1 mL saliva solution at 25 ^{0}C, respectively. However, films containing higher dose loaded films show a trend for faster disintegration time at the same temperature, which indicates the fast, water – solubility property of DCM. As evidence, films dissolved 19 ± 1.53 s, 19 ± 2.08 s and 17 ± 0.58 s in the test media. In this study, artificial saliva solution was used which clearly indicated that the distilled water and saliva solution is similar to each other and, hence, they both have no impact on disintegration profile of ODFs, whilst higher loading of drug has direct relationship with faster rate of disintegration at the higher temperature (maximum 15 seconds). Although, the artificial saliva used here is composed of 99 % water and other inorganic components (Humphrey and Williamson, 2001), whereas, in comparison, the actual saliva fluid consists electrolytes, enzymes, proteins and other antimicrobial

constituents that maintain the health of oral cavity (de Almeida *et al.*, 2008); all of which could further impact on disintegration time, so may require further investigation.

(A)



(B)

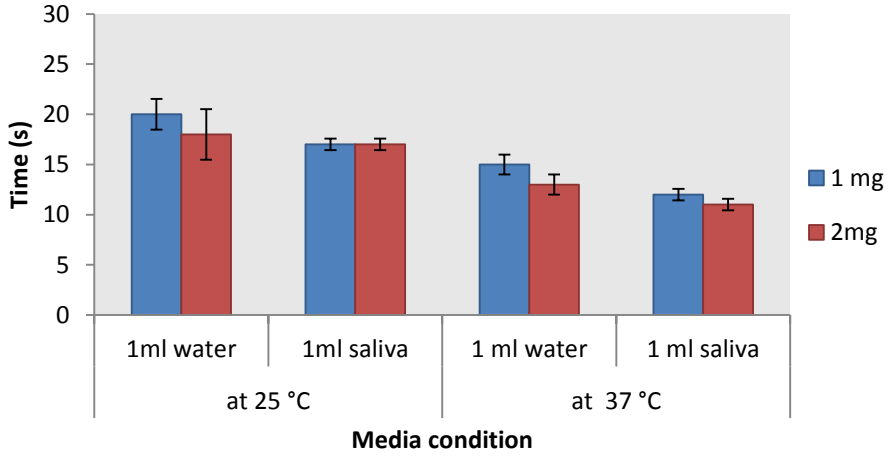


Figure 5.6- Disintegration time of ODFs loaded with dexchlorpheniramine maleate at 1 mg and 2 mg recorded by (A) dissolving in beaker and (B) use of texture analyzer with probe in distilled water and simulated saliva solution at 25 ^{0}C and 37 ^{0}C (mean ± SD, n=3).

5.2.6 Impact of test volume

Media volume is an important controlling factor that could influence the disintegration behaviour of the dosage form, especially to ODFs as they are quickly hydrolysed upon contact with the saliva. Both systems revealed almost similar results of disintegration times of ODFs loaded at two different dosage strengths (Figure 5.6.B). A small beaker was used as a test vessel, where films were placed into 1 mL or 25 mL of distilled water with gentle stirring. The disintegration time of films was recorded to be approximately 20 seconds, as a result of film swell rapidly when in contact with the large surface area of the beaker. As seen, the test volume did not have a strong impact on the disintegration time of ODFs. However, small volume of used media (1 mL) provide an accurate stimulation of the saliva production of the human mouth.

5.2.7 Impact of the method

The probe system generated shorter disintegration times compared to the beaker method. The disintegration times recorded for films loaded with 1 mg DCM were 17 ± 1.53 s in saliva and 20 ± 2.52 s in distilled water, while ODF loaded at 2 mg dose have disintegration times of 18 ± 2.52 s in water and 17 ± 0.58 s in saliva solution at 25 ^{0}C, suggesting that films were under additional force that mimics oral cavity pressure, where it appears to be reflective of the movable platform of the human mouth. Again, even shorter times were observed for both drug loaded films at two dose strength at 37 ^{0}C, e.g. 15 ± 1.00 s and 13 ± 1.00 s in distilled water; 12 ± 1.00 s and 11 ± 0.58 s in saliva for 1 mg and 2 mg, respectively.

As mentioned, both solutions were similar, which did not lead not to diverging results in those cases. Nevertheless, using small beakers as test vessels can be challenging for visual inspection, as it was subject to individual judgement of a defined end- point, which may lead to a bias in results. The probe method developed in this study provides clear end-point determination and is, therefore, more suitable for control performance, where it performs as a convenient operating structure that demonstrates a simulation of the dynamic conditions of the human mouth and deliver precise results in terms of reproducibility.

5.2.8 *In - vitro* drug release study

Both DCM as a pure powder form and loaded into ODFs exhibited rapid dissolution. Pure DCM released up to 90 % of the total amount of drug within 3 minutes in simulated saliva solution (pH 6.8) (Figure 5.7), as the drug itself is very hydrophilic, whilst the drug loaded films achieve a similar amount of release (90% of drug) after 4 minutes (Figure 5.7). There was no significant difference in release profile of pure drug between 2 and 3 minutes ($p > 0.05$, ANOVA followed by Tukey's test), but it was recorded significantly for drug loaded ODF at the same time points ($p < 0.05$ ANOVA followed by Tukey's test). The slightly delayed release of drug from the film formulation may be attributable to the drug molecules requiring some time to diffuse out from the polymeric films. The quick release of the drug was observed in PBS solution (Figure 5.7). The fast dissolution performance in both media conditions can be attributed to the relatively high solubility profile of the API in both conditions in a low amount of drug loading, as well as the hydrophilic nature of the film forming polymer or perhaps the components in the saliva.

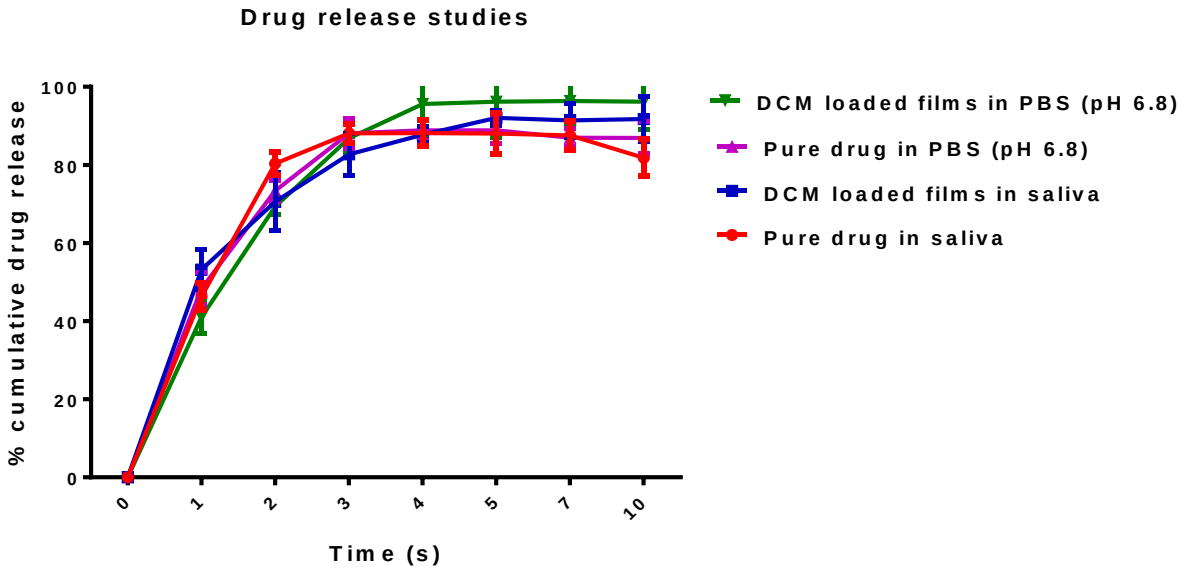


Figure 5.7- Dissolution profile of DCM and DCM - loaded films in simulated saliva solution (pH 6.8) and.in PBS solution (pH 6.8) (mean ±SD, n=3).

5.2.9 Drug content uniformity

It is essential to have uniformity of API throughout the polymeric films, which is vital for patient safety and efficacy, since a uniform dose will provide consistent dosing within the patient, thereby providing a more consistent and predictable therapeutic effect. According to the BP criteria, content uniformity for dosage units of DCM should be within the range of 85 - 115 % (BP, 2016). The drug content uniformity of the DCM loaded ODFs prepared here was recorded at 99.40 ± 4.79 % and 99.87 ± 5.15 % (expressed as mean ± RSD) for 1 mg and 2 mg dose, respectively, which is within the acceptance criteria, suggesting the uniform distribution of DCM within films.

5.2.10 Stability studies

Due to the fact that films can be susceptible to breaking/tearing and absorption of moisture from the environment, packaging also plays a crucial part in practical film performance and stability. According to the WHO guidelines on GMP for pharmaceutical preparations, the products must be kept in a well closed package or moisture - proof packs and protected from any sensitive environment, such as light, moisture and mechanical damage (WHO, 2002). In terms of stability, the packaging materials should have no interaction with the pharmaceutical products. Indeed, depending on the use and the route of administration of medicine products, different requirement of packaging materials are highly considered for maintaining the integrity of products (FDA, 1999). For example, the most common forms of packaging for solid dosage forms, such as tablets or capsules, are blister package and aluminium foil. Moreover, these materials provide supportive and barrier properties, which also have a use for film packaging. However, plastic/ paper provides more economical viability and is widely used for primary packaging (e.g ointment, tablet boxes), but they also can be used for strip packs (see Figure 5.9 A), whereas foil packaging offers a better protection than plastic for films. Nevertheless, the design of packaging from pharmaceutical compliance should be administrated in a safe manner (e.g delivery accurate dosing and compliance); ease of usage to the patients and economical (WHO, 2002). Based on these reflective properties of these packaging materials, the prototype packagings were chosen to study for investigation of a suitable packaging material for oral dissolving films.

Currently, there are various packaging systems for oral dissolving films, including foil-paper or plastic pouches. Peelable pouches are an example of a single pouch packaging

for oral fast dissolving films (Figure 5.9 A), in order to provide high protection from the moisture in the air. Whereas, other different packaging materials employed for ODFs are demonstrated as examples in Figure 5.9 B and C. Blister card can also be used for film packaging. The blister container composes of two parts: the blister and the lid stock. The blister material is generally plastic and the lid stock is sealed with paperboard or aluminium (Panda *et al.*, 2012). The blister packs are further divided as heat sealed blisters, which are laminated with thermoformed plastic mould, and cold blisters, which consist of cold forming of aluminium- based laminate film, where both are sealed with aluminium lid material. Besides that, aluminium foil is the most widely preferred choice used for packaging format, especially for packaging films, due to its great protection from moisture and humidity (Nagaraju *et al.*, 2013).

Figure 5.8- Examples of different types packaging for ODFs: A) peel pouch B) plastic blister C) paper

The results in this chapter, therefore, depict the use of prototype packaging as a potential approach for ODF packaging; indeed, a durable, airtight and tamper proof packaging is of significant importance for a single dosage form for a special purpose, in line with pharmaceutical industrial requirements.

These studies were carried out by storing drug-loaded films sealed by the prototype packaging in climate controlled stability cabinets, set to ICH conditions of either 25 ± 2 ^{0}C and 60 ± 5 % RH or 40 ± 2 ^{0}C and 75 ± 5 % RH. Films were wrapped either in aluminium foil or sealed in cold blister tablet packaging and stored for 90 days (See Figure 5.10).

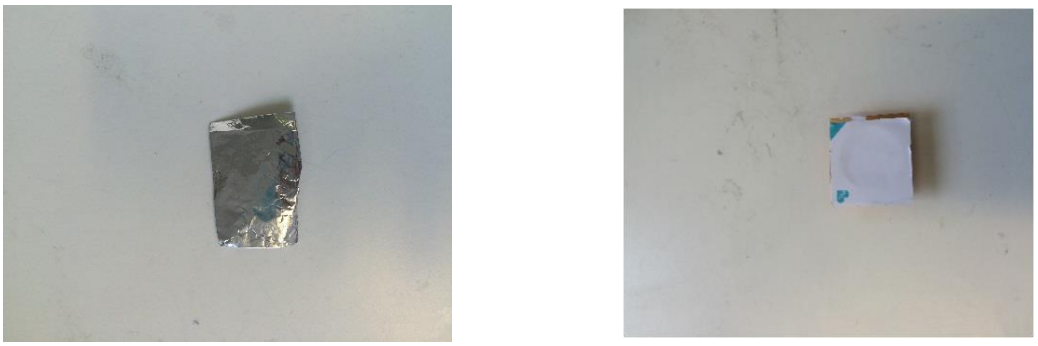


Figure 5.9– Packaging storage of films in aluminium foils and tablet blister for stability studies at 25 ± 2 ^{0}C and 60 ± 5 % RH and at 40 ± 2 ^{0}C and 75 ± 5 % RH.

5.2.10.1 *Visual inspection*

All film samples remain unchanged in colour in aluminium foil and blister when stored at both long term (25°C / 60 % RH) and accelerated (40°C / 75 % RH) conditions (Figure 5.11 and 5.12). Films were clear, transparent and non- sticky. Only slight changes in appearance could be observed with packaging in blister, since it was necessary to fold the

films to ensure that they would fit in the blister packs that were available, which led to the shape becoming distorted as a result of storage. Hence, in this instance, aluminium foil would appear to be the better choice for packaging of ODFs, which has previously been shown as the recommended packaging for oral films for protection of dosage forms from moisture (Heer *et al.*, 2013). However, it may be that a more suitable sized blister pack, or one that is bespoke for the dimensions of the film, could alleviate the slight deformations seen here.

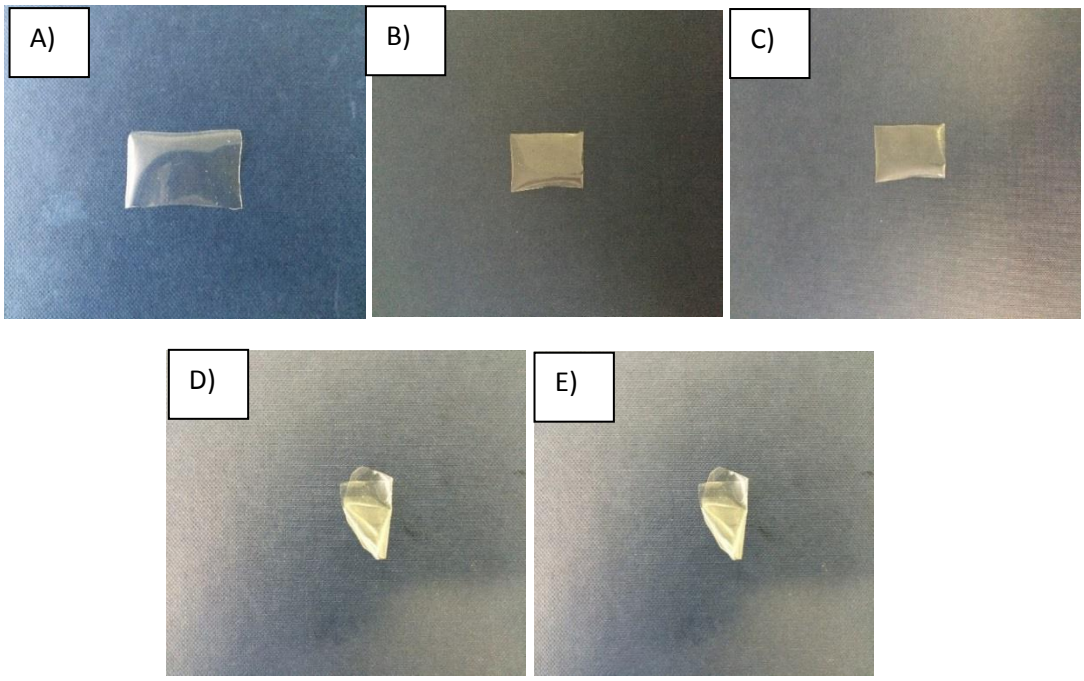


Figure 5.10- Visual appearance of DCM loaded films -1a) in foil & blister at day 1; 1b & 1c) in foil at 30 days and 90 days; 1d & 1e) in blister at 30 days and 90 days at long term storage humidity condition (25 ± 2 ^{0}C and 60 ± 5 % RH).

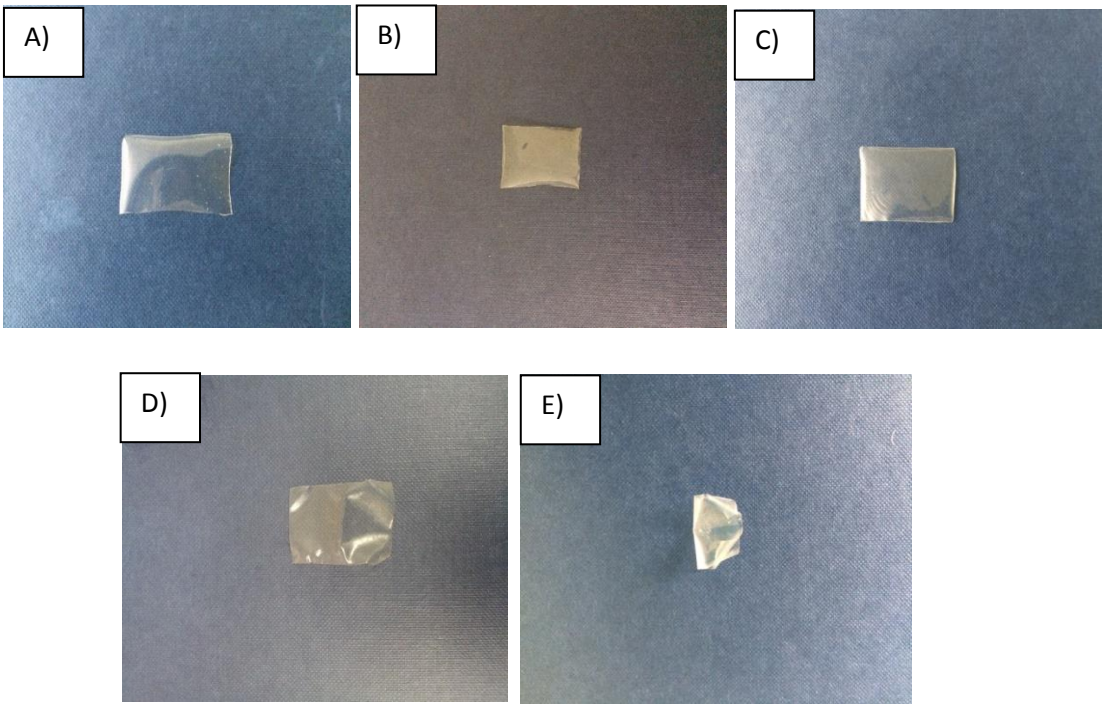


Figure 5.11- Visual appearance of DCM loaded films -1a) in foil & blister at day 1; 1b & 1c) in foil at 30 days and 90 days; 1d & 1e) in blister at 30 days and 90 days at accelerated storage humidity condition (40 ± 2 ^{0}C and 75 ± 5 % RH).

5.2.10.2 *Impact of storage conditions on mechanical properties of ODFs*

Mechanical strength is a vital parameter to evaluate the robustness and flexibility of ODFs, as it is essential for handling and storing, allowing consumers to take a safe, adequate level of medicine while maintaining the integrity of samples. When analysing the effect of types of packaging on the mechanical properties of ODF loaded with DCM (Figure 5.13), results showed that there was no significant difference in tensile properties of all film formulations at 25 ^{0}C, which indicated that films remains strong and flexible ($p > 0.05$, ANOVA followed by Tukey's test).

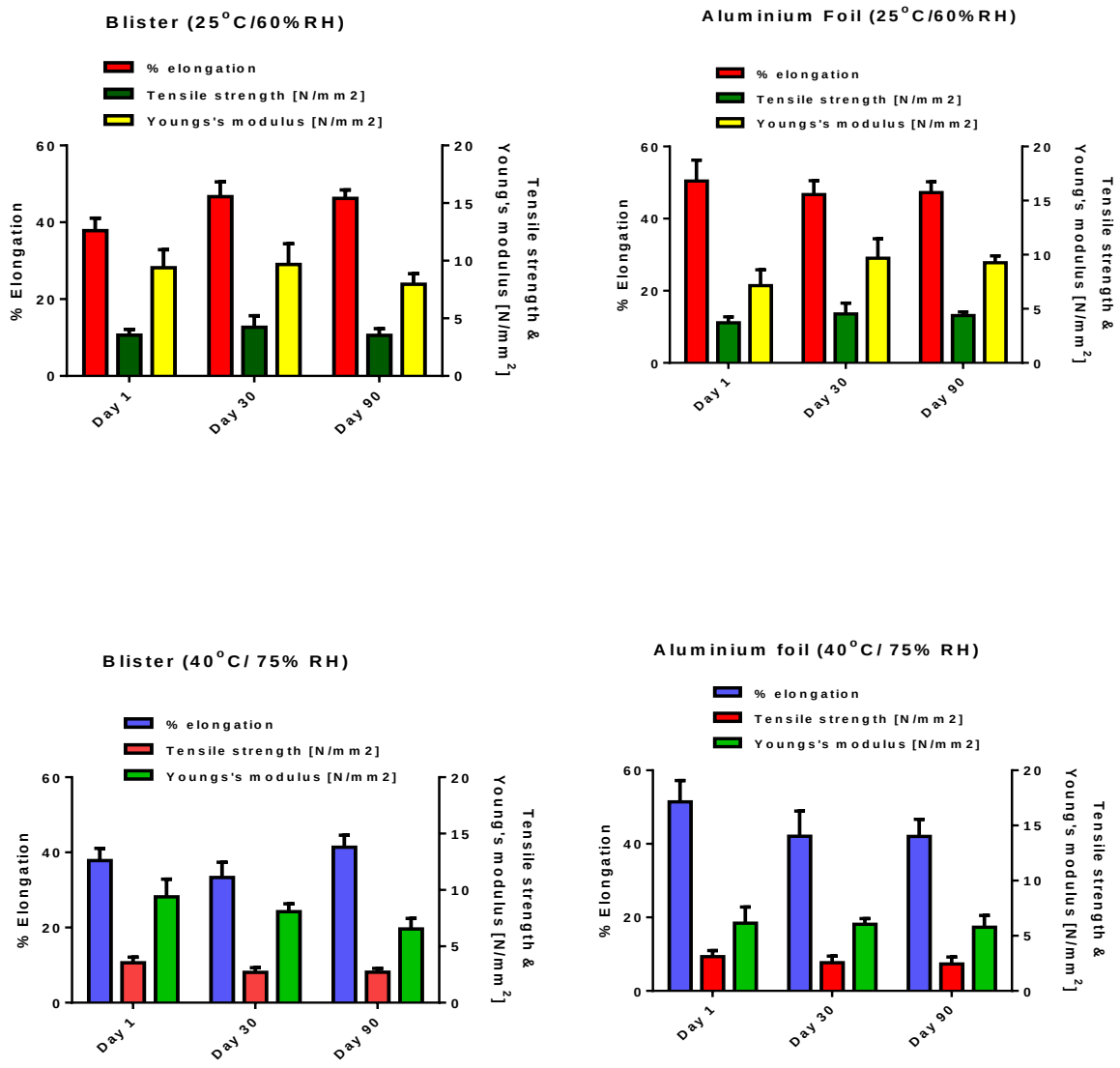


Figure 5.12- Mechanical properties of ODFs loaded with at 25 ^{0}C/ 60% RH and 40 ^{0}C/ 75% RH in blister and in in foil after 90 days (mean ± SD, n=3). No change in mechanical properties of OFDs at long term conditions, but films became weaker in the accelerated conditions after 90 days.

On the other hand, films became weaker after 90 days when stored at accelerated storage conditions, which resulted in a significant reduction in tensile strength ($p < 0.05$ ANOVA followed by Tukey's test) and a slight increase in percentage elongation of films. This change varied for blister and foil packaging, with the tensile strength reduced from 3.53 ± 0.50 N/mm^2 to 2.70 ± 0.33 N/mm^2 for ODFs in blister packaging, and a reduction from

3.11 ± 0.53 N/mm^2 to 2.45 ± 0.63 N/mm^2 for films packed in foil. This is possibly due to residual water absorbed on the film samples at the accelerated condition, as expected (see 5.7.3). As a result, the moisture could weaken the intermolecular forces between the polymer chains, whilst enhancing an increase in the water absorption through the hydrophilic property of the plasticiser; thus, films became softer (Bhattacharya *et al.*, 2014).

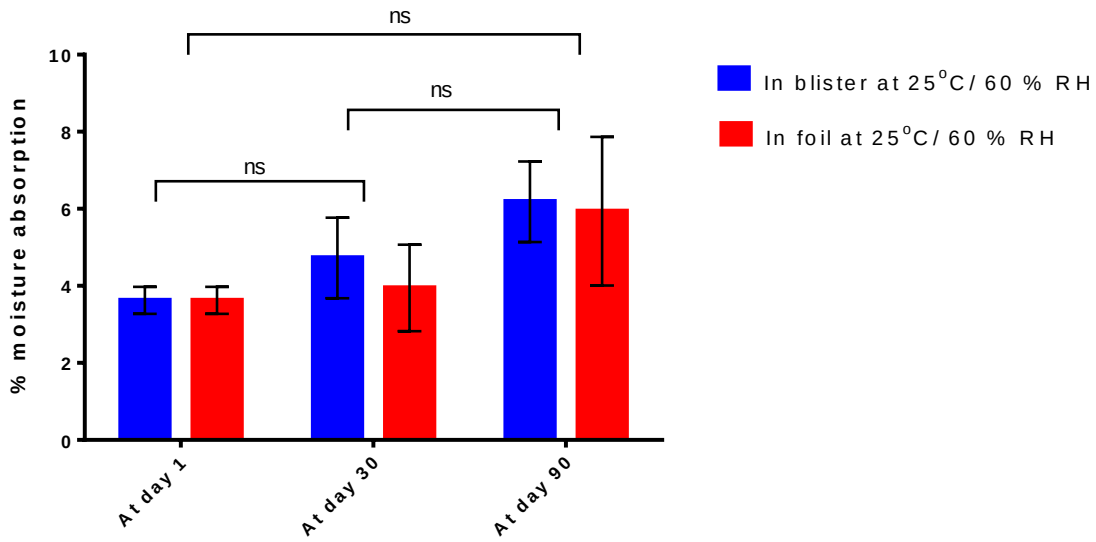
5.2.10.3 *Moisture content*

KP is a polymer with a strong barrier against water vapour (Yadav and Ansari, 2013). The percentage of moisture uptake of ODFs varied in both types of packaging, with an overall increase in moisture uptake when storing in both conditions up to 3 months. Film samples have a low percentage moisture uptake (up to 4 %) recorded at both humidity conditions at the initial day. However, the trend of increase in moisture observed varied between 6 % to 9 % for foil and blister at 25 ^{0}C / 60% RH and 40 ^{0}C / 75% RH, respectively, but recorded the highest value (up to 9%) at higher temperature (accelerated condition) after 3 months (Figure 5.14) (Singh *et al.*, 2013). It was observed that there was no significant difference impact on moisture studies after 90 days, regardless of types of packaging, at 25 ^{0}C / 60% RH ($p > 0.05$, ANOVA followed by Tukey's test). In contrast, results obtained were found to be statistically significant on the moisture content for ODF packed with blister at 40 ^{0}C / 75% RH ($p < 0.05$, ANOVA followed by Tukey's test). With the addition of plasticiser, plasticiser molecules interact with the polymer chains, which thereby weaken the intermolecular forces within the polymer molecules

and possibly integrating H-bonding of glycerol to polymers, that induces the higher exposure for moisture sorption (Fundo *et al.*, 2014)

Moreover, moisture analysis provides the information on the suitability of the type of packaging for particular dosage forms; it can be seen that the measured percentage moisture uptake was higher when films were packed in blisters, hence suggesting that blisters are not a suitable choice for packaging in this case. Having said that, the quality of the packaging studied here are not as the industrial standard quality, nor bespoke to the ODFs produced, which might lead to issues of moisture uptake. As a result, the need of airtight packaging of ODFs should be considered to prevent any change in physicochemical properties of ODFs.

(A)



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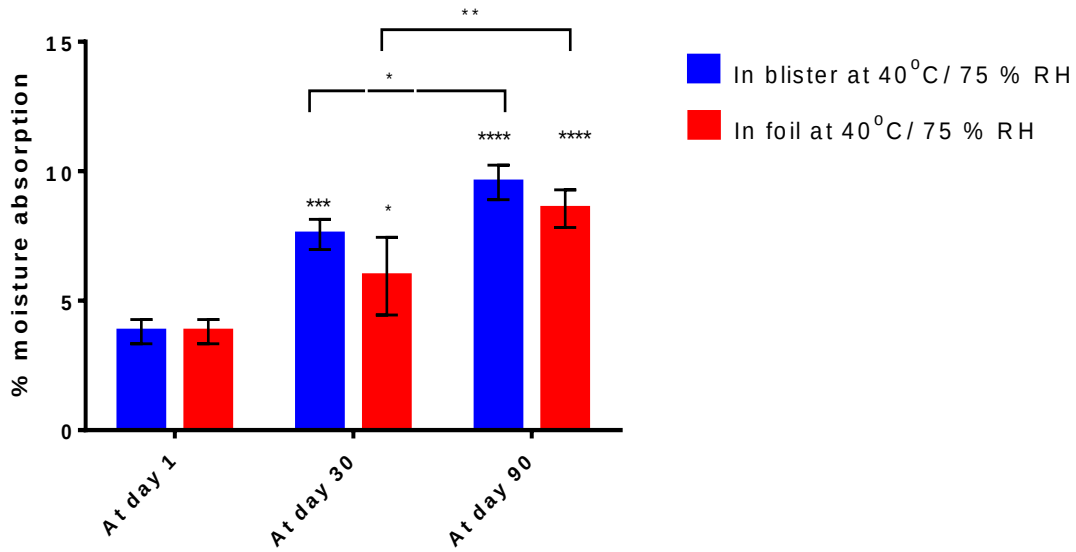
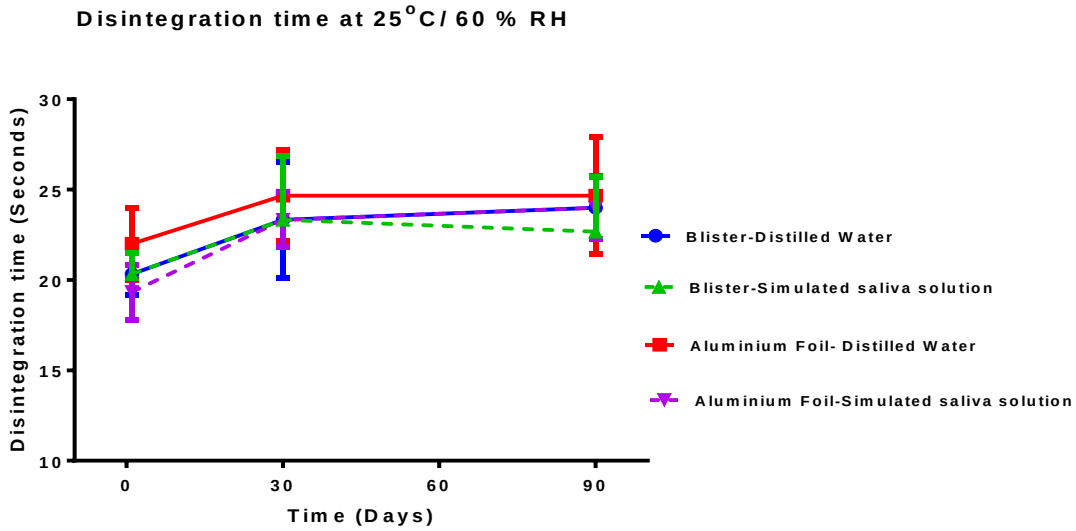


Figure 5.13- Moisture studies of DCM loaded ODFs packed in aluminium foil and blister. Films were stored at (A) 25 ± 2 ^{0}C and 60 ± 5 % RH and (B) at 40 ± 2 ^{0}C and 75 ± 5 % RH (mean ± SD, n=3).No significantly difference were observed for films stored at long term condition, but recorded significantly change in moisture uptake for films stored at the accelerated condition ($p < 0.001$, Tukey's test), from day 1 to day 90.

5.2.10.4 *Impact of storage conditions on disintegration time*

Despite perceived changes in mechanical properties and moisture uptake on storage, as described in the sections above, all films were still able to dissolve within 25 seconds either in distilled water or saliva solution after 3 months, regardless of packaging type or storage condition. Indeed, the type of packaging has no significant impact on the disintegration time of films when stored at 25 ^{0}C/ 60 % RH, since all samples barely changed on the disintegration behaviour ($p > 0.05$, ANOVA followed by Tukey's test) (Figure 5.15.A). However, the shift in longer disintegration time was observed in the accelerated condition at 40 ^{0}C/ 75% RH, which could be due to the hardening of the polymer following exposure to high temperature over a period of time, in addition to the increased moisture uptake (Figure 5.15.B). The moisture uptake could depolymerise and disrupt the chemical cross-links between the polymer chains, especially to those side chains group (-OH group) which are susceptible to the chemical degrading reaction, causing polymer degradation under the influence of moisture (Vogt *et al.*, 2004).

A)



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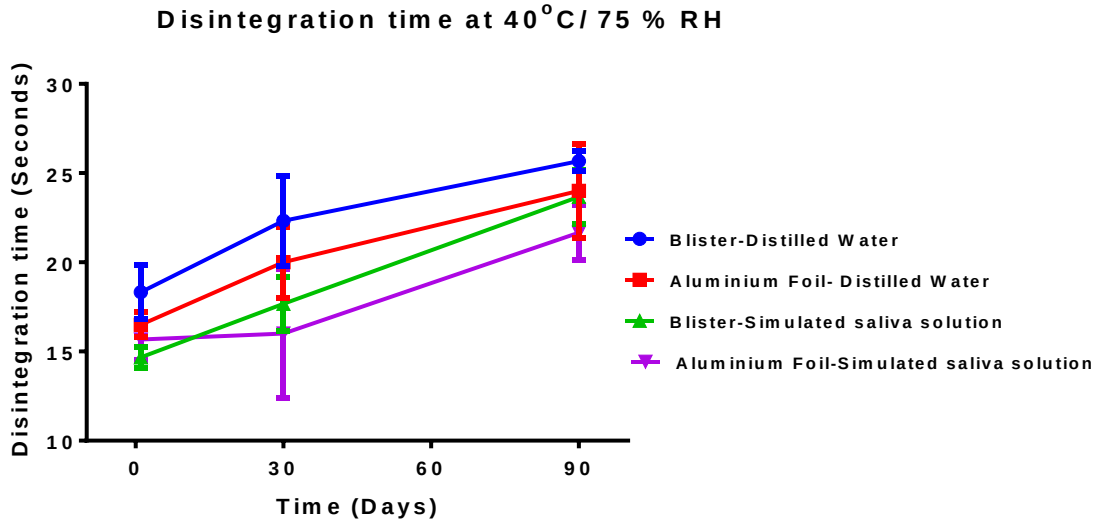


Figure 5.14- Disintegration time of ODFs loaded DCM at A) 25 ^{0}C/ 60% RH and B) 40 ^{0}C/ 75% RH in blister and in foil after 90 days (mean ± SD, n=3).

5.2.10.5 *Drug content uniformity*

In addition to ensuring that the drug remains uniformily dispersed in the polymeric films upon storage, it is also crucial that the degradation of the drug is minimised and remains within an acceptable range of the initial amount (85 -115 %) (BP, 2016). ODFs containing DCM showed the drug content was within the permissible criteria for those films placed in both blister and aluminium foil packaging films at long term (25 ± 2 ^{0}C and 40 ± 5 % RH) and accelerated stability condition (40 ^{0}C ± 2 ^{0}C and 75 ± 5 % RH). Therefore, the drug showed uniform distribution within films and retained its physically stability over the time period of stability studies (Figure 5.16).

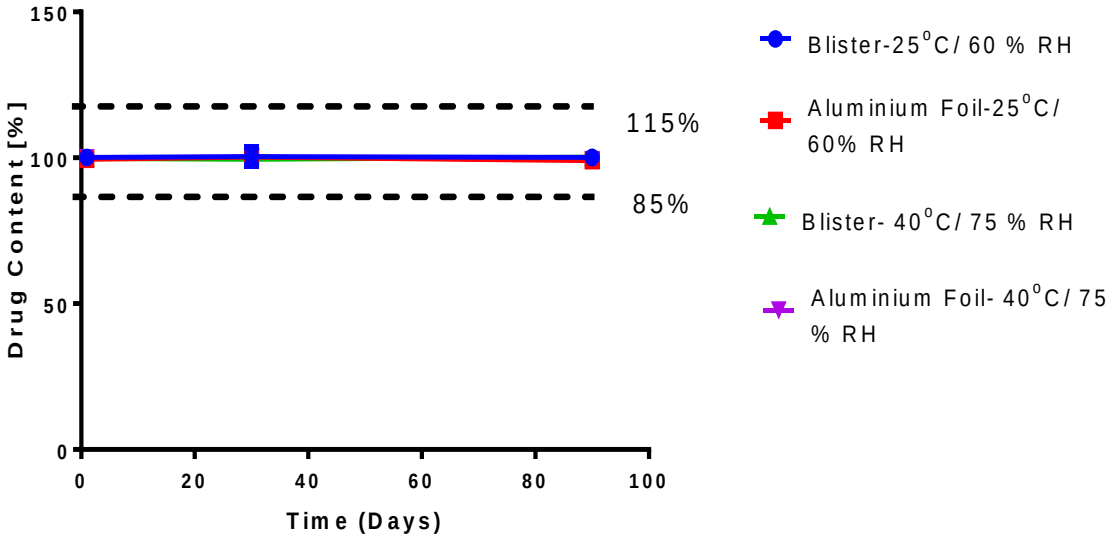


Figure 5.15- Drug content uniformity of ODF loaded DCM in blister and aluminium foil packaging films at long term (25 ± 2 ^{0}C and 60 ± 5 % RH) and accelerated stability condition (40 ^{0}C ± 2 ^{0}C and 75 ± 5 % RH).

5.3 Conclusion

The ODFs formulated here, loaded with antihistamic drug, dexchlorpheniramine maleate, at 1 mg and 2 mg dose, achieved desirable physical and mechanical properties, drug content uniformity with a suitable clinical use for quick onset of action, which is beneficial in management of allergic rhinitis with an ease of administration. The study initially aimed to focus on the development of a suitable disintegration test system to quantify the disintegration time for ODFs, since no standard pharmacopoeia disintegration test method for ODFs exists. It was seen that the use of distilled water and simulated saliva solution has no significant impact on the disintegration time of ODFs conducted by either a TA-CT3 texture analyser with probe or simply stirring in a beaker ($p > 0.05$, ANOVA followed by Tukey's test). In contrast, the amount of test media did not lead to crucial differences in disintegration time of ODFs, since they were carried out in a dynamic condition. The loading of drug has a direct relationship with lowering disintegration time, as does an increase in temperature. The measurement method using a small beaker as a test vessel can be challenging for visual inspection, as it was subject to individual judgement of a defined end- point, whereas the probe method developed in this study using a small media is more biorelevant to the oral cavity volume and provides clear end-point determination and it, therefore, is more suitable for quality control setting of ODFs. Pure DCM and drug loaded films released up to 90 % of the total amount of drug within 3 minutes in simulated saliva solution and PBS solution at pH 6.8. All films remained clear, transparent and non- sticky in blister and foil packaging over a period of three months. Only slight changes in the shape of films could be observed with packaging in blister, as a result of the effect of folding to accommodate the film within the available blister packaging. Hence, aluminium foil is the better choice for packaging of ODFs.

There was no significant difference in mechanical properties of all film formulations at both these two humidity conditions, which indicated that films remain strong and flexible. An increase in moisture uptake was observed for ODFs packaged in foil and blister at both storage conditions, but was higher when films were packed in blisters, hence suggesting that blisters are not a suitable choice for moisture protection for ODFs. Despite certain changes being perceived in terms of physical characteristics, the disintegration time for all ODFs remained less than 25 seconds, and all films achieved good content uniformity and remained stable at both storage conditions over a period of time, regardless of the type of packaging. Hence, these formulations are more robust and, therefore, potentially more viable industrially and clinically through the product developmental stages.

Chapter 6
Preparation and Characterisation of ODFs loaded with nanoparticles encapsulating a poorly soluble drug

6.1 Introduction

According to the BCS classification system, class II drugs are known for their poor solubility and high permeability, resulting in limited dissolution and poor oral bioavailability; hence, such drugs remain challenging for formulation of oral dosage forms. A variety of technological approaches have been utilised to enhance the dissolution profile of drug substances, such as pH adjustment (Vemula *et al.*, 2010), solid dispersion (Kakran *et al.*, 2012, Vo *et al.*, 2013) and cyclodextrin complexation (Loftsson and Duchene, 2007). Alternatively, one of the common technologies involves the reduction of particle sizes to overcome these issues. The use of nanoparticles, by dispersing preformed polymers or by polymerisation to produce the colloidal particles in the range of 10- 1000 nm, promotes the dissolution rate of poorly soluble drugs, enhances the drug loading capacity and reduces the toxicity level of the excipients (Rabinow, 2004, Vauthier and Bouchemal, 2009). Therefore, the field of polymer nanoparticles has been expanded to exploit their use as a particulate carrier system in a range of fields, based on their unique properties, ranging from medicine to biotechnology as a drug delivery system (Anton *et al.*, 2008, Ahlin Grabnar and Kristl, 2011).

Nanoparticles are defined as solid colloidal particles with a carrier system under sub-micron units that comprises nanospheres and nanocapsules (Mora-Huertas *et al.*, 2010). The nanoparticulated system has a polymeric matrix that encapsulates the API within the polymeric layer, which is advantageous for poorly water soluble drugs as this can increase the surface area to volume ratio of drug particles, whilst also improving the stability of the API and enhancing the uptake by the intracellular cells (Jung *et al.*, 2000, Reis *et al.*, 2006). For instance, this system has been extensively used in the delivery of both low

molecular weight drugs, as well as peptides and proteins (Ahlin Grabnar and Kristl, 2011).

Moreover, depending on the physico-chemical properties of the drug, the choice of preparation method of nanoparticles is considered by many features, including route of delivery, requirement of sizes, or site of target to achieve the efficient encapsulation of the API and, hence, improve the therapeutic performance of such problematic drugs.

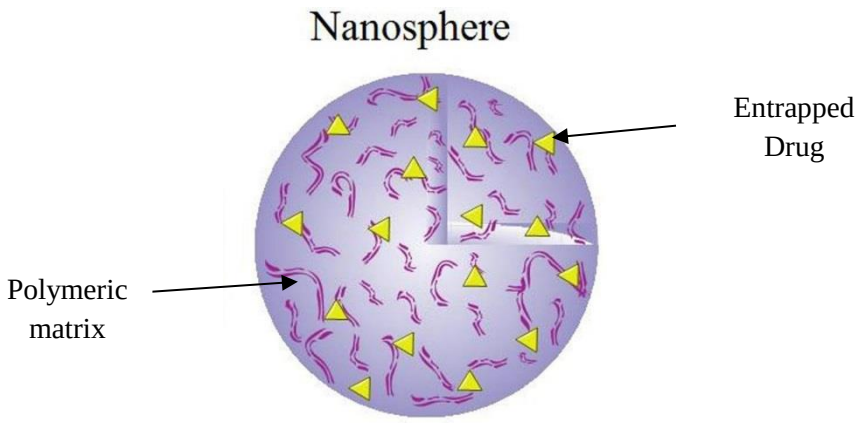


Figure 6.1- Schematic representation of nanosphere structure. Adapted from (Deda and Araki, 2015).

6.1.1 Nanoprecipitation method

Developed by Fessi (Fessi *et al.*, 1989), the nanoprecipitation method, also alternatively defined as solvent displacement method, is one of the most commonly reproducible techniques to prepare nanoparticles. The production of nanoparticles is based on two phases: the organic solvent phase (containing the polymer) and the aqueous phase. The organic phase consists of a solvent (e.g. acetone, ethanol, hexane) that is water miscibile and easily evaporates during the displacement process in the presence of polymer (either

natural, semi- synthetic or synthetic form). On the other hand, the aqueous phase contains non-solvent substance with the addition of surfactants. A literature review demonstrated the enhancement of both hydrophilic and hydrophobic drugs into nanoparticles using the nanoprecipitation method (Barichello *et al.*, 1999). For example, Cucurbitacin, a hydrophobic molecule that is being entrapped into poly (D,L-lactic-*co*-glycolic acid) using nanoprecipitation method, showed a great extent of drug loading of this drug (Alshamsan, 2014). Also, preparation of nanoparticles containing atorvastatin calcium, using Polycaprolactone (PCL), revealed high entrapment efficiency and a satisfactory drug stability (Ahmed *et al.*, 2014).

Moreover, the field of nanoparticles loaded ODFs remains as a new technology. Transformation of nanoparticles into films would overcome the drawback of poor dissolution and limited bioavailability, due to the great surface area to volume ratio of the drug particles. Previous studies have demonstrated that the incorporation of BCS class II drug nanoparticles, e.g naproxen, fenofibrate and griseofulvin, into the edible strips using hydroxypropyl methyl cellulose as a film former exhibited the enhancement of dissolution performance and bioavailability of those studied drugs (Sievens-Figueroa *et al.*, 2012). The loading of lercanidipine nanoparticles into oral films performed good *in vitro* dissolution and *ex vivo* permeation through buccal mucosa (Chonkar *et al.*, 2016).

6.1.2 Aim and Objectives

This chapter focuses on the incorporation of orally dissolving films with poorly water soluble drug loaded nanoparticles for enhancing the bioavailability of glipizide.

The objectives were:

- To investigate the feasibility of nanoparticles developed by nanoprecipitation method loaded into ODF formulations.
- To efficient delivery of hydrophobic drug, glipizide, in order to improve their dissolution performance.
- To investigate the capacity of drug loading using nanoparticles distribute within the ODFs.
- To enhance the perception of bitter taste of drug and uniformity distribution.

6.2 Results and Discussion

6.2.1 Effect of the amount of polymer used

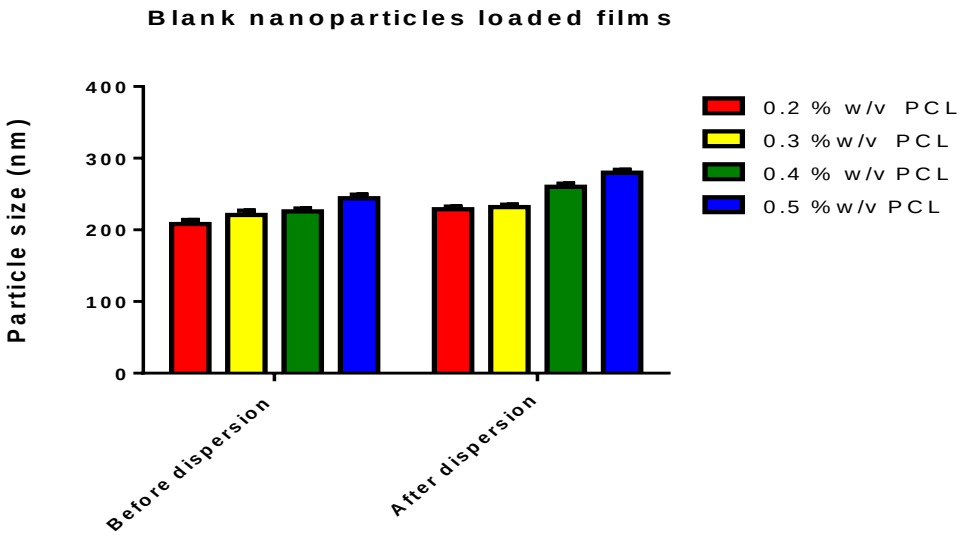
Varying concentrations of polymer were used for nanoparticle formulations (PCL) to investigate the impact on the distribution of nanoparticles and the behaviour of blank nanoparticles by dispersion from the films. It was very clear that the particle size is significantly influenced by the amount of PCL used in the preparation of these blank nanoparticles; the average particle size was observed to increase with an increase in PCL concentration (Figure 6.2) ($p < 0.001$, ANOVA followed by Tukey's test). In contrast, the changes in the polydispersity index (PDI) of the blank formulation was found insignificant ($p > 0.05$, ANOVA followed by Tukey's test). The average effective diameter of blank nanoparticles before incorporation into the ODF was measured at 208 ± 4.79 nm, 220 ± 3.53 nm, 225 ± 4.03 nm and 244 ± 5.02 nm and the polydispersity index of 0.102 ± 0.007, 0.098 ± 0.012, 0.106 ± 0.014, 0.101 ± 0.015 with an increase of concentration from 0.2 % w/v to 0.5 % w/v, respectively (Figure 6.2 A). Low values of

PDI indicate a narrow size distribution within the particles, which may be expected, since these were blank (i.e. drug-free) formulations (Figure 6.3 A). On the other hand, in case of blank nanoparticles dispersed from film, a significant change in diameter of those nanoparticles was recorded compared to that of before dispersion at the same polymer concentration ($p < 0.05$, ANOVA followed by Tukey's test), while there was no significant impact on the polydispersity index, despite a slight increase in PDI values ($p > 0.05$, ANOVA followed by Tukey's test). Following the incorporation and subsequent dispersion from films, the mean size was collected at 228 ± 3.49 nm, 231 ± 2.35 nm, 260 ± 1.34 nm and 279 ± 2.19 nm (Figure 6.2 A) and the polydispersity index of 0.119 ± 0.053, 0.102 ± 0.018, 0.111 ± 0.015, 0.109 ± 0.010 (Figure 6.3 A). This could be the extent of agglomeration of particles, due to particles getting into close contact with the film forming polymer during the drying process, and, consequently, larger particle sizes are formed (Susarla *et al.*, 2013). Another possible reason is that an increase in PCL concentration in the maintained volume of organic phase (10 mL), could increase the viscous forces, resisting the droplet breakdown as the viscosity of the organic phase is increased, forming larger size of particles (Steiner *et al.*, 2016). Also, the evaporation time is considered as an important parameter of particle sizes, as the process time for allowing the nanoparticles formation from emulsion droplet via the diffusion of organic solvent. As the evaporation time was carried out in a short period of time, the organic solvent did not have enough time to diffuse out of the emulsion droplets before the droplets are getting hardened, resulting in the larger particle sizes (Sharma *et al.*, 2016).

However, a similar observation was seen for the particle sizes and polydispersity index of drug loaded nanoparticles before and after dispersion into film solution. There was a significant increase in the size for drug-loaded nanoparticles before and after

incorporation into the film ($p < 0.001$, ANOVA followed by Tukey's test). The particle size increase from 184.89 ± 2.36 nm, 214.75 ± 1.43 nm, 232.19 ± 2.58 nm and 284.65 ± 3.89 nm before dispersion to 257.85 ± 2.31 nm, 276.70 ± 3.26 nm, 284.38 ± 4.61 nm and 291.45 ± 2.16 after dispersion to film, with the concentration PCL range of 0.2 to 0.5 % w/v, respectively (Figure 6.2 B). Increasing the amount of polymer concentration lead to an increase in viscosity of the organic phase, which enhances the entrapment of drug, resulting in the formation of larger nanoparticles. There was no significant difference recorded for polydispersity index of those nanoparticles either before mixing or after mixing, although there was an increase in polydispersity index ($p > 0.05$, ANOVA followed by Tukey's test) (Figure 6.3 B).

A)



B)

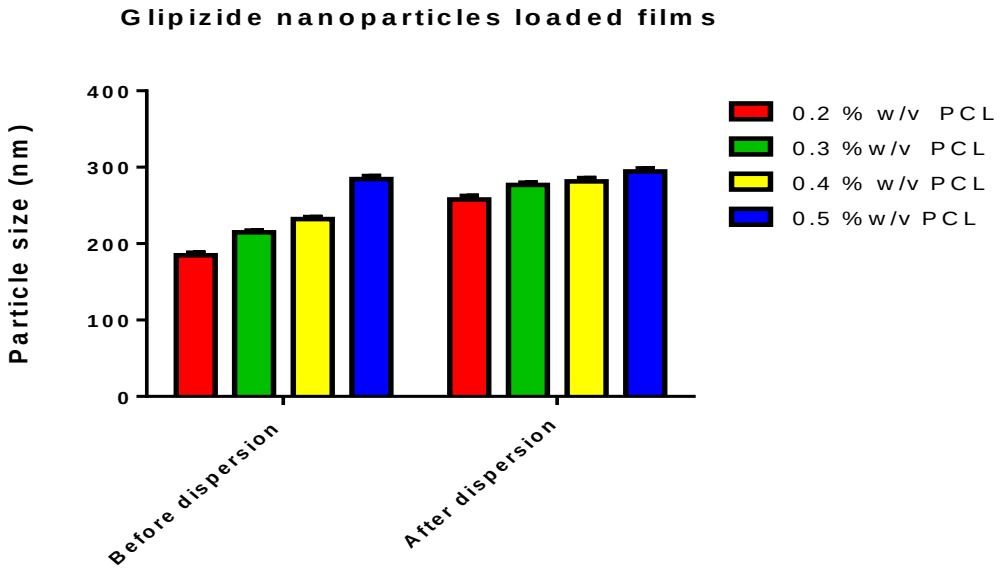


Figure 6.2- The effect of organic phase on the particle sizes of A) blank nanoparticles and B) glipizide loaded nanoparticles before and after dispersion to the film formulation. Error bars represent as standard deviation. Results express as mean ± SD, from three replicates. Statistically significant differences are noted as ($p < 0.001$) in particles sizes before and after dispersion.

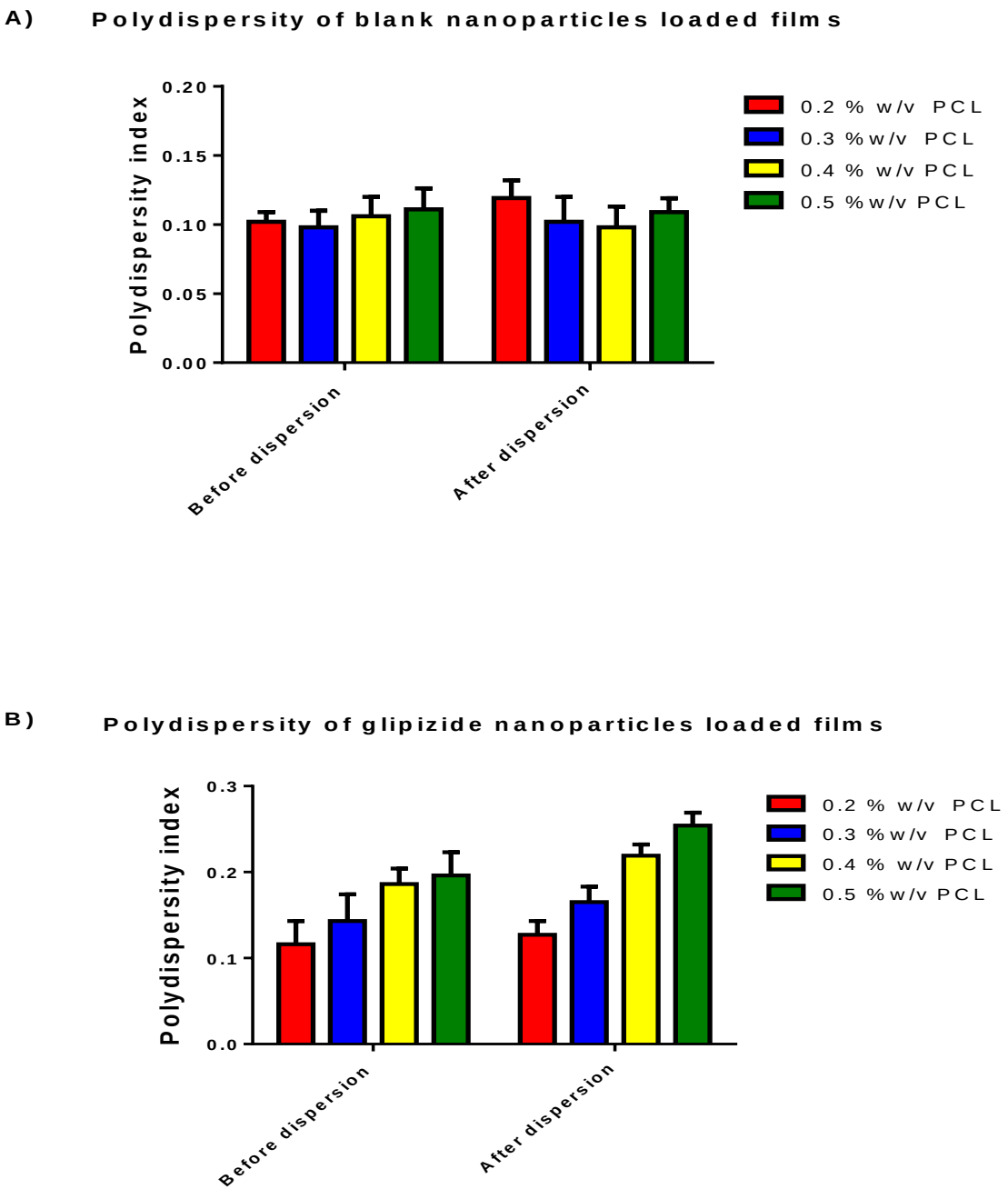


Figure 6.3- The effect of organic phase on the polydispersity index of A) blank nanoparticles and B) glipizide loaded nanoparticles before and after dispersion to the film formulation. Error bars represent as standard deviation. Statistically insignificant differences are noted as ($p > 0.05$) in polydispersity index of particles before and after dispersion. Results express as mean ± SD, from three replicates.

6.2.2 Zeta potential

The zeta potential of blank nanoparticles and drug loaded nanoparticles was measured before and after incorporation into the film. All the nanoparticles exhibited neutral zeta potential. The measurement of zeta potential of blank nanoparticles was varied from - 3.39 ± 0.64 mV to - 7.07 ± 0.35 mV before incorporation in the range of 0.2 – 0.5 % w/v PCL. There was a significant increase in zeta potential for blank nanoparticles in the presence of 0.4 and 0.5 % w/v PCL before incorporation (Figure 6.4 A). However, after dispersion of blank nanoparticles into KP films, it was observed that a significant reduction in zeta potential was apparent, in the same order as the film forming polymer and the surfactant molecules are located on the surface of the nanoparticles ($p < 0.05$, ANOVA followed by Tukey's test).

Moreover, the incorporation of drug also influenced the zeta potential of the nanoparticles. Results showed that a similar effect on the zeta potential was observed for the nanoparticles containing drug (Figure 6.4 B). It is possible that the presence of surfactant reduces the surface tension of the two phases. Since the measurement of zeta potential is based on the magnitude of electrostatic repulsion, it was clear that the reduction in zeta potential was obtained with the addition of surfactant as well as drug particles that might coat on the surface of nanoparticles.

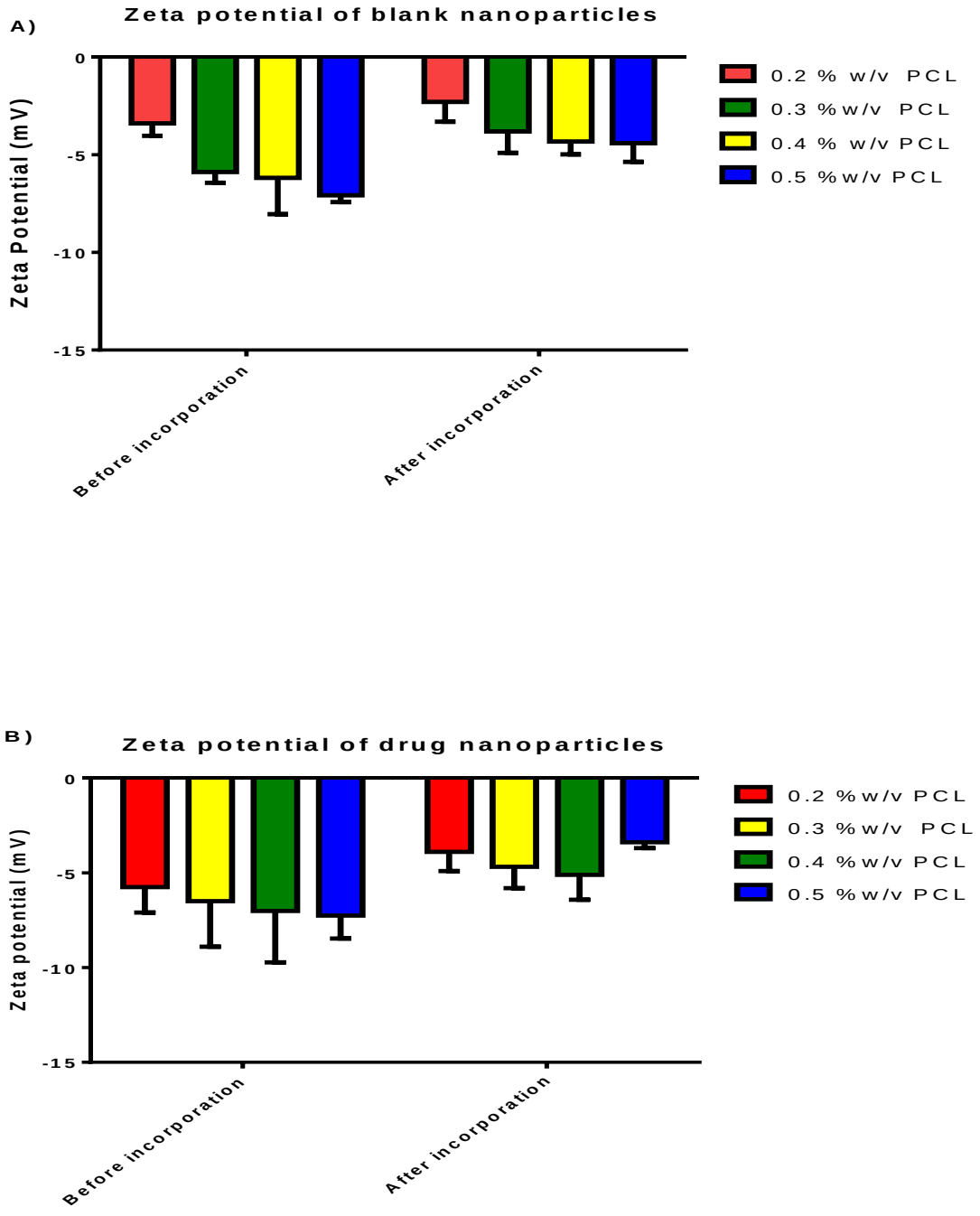


Figure 6.4- The effect of organic phase on the properties of blank and drug nanoparticles before and after dispersion from film formulations on zeta potential of A) blank nanoparticles and B) drug-loaded nanoparticles. Statistically significant differences are noted as ($p < 0.001$) in zeta potential before and after dispersion. Results are expressed as mean ± SD, from three replicates.

6.2.3 Disintegration time

The disintegration testing is carried out using the probe method (see chapter 5). The time taken for ODFs containing either blank nanoparticles or drug loaded nanoparticles to dissolve completely was less than 20 seconds, as films were under additional force that mimics oral cavity pressure (Figure 6.5). As seen, there was no significant difference in the disintegration time of the films containing blank nanoparticles varying at different concentration of PCL polymers in saliva solution ($p > 0.05$, ANOVA followed by Tukey's test). The times recorded for ODFs incorporating blank nanoparticles to completely dissolve were 11 ± 2.6 s, 12 ± 1.0 s, 12 ± 1.0, and 12 ± 1.0 s, from 0.2 % w/v to 0.5 % w/v PCL, respectively. However, there is a slight increase in disintegration time when drug-loaded nanoparticles were incorporated into the films; 11 ± 1.50 s, 12 ± 2.08 s, 14 ± 0.58 s and 14 ± 0.58 s from 0.2 % w/v to 0.5 % w/v PCL, respectively, but this was not deemed significant compared to the blank film ($p> 0.05$, ANOVA followed by Tukey's test). It is possible that, as more organic particles are embedded in the films with an increase in PCL concentration, more time is required to gain the access for water molecules for breaking the polymer matrix of the film forming polymer, hence a longer disintegration time (Steiner *et al.*, 2016).

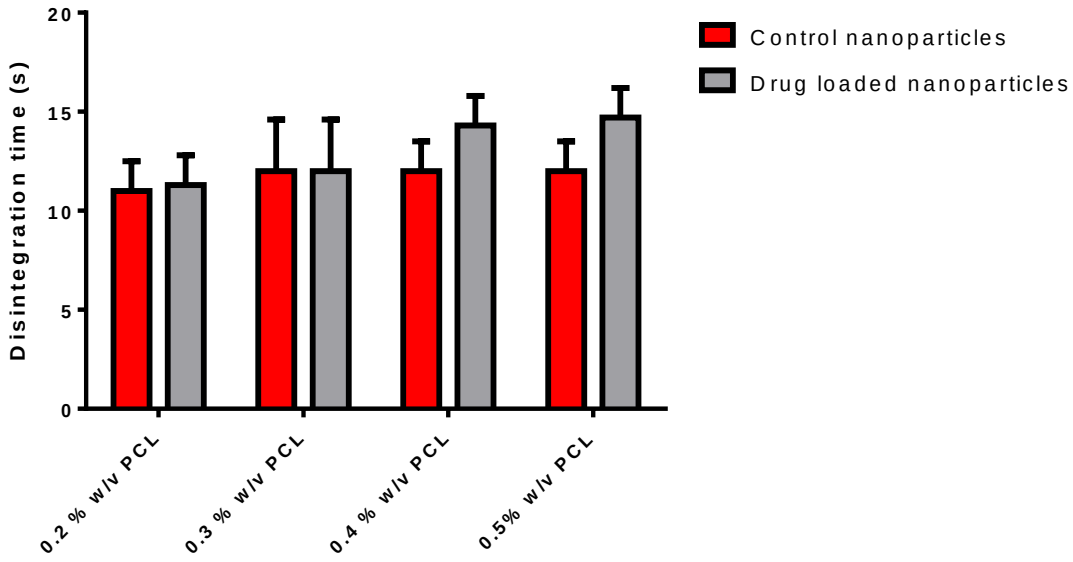


Figure 6.5- Disintegration time of KP film loaded blank nanoparticles and KP films containing drug – nanoparticles in 1ml saliva solution at 37 ^{0}C (n=3 ± SD). Statistical different was observed using one way ANOVA followed by Dunnett's post- test, between drug nanoparticles loaded films and the controls using different concentrations of PCL ($p > 0.05$) indicated that non significance.

6.2.4 Mechanical properties

The mechanical properties of films was measured to ensure the integrity and sufficient physical properties for handling and storage. The thickness of the films before and after nanoparticles loading was uniform (0.14 µm). When analysing the effect of nanoparticles, either with or without drug loading, on the mechanical properties of ODF, results revealed that the loading of nanoparticles seems not to have any effect on these properties of films with an increase in PCL amount ($p > 0.05$, ANOVA followed by Dunnett's test) compared to the control blank (Table 6.1). This is because, especially with the nanoparticles encapsulating with drug, they were further incorporated into ODFs.

Table 6.1- Comparison of mechanical properties of blank control film with ODF containing blank nanoparticles and drug nanoparticles a variable concentration of PCL (0.2 – 05 w/v) at size 3 x2 cm^2. Nanoparticles loading has no effect on the mechanical properties of film ($p > 0.05$, one way ANOVA, Dunnett's test).

	Mechanical properties		
Formulation	**Tensile strength (N/mm^2)**	**Elongation (%)**	**Young's modulus (N/mm^2)**
Control film	4.72 ± 0.53	51.08 ± 2.93	10.01 ± 0.03
Blank nanoparticles (0.2 % w/v PCL) loaded films	3.07 ± 0.82	50.44 ± 1.01	7.51 ± 2.05
Blank nanoparticles (0.5 % w/v PCL) loaded films	5.07 ± 0.77	61.00 ± 3.84	10.52 ± 1.09
Drug nanoparticle (0.2 % w/v PCL) loaded films	4.30 ± 0.79	52.68 ± 2.68	6.92 ± 1.01
Drug nanoparticle (0.5 % w/v PCL) loaded films	5.31 ± 0.107	63.09 ± 4.82	9.18 ± 1.01

Further examining the effect of nanoparticles on the mechanical properties of ODF formulation with an increase of PCL concentrations, results showed that there were no significant differences in tensile properties of all film formulations containing blank nanoparticles with increasing PCL concentrations (Figure 6.6). Despite a minor increase in mechanical properties (e.g. tensile strength, % elongation and Young's modulus), films remain strong and flexible ($p > 0.05$, ANOVA followed by Tukey's test). Similarly, a

small increase in mechanical properties of films observed when glipizide loaded nanoparticles were incorporated into films. There was no significant difference observed in those parameters with an increase in PCL amount ($p > 0.05$, ANOVA followed by Tukey's test). However, incorporation of poorly water soluble drug nanoparticles can be seen to influence the mechanical profile of those films compared to films with blank nanoparticles (Figure 6.7) ($p < 0.05$, ANOVA followed by Tukey's test). As evidence, the tensile strength of drug loaded nanoparticles increases; for example, it increases from 3.07 ± 0.82 N/mn^2 to 4.30 ± 0.82 N/mn^2 using 0.2 % w/v PCL. In addition, a similar effect was observed for other film formulations. It is possible that poorly water soluble drug nanoparticles, with glipizide as a model drug, could potentially perturb the particles in the film forming polymer, which retained less water upon drying due to a decrease in the water affinity through the hydrophilic property of the polymer, resulting in an increase in mechanical properties (Krull *et al.*, 2015).

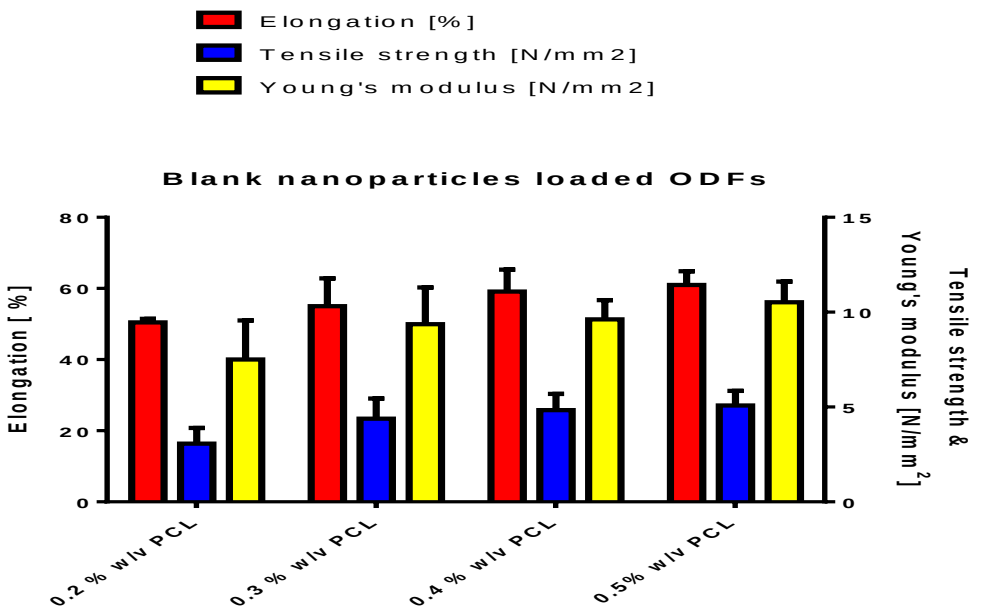


Figure 6.6- Mechanical properties of blank nanoparticles loaded ODF with a variable concentration of PCL (0.2 – 05 w/v). Results are expressed in triplicate, mean ± SD.

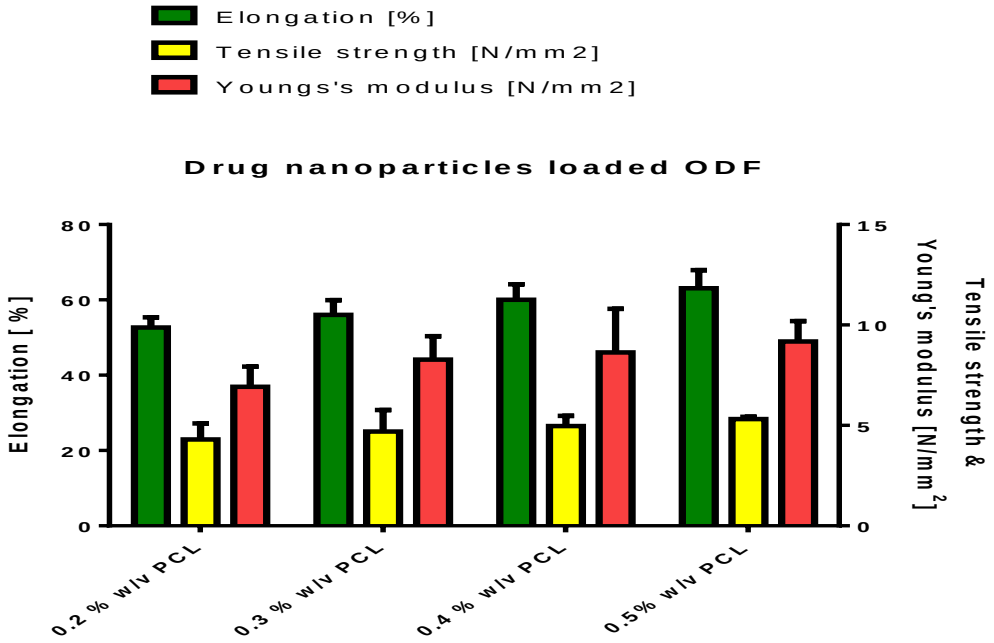


Figure 6.7- Mechanical properties of glipizide nanoparticles loaded ODF with a variable concentration of PCL (0.2 – 05 w/v). Results are expressed in triplicate, mean ± SD.

6.2.5 *In - vitro* drug release

The drug release profile of pure glipizide and drug loaded nanoparticles was studied in phosphate buffer solution as the medium at pH 6.8. Pure glipizide released 51 % of drug after 5 minutes. However, a significant increase was observed in drug release (up to 70 %) of total drug in phosphate buffer solution (pH 6.8) after 10 minutes (Figure 6.8) ($p < 0.05$, ANOVA followed by Tukey's test), as glipizide becomes more ionised in solution – the weakly acidic sulfonylurea groups of glipizide become protonated at higher pH, due to the delocalisation of the nitrogen electron pair from the sulfonyl group (Jamzad and Fassihi, 2006). In contrast, the slightly delayed release of drug from nanoparticles may be attributable to the drug molecules requiring some time to diffuse out from nanoparticles. Similar observation for a burst of drug release after 10 minutes for films containing nanoparticles ($p < 0.05$, ANOVA followed by Tukey's test). Due to the film forming polymer (KP) being very hydrophilic and after slow release of drug from nanoparticles, the burst effect was triggered by the pH condition of the medium (pH 6.8). Also, the rapid dissolution profile after 10 minutes was attributed to the small size of nanoparticles, making a large surface area available for wetting the films (Pandya *et al.*, 2011, Saharan *et al.*, 2015). Thus, ODF could be the potential carrier of nanoparticles to enhance the bioavailability of poor soluble drug,

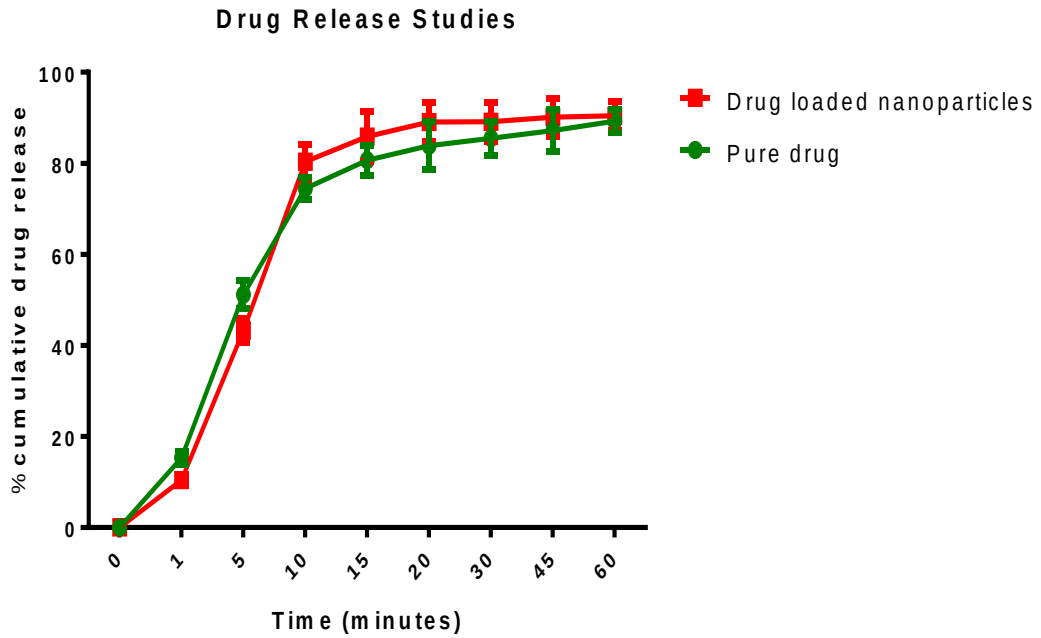
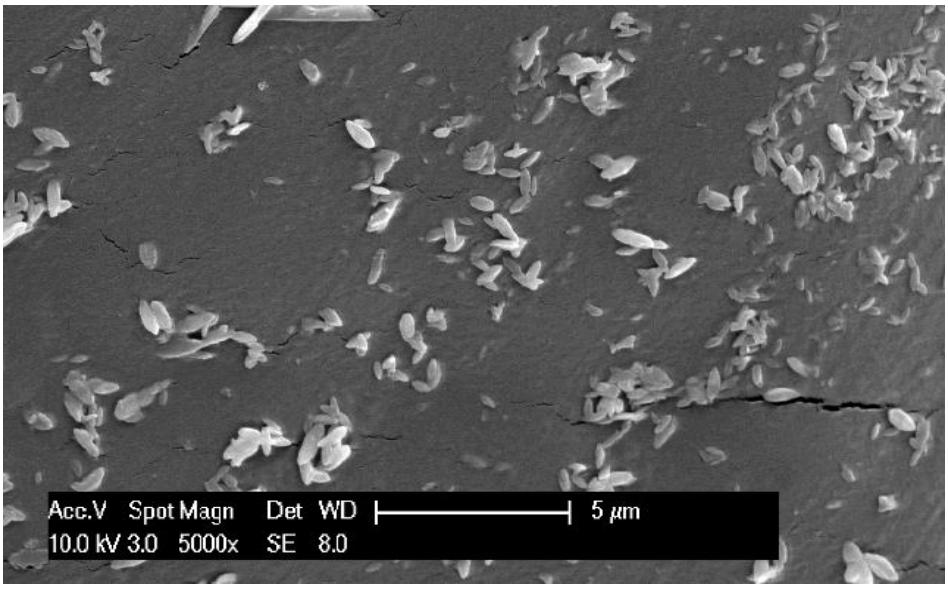


Figure 6.8- Drug release studies of pure glipizide and KP film consisting of glipizide loaded nanoparticles in PBS (pH 6.8). Results as express of mean ± SD, n=3.

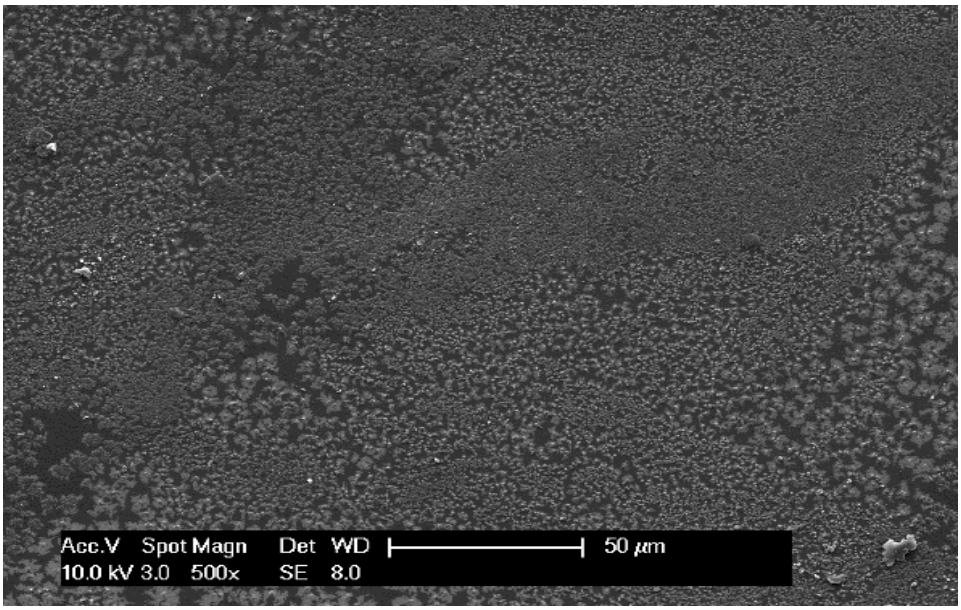
6.2.6 SEM

SEM was carried out to investigate the surface morphologies of films. As shown, different morphologies were captured for the different film samples (Figure 6.9). Pure glipizide exhibited a smooth surfaced, rectangular crystal into films (Figure 6.9 A) (Kushare and Gattani, 2013). However, blank nanoparticle loaded films revealed a homogenous distribution of nanoparticles, with a slight adhesion to each other (Figure 6.9 B). Compared with the morphology of films containing blank nanoparticles, the surface morphology of drug loaded nanoparticles showed the absence of glipizide, which suggested that the drug was successfully incorporated and molecularly dispersed inside the polymer matrix (Figure 6.6 C). These results could be further confirmed by additional studies such as Raman spectroscopy.

A)


Acc.V Spot Magn Det WD 5 µm
10.0 kV 3.0 5000x SE 8.0

B)


Acc.V Spot Magn Det WD 50 µm
10.0 kV 3.0 500x SE 8.0

C)

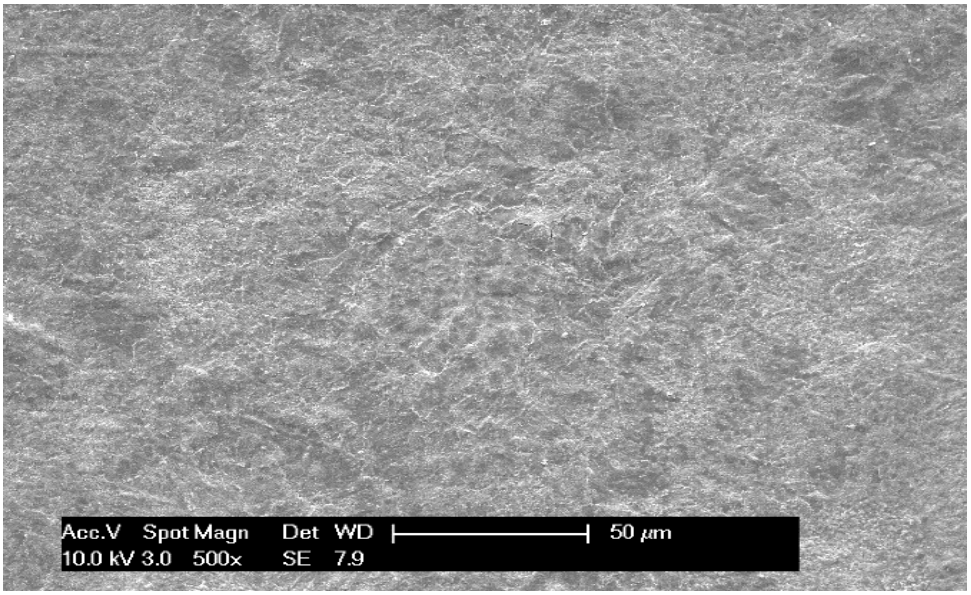


Figure 6.9- SEM images of A) pure glipizide loaded film B) blank nanoparticles loaded ODF and C) drug loaded nanoparticles incorporated into ODF.

6.2.7 Drug entrapment efficiency

The drug entrapment efficiency is the evaluation of the amount of drug that can be embedded into the prepared nanoparticles. The studies were carried out by loading glipizide at 20 mg and 40 mg in the same volume of organic phase (10 mL) at the polymer solvent of 0.2 % w/v and 0.5 % w/v PCL. Results showed that the entrapment efficiency was in the range of 62.12 ± 4.15 % to 80.55 ± 3.25 % (Table 6.1). The solubility of drug in the organic phase and aqueous phase could influence the entrapment efficiency. As seen, the higher entrapment efficiency was found in case of an increase of polymer concentration, as glipizide is soluble in the acetone as an organic solvent Also, an increase in polymer concentration creates more polymer available for the formation of nanoparticles that allows more spaces for drug entrapment. A similar observation for the effect of polymer used was studied (Youan *et al.*, 2001). Although there was an increase

in entrapment level at both doses, but recorded significantly difference for the individual dose of glipizide ($p < 0.05$, ANOVA followed by Tukey's test). This is possibly due to high polymer concentration lead to an increase in viscosity of the organic phase, resulting in increase in larger size of particles that creates diffusional resistance of drug molecules into the aqueous phase, thereby enhancing the entrapment of drug.

Table 6.2- Entrapment efficiency of drug loaded nanoparticles at different solvent concentration. Result are expressed in triplicate, mean ± SD.

Dose of glipizide	Volume of organic phase	Polymer solvent concentration	Entrapment efficiency (%)
20 mg	10 mL	0.2 % w/v PCL	62.12 ± 4.15
20 mg	10 mL	0.5 % w/v PCL	70.55 ± 2.65
40 mg	10 mL	0.2 % w/v PCL	72.12 ± 2.65
40 mg	10 mL	0.5 % w/v PCL	80.55 ± 3.25

6.3 Conclusion

With a low capacity of drug loading into the ODF formulation, the use of nanoparticles was prepared by incorporation of the drug nanoparticles into films with the aim to enhance the bioavailability of a poorly soluble drug, glipizide and potentially improve the drug loading efficiency. The blank nanoparticles and drug nanoparticles were prepared by the solvent replacement method in the range of 0.2 to 0.5 % w/v of the organic phase, which were then mix with film forming polymer, KP, for casting and drying. Films developed with either blank or drug nanoparticles were flexible and transparent. Regarding the amount of PCL used, the particle sizes of blank nanoparticles was recorded as 208 ± 4.79 nm, 220 ± 3.53 nm, 225 ± 4.03 nm and 244 ± 5.02 nm and the

polydispersity index of 0.102 ± 0.007, 0.098 ± 0.012, 0.106 ± 0.014, 0.101 ± 0.015, in the range of 0.2 to 0.5 % w/v, respectively. Furthermore, the particle sizes observed a significant increase after dispersion with the KP solution with the same range of PCL used, possibly as a result of the extent of agglomeration of particles, due to particles getting in close contact with the film forming polymer during the drying process. The results showed that not only the polymer solvent, but also incorporation with film solution potentially affects the particle size and polydispersity index. All films dissolved in less than 20 seconds, and there was no significant difference in the disintegration time of the films containing blank nanoparticles or drug loaded nanoparticles; however, there was a slight delay for films to completely dissolve due to more time required for water molecules to gain access for breaking the polymer matrix of nanoparticles and the film forming polymer. In term of mechanical properties, blank nanoparticles showed no effect on the properties of films, but recorded a minor increase in mechanical properties of films loaded with drug nanoparticles, which could potentially perturb the particles in the film forming polymer, hence the distribution of nanoparticles have no impact on the mechanical properties of films, which indicates films remained strong and flexible. Pure glipizide and drug nanoparticles loaded films released an initial burst up to 70 % of the total amount of drug after 10 minutes in PBS solution at pH 6.8, due to large surface area and small particle size available, which reinforces the ability of ODF to enhance the dissolution rate of poorly water soluble drug via nanoparticles production. No drug particles were observed on the surface morphology of films, which indicated that drug was incorporated successfully inside film. High drug loading was obtained with an increase in polymer concentration used, which demonstrated the potential use of nanoparticles for poorly water soluble drugs with the aim to enhance the drug loading

into films. Overall, ODFs containing nanoparticles is a novel approach to fast release of BCS class II drug.

Chapter 7
General Conclusion and Future Work

7.1 General Conclusion and Future work

Developing formulations for children with the appropriate adapted doses is a particular challenge in drug development, since children are not small adults and they go through different stages of growth and development. As administration of the APIs via the oral route is the most popular choice, most are generally available in solid (e.g. tablets, capsules) or liquid (e.g solutions, suspensions and emulsions) formylations. However, the traditional dosage forms are limited by the dose rigidity, risk of choking and some adverse effects associated with the excipients.

ODFs have gained attention and are acknowledged as a new potential dosage form for paediatric use, which is more advantageous over traditional dosage forms, in terms of improved patient compliance and convenience. Yet, although there has been extensive interest in developing this technology, they are associated with certain limitations as a new, dosage form undergoing development; low dose capacity (less than 40 mg API), since the small, thin size is the limiting factor, therefore restricting their use to potent drugs that are clinically efficacious at lower doses.. Moreover, the currently available methods for preparation of ODFs are generally based on the solvent evaporation method, where the manufacturing process is a controlling parameter to produce uniformity in thickness, which also makes achievement of drug content uniformity challenging, especially for poorly water soluble drugs.

The formulation of ODFs is highly dependent on the selection of the key component: the film-forming polymer. Given that ODFs target fast release and rapid onset of action, the appropriate selection of type and concentration of film forming polymer is a critical parameter for forming a film forming polymer that can be easily removed without damage or rupture, as it is important for storing and handling, achieve efficient mechanical properties, disintegration time, as well as the drug loading of the ODFs. In order to optimise the formulation of ODFs, screening of a range of polymers was initially carried out using the baking tray method (Chapter 1). SA, pectin, and KP were selected as film forming polymers, as they exhibited good texture with excellent film forming capacity; other polymers were excluded from further studies, as they were difficult to peel or no film developed. Also, the use of plasticiser is essential for film formulation, as it helps to improve the flexibility and reduce brittleness of the film. Types of plasticiser and the amount of plasticiser concentration must be compatible with the polymer and appropriately used to enhance the strength of the ODF. Among the selected polymers, Kollicoat protect (KP) was the appropriate polymer candidate for extensive studies in the development of ODFs, due to its instant release profile, great flexibility, good film forming agent with taste masking and moisture barrier properties, as well as great compatibility with the selection of plasticiser (glycerol) and a variety of loaded drug. However, when incorporating drug into films, although the uniformity in weight was achieved, the uniformity of drug failed due to uneven distribution of drugs in the films using this baking tray technique. Since the ODFs have a critical issue to obtain a uniform dose for the individual unit of films, a new alternative method has been approached for further ideal film development in order to achieve content uniformity. Following on from the optimisation process above for the content uniformity of films, the Elcometer Film Applicator, which is equipped for applying uniform and reproducible film products, based

on the principal application of solvent casting technology, was ideally selected for further study of film formulation.

Formulation for poorly water soluble drugs remains a challenging task for the design of oral dosage forms, as most of the drugs being developed in the pipeline today are poorly water soluble. The associated low aqueous solubility and slow release of drugs in the gastrointestinal tract results in low oral bioavailability, leading to consequences such as ineffective treatment and frequent dose escalations to achieve therapeutic effects. In addition, most drugs have a bitter taste and it becomes a serious problem affecting patient compliance and acceptability, particularly to paediatric patients. Besides that, the high drug loading becomes a challenge as a result of poor powder flowability and sticky tendency. Inclusion of BCS class II drug, glipizide, with different types of natural and synthetic cyclodextrins (CDs) was investigated for improving the aqueous solubility and bioavailability of this drug via the formation of inclusion complexation, as well as to taste mask this bitter drug. The solubility of glipizide was enhanced by incorporating within the complexes in both water and PBS solution. Also, the stability constant of complexation is strongly dependent on the strength of the interactions of the guest molecule and the CD cavity, which show that a typical A_L type was observed for glipizide–CD complexation. The incorporation of taste masking agents may affect the mechanical properties of ODFs, as they could aggregate and break down the polymer matrix. Other physicochemical properties of films, including moisture content and disintegration time, were not influenced by the CD complexation. Although there was an improvement in the solubility of poorly water soluble drug and drug uniformity by the

CD complexation, there still remains a low drug loading efficiency due to poor loading capacity of cavity volume of CDs.

Furthermore, the use of polymeric nanoparticles have been extensively used as particulate carrier systems, which is advantageous for poorly water soluble drugs, due to the increase in the surface area to volume ratio of drug particles, as well as improved stability of the API. The exploration of nanoparticles into ODFs, with the aim to enhance the bioavailability of poorly soluble drug, glipizide, and potentially improve the drug loading efficiency was investigated. Nanoparticles prepared by the nanoprecipitation method gave an average size from 180 to 300 nm. Results showed that the particle size of blank or drug nanoparticles, before and after dispersion with polymer solution, increased with an increase in polymer concentration, as it is clear that not only the polymer solvent, but also incorporation within ODFs causes an extent of agglomeration of particles, due to particles being in close contact with the film forming polymer during the drying process. The mechanical properties of films either loaded with blank or drug-loaded nanoparticles produced clear, flexible films; however, when the drug-loaded nanoparticles were incorporated into the ODF, there was a slight delay in disintegration time of film observed, but this remained within the recommended time (30 seconds). Moreover, the dissolution profile of drug was also improved by the use of nanoparticles, due to the availability of a large surface area and small particle size. High entrapment efficiency of drug (up to 80 %) indicated the potential use of nanoparticles for incorporation of poorly water soluble drugs, with the aim to enhance the drug loading into films.

As this is a new innovative technology, a set of associated limitations and challenges arise in the quality control of ODF formulations. ODFs are designed for quick release purposes when applied onto the tongue. Following the European Pharmacopeia, orodispersible tablets (ODTs) should disintegrate in less than 180 seconds (European Pharmacopoeia Commision, 2013) and less than 30 seconds by FDA guidance (FDA, 2008); whereas ODFs, recently subordinates to the "oromucosal preparations" monographs, only stated as "dissolve rapidly" without a defined time limit (European Pharmacopoeia Commision, 2013), although the disintegration time limit of 30 seconds or less is a recommended value for ODFs (Barnhart *et al.*, 2008). However, no standard pharmacopoeia disintegration test method for ODFs exists. As such, the studies described here took a developmental approach to a more relevant and suitable testing method for the disintegration time of ODFs, by dissolving films into a beaker with gentle stirring and through the use of a texture analyser, in order to better reflect the dynamic physiological environment of the human mouth. Media volume is an important controlling factor that could influence the disintegration behaviour of the dosage form, especially to ODFs, as they are quickly hydrolysed upon contact with the saliva. Results showed that the amount of test media and the media itself (e.g. distilled water or saliva solution) did not lead to crucial differences in disintegration time of ODFs, conducted by either a TA-CT3 texture analyser with probe or simply stirring in a beaker. However, the probe method developed using a small volume of media is more biorelevant to the oral cavity volume and provides a clear end-point determination and is, therefore, more suitable for control performance in terms of reproducibility compared to the beaker method. Furthermore, the prototype packaging was used as an alternative approach for ODF packaging, where films were packed either in a cold sealed blister or aluminium foil. As a result, aluminium foil was considered to be the better choice for packaging of ODFs, as expected, due to its great

protection from moisture. It also is important to maintain the integrity of products and stability profile of ODFs, since they are easily to exposed to the environment; stability studies were carried out for films packed by these prototypes packaging at long term (25 ± 2 ^{0}C and 60 ± 5 % RH) and accelerated stability conditions (40 ^{0}C ± 2 ^{0}C and 75 ± 5 % RH). Films exhibited strong, flexible properties over a period of 3 months, with no change in the mechanical properties, retaining its instant release (less than 25 seconds), with good uniformity of dosage, which is available at such low dose, and therefore, those formulation could more viable industrially and clinically through the product developmental stages.

7.2 Future work

- Investigation of permeability of drug through the buccal cells to predict the processing of drug transport across the buccal mucosa membrane and in terms of toxicity.

- Exploring different manufacturing methods for preparation of nanoparticles in order to enhance drug loading and drug content uniformity, especially for poorly water soluble drug.

- Optimisation the suitable appropriate volume of media for dissolution studies and standardised packaging for further improvement in performance of ODFs.

- Conduction of clinical survey to predict the potential use of ODFs in the children.

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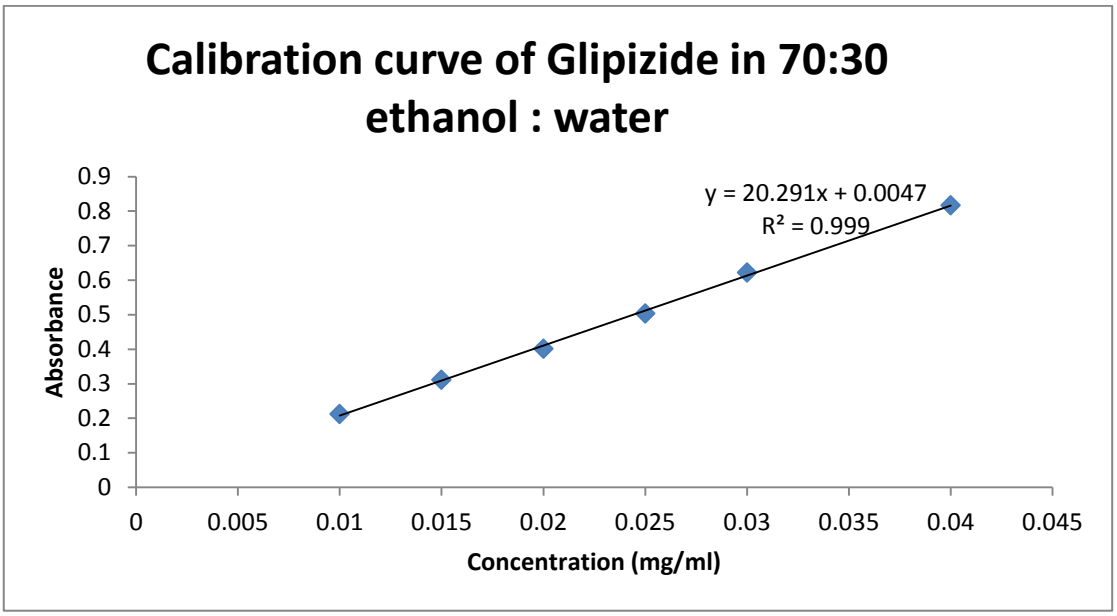
Appendix 1



Appendix 1- Calibration curve of glipizide in 70: 30 v/v ethanol: water