Aston University

Some pages of this thesis may have been removed for copyright restrictions.

If you have discovered material in AURA which is unlawful e.g. breaches copyright, (either yours or that of a third party) or any other law, including but not limited to those relating to patent, trademark, confidentiality, data protection, obscenity, defamation, libel, then please read our Takedown Policy and contact the service immediately

Polymeric Films for Buccal Drug Delivery

Songjie Chen

Doctor of Philosophy

ASTON UNIVERSITY

June 2009

This copy of the thesis has been supplied on condition that anyone who consults it is understood to recognize that its copyright rests with its author and that no quotation from the thesis and no information derived from it may be published without proper acknowledgement.

Aston University

Polymeric films for Buccal Drug Delivery

A thesis submitted by Songjie Chen for the degree of Doctor of Philosophy

June 2009

Summary

Buccal is an attractive site for drug delivery due to ease of administration and avoidance of possible drug degradation in the gastrointestinal tract and first-pass metabolism. Bioadhesive polymeric films are the formulations which have received the most attention for buccal drug delivery because they present a great patient compliance and cause minor discomfort to patient due to their better flexibility than tablets. Pullulan as a biodegradable polysaccharide, is widely used in pharmaceutical, clinical, health care and food industry owning to its highly water-soluble and good film-forming ability.

The aim of this research project is to evaluate whether or not pullulan films are suitable to buccal drug delivery of a phosphodiesterase5 (PDE5) inhibitor yonkenafil, which was discovered in our research group and currently is under phase II clinical trial for treatment of erectile dysfunction (ED). Variable formulations of pullulan films were designed and the films were prepared in laboratories. Mechanical properties of the films, in vitro drug release and polymer dissolution, *in vitro* drug penetration through porcine esophageal mucosa were investigated in this project. The plasticization effects of solvents, polyols and acids to the films were studied by tensile test, and differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), fourier transform-infrared (FT-IR), scanning electron microscopy (SEM), optical microscopy was applied to analyse of the structure and chemical-bonding between pullulan and the additives within the films. Release mathematics models were used in the study of the mechanism of drug releases and polymer dissolutions. Ethanol, menthol, fatty acids, and sodium dodecyl sulfate (SDS) were employed as penetration enhancers to pretreat the tissue, and drug penetration rate through the pretreated membrane were calculated to estimate the enhancing effect of each penetration enhancer.

Various plasticizers and acids were applied into the films and the result showed polyethylene glycol (PEG) 400 and 600 had the excellent plasticization effect on the drug-free pullulan films, while lactic acid was the best plasticizer for the drug-loaded films. Both PEG400 and lactic acid had a great effect on the drug release from the films *in vitro*, and all the results indicated that the hydroxyl and carboxyl groups of pullulan and the additives influenced the mechanical properties of the films significantly, and also altered drug release mechanisms. Ethanol shows the greatest enhancing ability on the drug permeation through the porcine esophageal mucosa. A possible mechanism for this is that ethanol interferes structures of the lipids in the mucosa, resulting in increased partitioning of the drug into the membrane.

Keyword: Polymeric film, buccal drug delivery, pullulan, yonkenafil

Acknowledgements

I would like to thank my supervisor, Dr. Yongfeng Wang and Dr. Barbara R. Conway, for their great support and encouragement in past 3 years.

I would like to thank Dr. Kejun Zhao and Dr. Dan Fei, for their great advice during my first year report. A special thank to Dr. Mike Davis, Mr. Jiteen Kanasara for their technical support and assistance. Thanks to all the staff and students in LHS School for their help and cooperation.

Thanks to my parents for their financial support and encouragement.

Contents

List of Figures

List of Tables

Chapter One

Introduction

1.1 Intraoral delivery systems (IDS)

Intraoral delivery systems (IDS) which can produce desirable drug exposure for optimum therapeutic effects have been being developed over the last few decades. Nontraditional oral dosage forms, such as buccal and sublingual, have become acceptable, and in some cases are the preferred alternative to conventional tablets and capsules. Pregastric absorption by various tissues of the oral cavity can avoid first-pass and gut-wall metabolism, enhance bioavailability, or improve convenience of dosing. Moreover, utilizing the buccal and sublingual mucosa as an absorption site is a promising drug delivery route which can promote rapid absorption. Therefore, there is growing interest in developing new, non-parenteral, reliable and convenient dosage forms using administration routes, where a rapidly dissolved drug is immediately absorbed into the systemic circulation. It has long been evident to drug delivery scientists that achieving therapeutically effective plasma levels with intraoral drug delivery systems can be a major challenge, because the relatively small surface area of oral mucosa and significant loss of the applied dose due to swallowing and salivary flow are the major limitations for intraoral drug delivery.

1.1.1 Routes of administrations

Drug delivery via the membranes of the oral cavity can be classified as follows, by drug administration sides:

Sublingual delivery: administration of a drug via the sublingual mucosa, the membrane of the ventral surface of the tongue and the floor of the mouth, to the systemic circulation systems.

Buccal delivery: administration of a drug via the buccal mocusa, the lining of the cheek and area between the gums and upper and lower lips, to the systemic circulation systems.

Periodontal, gingival, and odontal delivery: local treatment of conditions of the oral cavity, principally aphthous ulcers, bacterial and fungal infections, and periodontal disease.

1.1.1.1 Sublingual route

The sublingual route of drug absorption is the most widely studied of these sites of the drug delivery via oral cavity, for being relatively permeable. The sublingual route can provide a rapid absorption and easy accessibility to the drug for systemic delivery, especially for quick-dissolving dosage forms (*Das, 2004*). However, the sublingual region lacks an expanse of smooth muscle or immobile mucosa and is constantly washed by saliva making it difficult to place a drug loaded device for sustained drug delivery (*Harris et. al, 1992*).

1.1.1.2 Buccal route

The buccal mucosa is somewhat less permeable than the sublingual mucosa, and is generally not the perfect delivery side for rapid absorption and does not exhibit as good a bioavailability as sublingual. However the buccal route provides the opportunity for drug absorption through the buccal epithelial lining of the oral cavity (mucosa and cheek) for local or systemic action (*Shojaei, 1998*).

1.1.1.3 Other routes

There are a number of terms that have been defined by the Food and Drug Administration (FDA) to describe routes of administration for all approved dosage forms and drug products. Table 1.1 shows the 15 routes related to pregastric intraoral delivery (*http://www.fda.gov*).

Table 1.1 Pregastric routes of drug administration

Delivery Route Name	Definition	Short Name
Buccal	Administration directly toward the cheek, generally from within the mouth	Buccal
Dental	Administration to a tooth or teeth	Dental
Endotracheal	Administration directly into the trachea	E-trache
Intracoronal, dental	Administration of a drug within a portion of a tooth covered by enamel and separated from the roots by a slightly constricted region known as the neck	I-Coronal
Intraesophageal	Administration within the esophagus	I-eso
Intragingival	Administration within the gingival	I-gingiv
Laryngeal	Administration directly upon the larynx	Laryn
Oral	Administration to or by way of the mouth	Oral
Oropharyngeal	Administration directly to the mouth and pharynx	Oro
Periodontal	Administration around the tooth	P-odont
Sublingual	Administration beneath the mucous membrane	SL
Submucosal	Administration beneath the mucous membrane	S-mucos
Transmucosal	Administration across the mucosa	T-mucos
Transtracheal	Administration through the wall of the trachea	T-trache
Intratracheal	Administration into the trachea	-

1.1.2 Classification of intraoral dosage forms

According to the dissolution or disintegration kinetics of the dosage forms, they can be classified as quick-dissolving (QD), slow-dissolving (SD), or nondissolving (ND).

1.1.2.1 Quick-dissolving delivery systems (QD)

The QD systems release the drug into the oral cavity over a period of seconds up to 1 minute and the delivery devices disintegrate or dissolve in the saliva. The QD systems are suitable for the treatment requiring a quick onset action of some drugs. They can be applied in supervised administration, buccal or sublingual absorption, systematic or local therapy of the oral mucosa. The technical advantages of the QD systems include ease of swallowing, administration without water anywhere and anytime, enhanced efficacy.

1.1.2.2 Slow-dissolving delivery systems (SD)

The SD systems release the drug into oral cavity over a period of 1 to 10minutes and the drug-loading vehicle are generally dissolved in the oral cavity, such as chewable tablets, sublingual tablets and mucoadhesive tablets. More and more researchers are designing bioadhesive and biodegradable polymeric delivery systems for drug delivery via the buccal mucosa.

1.1.2.3 Nondissolving delivery systems (ND)

The ND systems release the drug into oral cavity over a period over 10 minutes up to hours, and the dosage forms do not dissolve entirely when they are placed in the

mouth. They are usually designed for controlled drug delivery, the dosage forms including: chewing gum, buccal and gingival patches, periodontal fibers etc.

1.2 Buccal drug delivery systems

Buccal mucosa allows drug delivery for both local and systemic therapies. Local delivery to tissue of the oral cavity has a number of applications, including treatment of local conditions such as periodontal disease, bacterial and fungal infections, aphthous stomatitis and vesiculo bullous diseases, toothache, and in facilitating tooth movement with prostaglandins (*Nagai et. al., 1987; Ishida et. al., 1983; Nagai 1985; Gallagher et. al., 1985; Lamb et. al., 1988*).

1.2.1 Buccal absorption

The anatomy of oral mucosa, barrier nature of buccal mucosa, routes of drug transport through buccal mucosa, will be introduced in Chapter Four, section 4.1.1.

1.2.2 Buccal drug delivery dosage forms

Many dosage forms for buccal administration have been developed in the past decades, including matrix tablets, patches, lipophilic gels, transfersomes, bioadhesive films (*Rossi et. al., 2005*), and more recently, an integrated device that comprised a drug reservoir, sensors, pressure pump, microvalve and control electronics was introduced (*Kohnle et. al. 2007*).

Medicated chewing gums have some advantages as drug delivery devices, particularly in the treatment of diseases in the oral cavity and in nicotine replacement therapy

although they pose difficulties in regulating the dose administered (*Hao & Heng 2003*). Some commercial products are available in the market. Caffeine chewing gum, Stay Alert®, was developed recently for alleviation of sleepiness (*Kamimori et al., 2002*). It is absorbed at a significantly faster rate and its bioavailability was equivalent to that in capsule formulation (*Hao & Heng 2003*). Nicotine chewing gums (e.g., Nicorette® and Nicotinell®) have been marketed for smoking cessation. The permeability of nicotine across the buccal mucosa is faster than across the skin (*Nielsen & Rassing, 2002*). However, chewing gum slowly generates a steady plasma level of nicotine rather than a sharp peak as experienced when smoking. Possible swallowing of considerable amount of nicotine during chewing may lead to decreased effectiveness of the chewing gum due to first-pass metabolism and gastrointestinal discomfort. It is a major challenge to optimize the dose-response relationship of nicotine administered in a chewing gum (*Nielsen & Rassing, 2002).*

Hydrogel-based bioadhesive tablets are bioadhesive to the buccal mucosa, when the device is hydrated, and the drug is released and form a hydrogel. Single-layer buccal tablets which lack an impermeable backing layer on the tablet buccal, cause a significant amount of dose lose due to being swallowed into the stomach, and finally have a low bioavailability (*Voorspoels et al., 1996*). To overcome the drawback of dose lose, tablets of triamcinolone acetonide (Aftach®), developed for local treatment of aphthous ulcers, consist of a bioadhesive hydroxypropyl cellulose/polyacrylic acid layer and a lactose nonadhesive backing layer (*Nagai & Machida, 1993*). Nifedipine/propranolol hydrochloride double-layer tablets for systemic delivery with prolonged drug release and adequate adhesiveness were developed (*Remuñán-López et al., 1998*). Nicotine replacement therapy requires a fast release of nicotine followed by a prolonged release of nicotine for maximal efficacy. A bilayer buccal adhesive nicotine tablet provided a drug release pattern combining fast release and prolonged release profiles and resulted in improved smoking cessation rates (*Park & Munday, 2002*). A problem associated with the double-layer tablet was separation of the two layers. This may be overcome by modifying the device so that there is a gradient in hydrophilicity from one side to the other (*Bologna et al., 2001*).

To use flexible adhesive films and laminated patches as buccal delivery systems (*Hao*

& Heng 2003) that requires:

(1) a bioadhesive to facilitate intimate contact with the mucosa and increase residence time
(2) a vehicle that releases the drug at an appropriate rate
(3) additives such as penetration enhancers and/or enzyme inhibitors.

The films generally dissolve rapidly to release the active agent and developed as quick-dissolving delivery system. However, they also can be tailored to release the drug more slowly as well, depending upon their thickness, and composition of the polymer matrix.

In the 1970s, using thin films or strips to delivery lidocaine for dental applications was examined. More recently, films dosage forms containing dibucaine and buprenorphine have been reported (*Danjo et. al., 1995; Ishida et. al., 1982; Roller et. al., 1975; Brook et. al., 1989; Yamamura et. al., 1998*). Bioadhesive chitosan film of chlorhexidine gluconate showed characteristics of increased residence time of drug and prolonged antimicrobial action (*Şenel I et al., 2000*). A novel bilayer bioadhesive film of testosterone is composed of a pH-sensitive bioadhesive layer containing polycarbophil/Eudragit S-100 and a pharmaceutical wax as the impermeable backing layer (*Jay et al., 2002*). The adhesion time of these films to rabbit buccal pouch was affected by the ratio of these two polymers. The presence of the wax-backing layer greatly enhanced the adhesion time of the bioadhesive layer and bioavailability by retarding the diffusion of saliva into the drug layer and drug loss into mouth. These bilayer bioadhesive buccal patches containing plasmid DNA were also explored for mucosal immunization in rabbits (*Cui & Mumper, 2002*). Table 1.2 shows the investigation of the buccal mucoadhesive films.

Table 1.2 List of investigated buccal mucoadhesive films

Active ingredient	Polymers used	Reference
Acyclovir	Chitosan HCl and PAA sodium salt	*Rossi et al., 2003*
Caffeine	PVA, Plastoid E35H	*Nicoli et al., 2005*
Chitosan	Chitosan	*İkinci et al., 2002*
Chlorhexidine diacetate	EC	*Jones & Medlicott, 1995*
Chlorhexidine digluconate	Chitosan	*Şenel et al., 2000*
CMV-β-gal plasmid DNA or β-gal protein	PC and Eudragit® S-100	*Cui & Mumper, 2002*
Dipotassium glycyrrhizate	PC, HPC, and EC	*Rhee et al., 1999*
Fentanyl	PVP	*Diaz del Consuelo et al., 2007*
Glibenclamide	Chitosan and PVP	*Ilango et al., 1997*
Glipizide	HPMC-E15, CP 934P, Eudragit RL-100 SCMC	*Semalty et al., 2008*
Ibuprofen	PVP, NaCMC	*Perioli et al., 2004*
Insulin	Gelatin and CP 934P	*Ritschel wt al., 1989*
Lidocaine	HPC	*Okamoto et al., 2001, 2002*
Nifedipine	Sodium alginate, MC, PVP, and PEG	*Save et al., 1994*
Nifedipine or Propranolol HCl	Chitosan with or without an anionic crosslinking polymer (PC, sodium alginate, gellan gum)	*Remuñán-López et al.,1998*
Salmon calcitonin	PC and Eudragit® S-100	*Cui & Mumper, 2002*
Testosterone	PC and Eudragit® S-100 (polymethacrylic acid-co-methyl methacrylate)	*Jay et al., 2002*
Tetracaine, ofloxacin, miconazole, guaiazulene, and triacetin	HPC	*Oguchi et al., 1998*
Tetracycline	Atelocollagen	*Minabe et al., 1989*

Bioadhesive films/patches are commonly manufactured by solvent evaporation process, in which the polymeric material, with or without plasticizer, is dissolved in a solvent or solvent mixture and into which the active constituent is dissolved or dispersed. This solvent is then cast onto a suitable substrate and the solvent is allowed to evaporate, leaving a solid polymeric film containing the drug (Jones & Medlicott, 1995). More recently, a hot-melt extrusion method was reported to fabricate hot-melt extruded films for buccal delivery, which overcomes the disadvantages associated with a solvent casting method such as environmental concerns, long processing times, and high costs (*Repka et al., 2002*).

1.3 Bioadhesion and mucoadhesion

1.3.1 Definition

The term bioadhesion refers to any bond formed between two biological surfaces or a bond between a biological and a synthetic surface. When the bonds formed between mucus and polymer, the term mucoadhesive is used synonymously with bioadhesion. Generally, bioadhesion is used to describe adhesive interactions with any biological or biologically derived substance, while mucoadhesion is used to only when describing a bond involving mucus or a mucosal surface.

1.3.2 Mechanism of bioadhesion

The mechanism of the formation of bioadhesive or mucoadhesive bonds is not fully clear. The process of forming bioadhesive bonds can be described as following steps (*Duchêne et. al., 1988; Ponchel et. al., 1991*):

(1) the polymer is wet and swells to contact with the biological surface
(2) interpenetration of bioadhesive polymer chains and entanglement of polymer and mucin chains
(3) formation of chemical bonds between entangled chains

In addition, the polymer characteristics are required to be bioadhesive (*Peppas et. al., 1985*):

(1) sufficient hydrogen-bonding function groups, such as -OH and –COOH
(2) anionic surface charges
(3) high molecular weight
(4) high chain flexibility
(5) surface tensions

1.3.3 Theories of bioadhesion

1.3.3.1 The electronic theory

On this hypothesis, bioadhesive materials and biological tissues have different electronic structures. When they contact each other, electron transfer happens and results in the formation of a double layer of electronic charge at the surface of bioadhesive-biologic material. The attraction force across this electric double layer is believed to be the bioadhesive force (*Derjahuin et. al., 1966*).

1.3.3.2 The absorption theories

These theories assume that bioadhesive materials and tissues or mucosa form the bioadhesive bonds via van der Waals interactions, hydrogen bonds, and relative forces (*Good, 1977; Tabor, 1977*).

1.3.3.3 The wetting theory

It is an important factor in bond formations that the bioadhesive materials or mucus are able to spread and develop intimate contact with its corresponding substrates. This theory is using interfacial tensions of liquid to predict spreading and in turn adhesions (*Peppas, 1985; Mikos et. al., 1989; Baszkin et. al., 1990*). As a result, the researchers have paid more attention to the surface energy of both polymers and tissues, to predict the bioadhesive performance (*Lehr et. al., 1993; Lehr et. al., 1992; Spychal et. al., 1989; Kaelble et. al., 1977*).

1.3.3.4 The diffusion theory

This theory proposes that the bond strength formed by the biological tissues and the bioadhesive materials increases with the degree of penetrations of the polymer chains into the mucosa layer. The penetration of polymer chains into the mucus network is dependent on concentration gradients and diffusion coefficients (*Voyutskii, 1963*).

1.4 Mucoadhesive polymers

1.4.1 Desired characteristics

An ideal mucoadhesive polymers used in buccal drug delivery should have such necessary structural characteristics:

(1) molecular weight: above 100,000 (*Chen et. al., 1970*)

(2) flexibility: a substantial degree of flexibility to achieve the desired entanglement with the mucus (*Gu et. al., 1998*)

(3) hydrogen bonding capacity: must have functional groups that are able to form hydrogen bond with the mucus (*Park et. al., 1987*)

(4) cross-linking density: when the crossing-linking of the polymer network is high, diffusion of water into the network is weakened and causes an insufficient swelling of the polymer and a decreased rate of interpenetrations between polymers and mucin (*Gu et. al., 1998*)

(5) charge: both anionic and cationic polymers may have a good bioadhesion (*Peppas et. al., 1985; Park et. al., 1989; Lehr et. al., 1992*)

(6) concentration: when the polymer concentration is low, the interaction between polymer and the mucus is unstable (*Peppas et. al., 1985*)

(7) hydration (swelling): to expand the polymer on the surface of mucosa and induce mobility in the polymer chains (*Gu et. al., 1998*)

1.4.2 Classification of new generation of mucoadhesive polymers

1.4.2.1 First generation of mucoadhesive polymers

The first generation of mucoadhesive polymers was lack of specificity and targeting capability. They adhere to the mucus non-specifically and suffer a short retention time. The bonds they formed with the mucus or tissue are generally non-covalent in nature, and are classified as consisting mostly of hydrogen bonds, hydrophobic and electrostatic interactions (*Lee et. al., 2000*).

1.4.2.2 Second or modern generation of mucoadhesive polymers

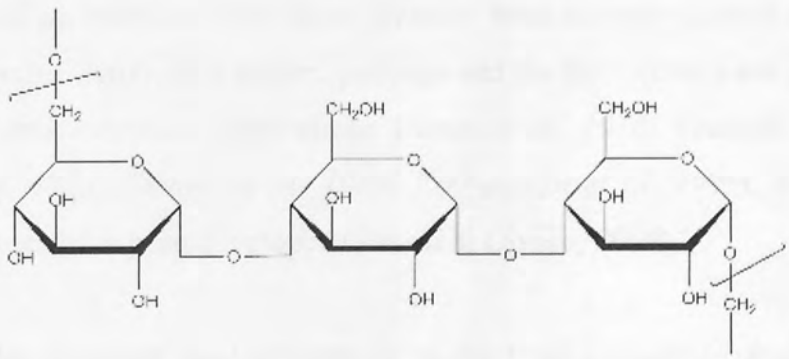
The new generation of mucoadhesive polymers can adhere directly to the cell surfaces, rather than to mucus. Instead of non-specific bonding, they interact with the cell surfaces by means of specific receptors or covalent bondings. The second generations, such as thiolated mucoadhesive polymers, target-specific, lectin-mediated bioadhesive polymers, bacterial adhesion polymers, mucoadhesive polymers as enzyme inhibitors and permeation enhancers, have created new possibilities for more specific drug–receptor interactions and improved targeted drug deliveries (*Nazila et. al., 2005*).

1.4.3 Pullulan

1.4.3.1 Physical and chemical properties of pullulan

Pullulan is a bioadhesive and biodegradable polysaccharide which has been in commercial production for more than 30 years. However, its high price has limited its potential commercial applications into food, even drug deliveries. Nevertheless, its excellent film-forming ability, highly water-soluble, stable physiochemical properties make pullulan the best choice in producing a polymeric matrix for film dosage forms for drug delivery. The chemical structure of pullulan is shown in Figure 1.1.

Figure 1.1 Chemical structure of Pullulan



Dry pullulan powder is white, non-hygroscopic and dissolve readily in hot or cold water, and it is non-toxic, non-mutagenic, odorless, tasteless, and edible (*Fujii et. al., 1986; Kimoto et. al., 1997*).

Pullulan solutions are of relative low viscosity, resembling gum Arabic (*Tsujisaka et. al., 1993*). The viscosity of the pullulan solution is not subject to the trend of heating,

to pH changes, and is tolerating with presence of most common metal and non-metal ions, including sodium chloride (*Nakashio et. al., 1976*).

Pullulan films are formed by drying a pullulan solution (usually 5-10%) on an appropriate smooth surface. Films can be as thin as 5-60 μm. The films are transparent, highly oxygen-impermeable (*Yuen, 1974*). Underivatized films are easily dissolve in water and are a good material to be used as a coating which can be rapidly dissolved in mouth (*Conca et. al., 1993*).

1.4.3.2 Applications of pullulan

Pullulan and its derivatives have many potential pharmaceutical, clinical, and health uses. Pullulan can be used in pharmaceutical coatings, including sustained-release formulations (*Miyamoto et al. 1986; Izutsu et al. 1987; Childers et al. 1991*). Oral care products based on pullulan films have recently been commercialized (*Leung et al. 2000; Anonymous 2001*). In addition, pullulan and its derivatives have photographic, lithographic, and electronic applications (*Sano et al. 1976; Tsukada et al. 1978; Shimizu et al. 1983; Sasago et al. 1988; Vermeersch et al. 1995*). Recently, hard pullulan capsules have been developed (*Sakata & Otsuka, 2009*).

Pullulan is also currently used extensively in the food industry (*Rekha & Chandra, 2007*). The pullulan edible films used in food preservation are colorless, tasteless, resistant to oil and heat-sealable, have very low oxygen permeability and are amenable to changes in mechanical and gas barrier properties on mixing with other biopolymers and plasticizers (*Yuen, 1974; Desphande et al., 1992; Gibbs & Seviour, 1996; Biliaderis et al., 1999*).

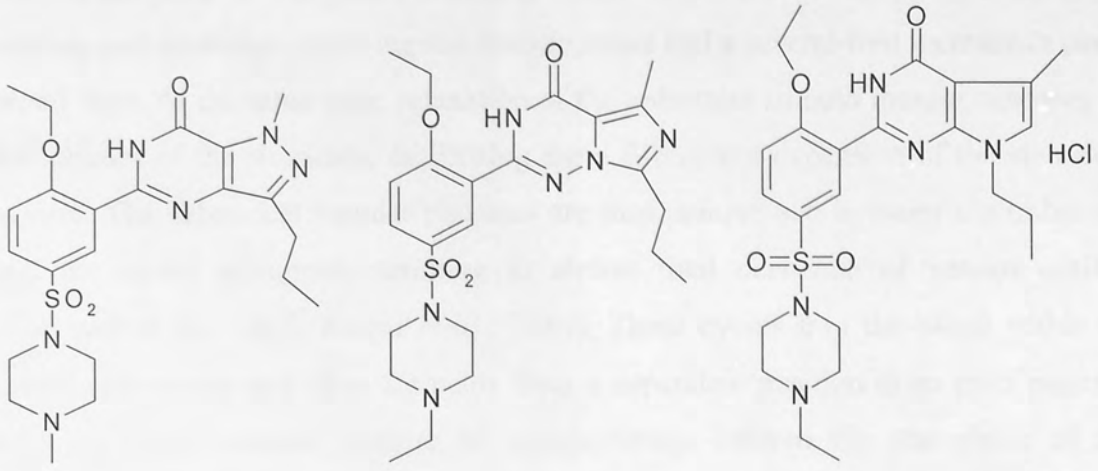
Pullulan has demonstrated uses in cosmetics, lotions, and shampoos (*Nakashio et al. 1976b*). Pullulan and its derivatives exhibit adhesive properties (*Hijiya and Shiosaka 1975a*) and can be used in wound-healing compositions (*Leung et al. 2001*). Pullulan

can be used as a denture adhesive, a binder and stabilizer in food pastes, and to adhere nuts to cookies.

1.5 Yonkenafil

The drug used in this study is yonkenafil (2-[2-ethoxyl-5-(4-ethylpiperazinyl -1-sulfonyl)phenyl]-5-methyl-7-n-propyl-3,7-dihydropyrrolo[2,3-d]pyrimidin-4-one,hydrochloride), a phosphodiesterase5 (PDE5) inhibitor discovered in our research group. The structurally yonkenafil is a me-too drug to sildenafil and vardenafil (Figure 1.2), which are world-wide used drugs for treatment of erectile dysfunction (ED). The major structural differences of yonkenafil from sildenafil and vardenafil are the numbers of nitrogen atoms and their positions on the five fused six membered heterocyclic rings, which are the core structure in sildenafil, vardenafil and yonkenafil. The bioactivity studies with phosphodiesterases revealed yonkenafil possessed a good PDE5 inhibiting activity and selectivity. The overall profile of yonkenafil against PDE1-6 is better than sildenafil and vardenafil (Table 1.3). The PDE5 inhibiting activity and PDe5 selectivity of yonkenafil were further proved on mice, rabbit and dog models (*Wang, 2009*). In the following pre-clinical studies, yonkenafil demonstrated its druggable nature, such as low toxicity LD50 >2000mg/kg and a good pharmakinetics. Therefore, yonkenafil was chosen as a candidate into development. Now the tablet dosage has come into phase II clinical trials in China. We felt that a drug for treatment of ED had better have some attracting properties, such as packaging stimulating, pleasant administration, fast onset, etc. A film dosage form meets all these factors, therefore we decided to apply pullulan as a vehicle to develop a yonkenafil film formulation and dosage form.

Figure 1.2 The chemical structure of sildenafil, vardenafil and yonkenafil



Sildenafil Vardenafil Yonkenafil

Table 1.3 Comparison of inhibiting activities against PDE1-6 of yonkenafil, vardenafil and sildenafil

Phosphodieserases type	Yonkenafil (nmol)	Vardenafil (nmol)	Sildenail (nmol)
PDE1	>10000	70	281
PDE2	>10000	6200	>30000
PDE3	>10000	>1000	16200
PDE4	>10000	6100	7680
PDE5	2.01-14	0.14	3.5-8.5
PDE6	266	3.5	37

1.5.1 Physiology of penile erection

Penile erection is a neurovascular event modulated by psychological factors and hormonal status. On sexual stimulation, nerve impulses cause the release of

neurotransmitters from the cavernous nerve terminals and of relaxing factors from the endothelial cells in the penis, resulting in the relaxation of smooth muscle in the arteries and arterioles supplying the erectile tissue and a several-fold increase in penile blood flow. At the same time, relaxation of the trabecular smooth muscle increases the compliance of the sinusoids, facilitating rapid filling and expansion of the sinusoidal system. The subtunical venular plexuses are thus compressed between the trabeculae and the tunica albuginea, resulting in almost total occlusion of venous outflow (*Fournier et al., 1987; Banya et al., 1989*). These events trap the blood within the corpora cavernosa and raise the penis from a dependent position to an erect position, with an intracavernous pressure of approximately 100mm Hg (the phase of full erection).

Nitric oxide released during nonadrenergic, noncholinergic neurotransmission and from the endothelium is probably the principal neurotransmitter mediating penile erection (*Saenz et al., 1989; Ignarro et al., 1990*). Within the muscle, nitric oxide activates a soluble guanylyl cyclase, which raises the intracellular concentration of cyclic guanosine monophosphate (GMP). Cyclic GMP in turn activates a specific protein kinase, which phosphorylates certain proteins and ion channels, resulting in the opening of potassium channels and hyperpolarization of the muscle-cell membrane, sequestration of intracellular calcium by the endoplasmic reticulum, and blocking of calcium influx by the inhibition of calcium channels. The consequence is a drop in cytosolic calcium concentrations and relaxation of the smooth muscle. During the return to the flaccid state, cyclic GMP is hydrolyzed to GMP by phosphodiesterase type 5. Other phosphodiesterases are also found in the corpus cavernosum, but they do not appear to have an important role in erection. Communication among smooth-muscle cells takes place through gap junctions in the membranes of adjacent cells, which allow the passage of ions and second messengers to synchronize muscle activity (*Christ et al., 1993*).

After released from nerve endings and vascular endothelial cells, NO diffuses to neighboring vascular and trabecular smooth muscle cells and binds to guanylate cyclase. This induces a conformational change in the enzyme and subsequent catalytic

production of 3',5'-cyclic guanosine monphosphate (cGMP) from guanosine 5'-triphosphate.

The intracellular cGMP and cAMP levels are finely tuned by a family of enzymes termed phosphodiesterases (PDEs) (*Beavo et al., 1994*) This family of enzymes is composed of at least nine different gene groups, that differ for substrate specificity, sensitivity to inhibitors and organ localization.

1.5.2 PDE5 inhibitor

PDE5 is abundant in the corpus cavernosum and is the predominant PDEs in this tissue (*Gopal et al., 2001; Boolell et al., 1996*). Although other PDEs are present in the corpus cavernosum (*Beavo, 1995; Ballard et al., 1998*), they do not appear to significantly modulate changes in cGMP levels associated with the ability to achieve penile erection (*Boolell et al., 1996*). Immunohistochemical studies have demonstrated the presence of PDE5 in vascular and bronchial smooth muscle and in platelets, whereas negligible amounts of PDE5 have been detected in myocardial contractile cells and cardiac conducting tissue, although this remains under some debate (*Beavo, 1995; Wallis et al., 1999; Sugiyama et al., 2001; Stief et al., 2000; Senzaki et al., 2001*).

PDE5 inhibitors are structurally similar to cGMP and compete with cGMP at the catalytic site of PDE5 (*Corbin & Francis, 1999*). Elevation of cGMP by PDE5 inhibitors occurs secondary to reduced cGMP degradation by PDE5 when cGMP synthesis is concomitantly increased. This is the rationale for pharmacologic inhibition of PDE5 as a therapeutic approach for inducing penile erection in men with ED.

Chapter Two

Mechanical Properties of Polymeric Films

2.1 Introduction

The use of polymeric films for buccal drug delivery as a novel and exciting dosage form, has attracted more and more researchers' attention and interest (*Giannola et al., 2008; Harris & Robinson, 1992*). Drug loaded mucoadhesive films can be produced by simply using bioadhesive and biodegradable polymers. Kurosaki (*Kurosaki et. al., 1988*) reported the application of a polymeric film made of hydroxypropyl cellulose to deliver propanolol. A mucoadhesive film can be designed either for systemic or oral cavity drug delivery, and the release direction can be unidirectional to the buccal mucosa or bidirectional. In this our research, pullulan was used as the polymer and Yonkenafil as the model drug, for systemic delivery to treat men's erectile dysfunction. This chapter discusses the relationship between the film composition and the mechanical properties of the polymeric films for a range of formulations.

2.1.1 Polymers for film matrix

Polymers selected in this study have to meet certain criteria, such as possessing film forming ability, bioadhesive, biodegradable, water soluble, and non irritant to the buccal mucosa. Pullulan and HPMC (6 cps) were chosen as our investigated objects.

2.1.1.1 Pullulan

Pullulan is a neutral linear polysaccharide composed of (1→6)-linked α-D-maltotriose residues and is synthesized from starch or sugar by *Aureobasidium pullulans* (*Wallenfells, K., 1961*). The white pullulan power is odorless, flavorless, and highly stable, soluble in water to form a clear and viscous solution. Edible films made from pullulan are transparent, tasteless, resistant to oil and heat-sealable. The α(1→6) linkages that interconnect the maltotriose units are responsible for the irregularly ordered chains and for the resulting amorphous character of this polysaccharide. At present, pullulan can be used as to make films or granules for tabletting, pan coating, agglomeration, sauces and dressings, innovative soft candy, coatings, and capsules (*HEO et al., 2003*).

2.1.1.2 Hydroxypropyl methylcellulose (HPMC)

Hydroxypropyl methylcellulose (HPMC) is a semi-synthetic, inert, viscoelastic polymer used as an ophthalmic lubricant, as well as an excipient and controlled-delivery component in oral medicaments found in a variety of commercial products (*de Silva et al. 2005; William et al., 2001).* It is an non-ionic ingredient of a water-soluble cellulose ether derivative (cellulose hydroxypropyl methyl ether) for use in controlled-release preparations. It is available in grades containing 16.5– 30% of methoxy and 4.0–32.0% of hydroxypropoxy groups, with specified viscosities for specific concentrations.

Hypromellose (short for hydroxypropyl methylcellulose) in an aqueous solution, unlike methylcellulose, does not exhibit thermal gelation. That is, when the solution heats up to a critical temperature, the solution congeals into a non-flowable but semi-flexible mass. Typically, this critical (congealing) temperature is inversely related to both the solution concentration of HPMC and the concentration of the methoxy group within the HPMC molecule, which in turn depends on both the degree of substitution

of the methoxy group and the molar substitution; the higher the concentration of the methoxy group, the lower the critical temperature. The inflexibility/viscosity of the resulting mass, however, is directly related to the concentration of the methoxy group. The higher the concentration, the more viscous or less flexible the resulting mass (*Der Marderosian, 1990*).

2.1.2 Plasticiser

Plasticisers are additives used to increase the flexibility or plasticity of polymers, and occasionally they are used to facilitate polymer processing (*Lourdin et al., 1997*). Several theories have been proposed to explain the mechanisms of plasticization actions (*di Gioia et al., 1999*).

- The lubrication theory postulates that plasticisers, by interspersing themselves, act as internal lubricants by reducing frictional forces between polymer chains.
- The gel theory postulates that the rigidity of polymers comes from three-dimensional structures, and plasticisers take effect by breaking polymer-polymer interactions (e.g., hydrogen bonds and van der Waals or ionic forces)
- The free volume theory describes plasticization as increasing free volume and is useful in explaining the lowering of the glass transition temperature (T_g), by a plasticiser (*Nugraha et al., 2005*).

2.1.3 Polymeric films for drug delivery

Physiologically acceptable films, including edible films, which can be used as carrier for oral drug delivery, are an interesting dosage form. A fast-dissolving polymer film embedded with drug swells and dissolves in the saliva of the oral cavity quickly and completely, releasing the drug for absorption through the oral mucosa. A fraction of the drug will be swallowed with the saliva and absorbed along the length of the GI

tract. An ideal buccal film should be flexible, uniform, and strong enough to withstand breakage caused by production (cutting, package, etc.) and usage (holding with hands, mouth activities, etc.). Moreover, it should possess moderate bioadhesive strength so that it can stick on the epithelium for a desired duration and release drug.

As the pullulan film is rigid and tough, a plasticiser is required to increase film flexibility (Sakata & Otsuka, 2009; Diab et al., 2001). Water, oligosaccharides, polyols, and lipids are different types of plasticisers widely used in hydrocolloid-based films (*Nugraha. et al., 2005*). Because pullulan is a water-soluble polysaccharide, the films were made by using a solvent evaporation method, and a series of polyols were added as plasticisers.

2.1.4 Mechanical strength study

2.1.4.1 Tensile Strength (TS)

Tensile strength measures the force (F) required to pull something such as rope, wire, or a structural beam to the point where it breaks, in units of force per unit area (A) Specifically, the tensile strength of a material is the maximum amount of tensile stress that it can be subjected to before failure (*Mashru, et al., 2005*).

Tensile strength = F/A (Eq. 2.1)

Stress is denoted by Greek letter σ, as the force normalized by the cross-sectional area of the material:

σ = F/A (Eq. 2.2)

2.1.4.2 Elongation

Elongation measured the change in length (L) of the sample undergoing stress.

$$\textbf{Elongation} = (L/L_0) \times 100\% \quad \textbf{(Eq. 2.3)}$$

L: the extension of length after stretching

L_0: the original length of the sample

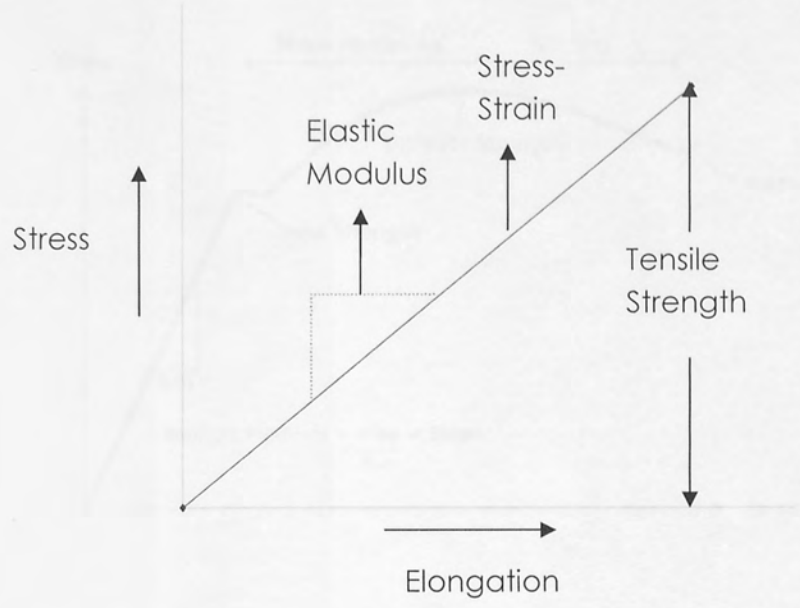
Like tensile strength and stress, elongation and strain have the same formula and definition.

$$\textbf{Strain} = (L/L_0) \times 100\% \quad \textbf{(Eq. 2.4)}$$

2.1.4.3 Elastic Modulus (EM)

An elastic modulus, or modulus of elasticity, is the mathematical description of an object or substance's tendency to be deformed when a force is applied to it. The elastic modulus of an object is defined as the slope of its stress-strain curve.

Figure 2.1 The definition of TS, Elongation and EM (*Mashru, et al., 2005*).



When the polymeric films are plastic enough and plastic deformation will happen after the elastic deformation (see Figure 2.2.).

Figure 2.2 Definition of elastic deformation and plastic deformation (*http://en.wikipedia.org/wiki/Elastic_deformation*)

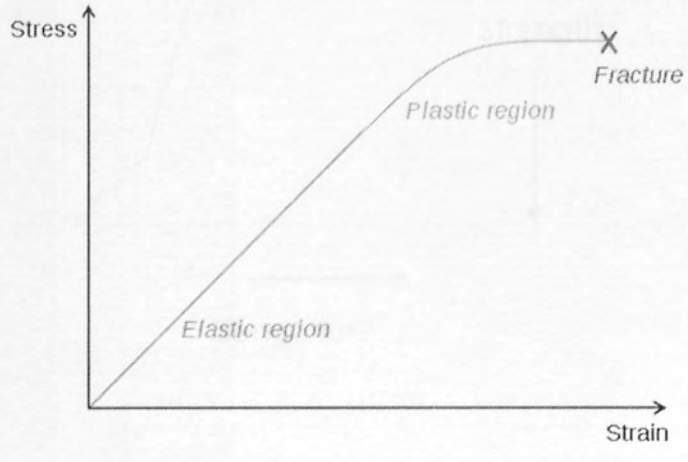


Figure 2.3 Various stages of deformation
(*http://en.wikipedia.org/wiki/Elastic_deformation*)

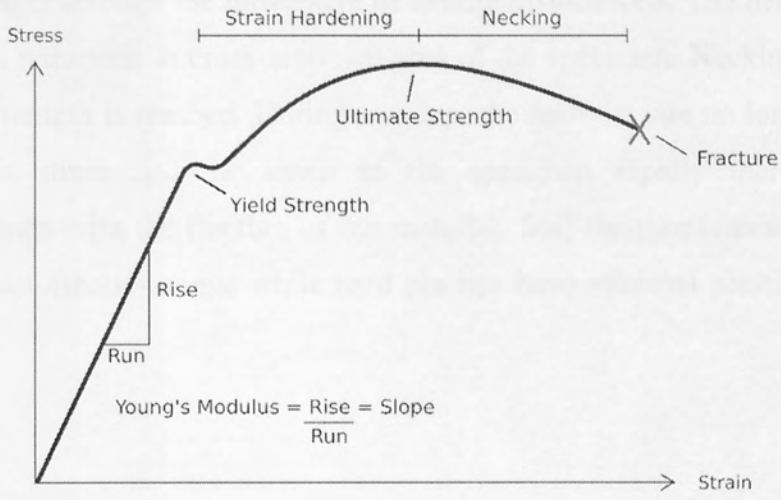
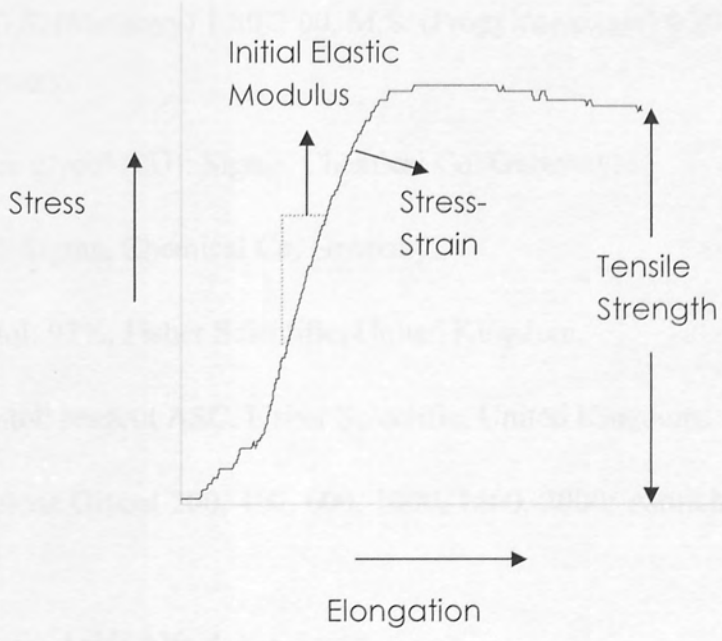


Figure 2.4 Definition of TS, Elongation and EM in plastic deformation (*Mashru, et al., 2005*).



Elastic deformation is reversible. The material returns to its original shape once the applied force is diminished. Plastic deformation is not reversible. Under tensile stress, plastic deformation is characterized by a strain hardening region and a necking region and finally, fracture (also called rupture). During strain hardening the material becomes stronger through the movement of atomic dislocations. The necking phase is indicated by a reduction in cross-sectional area of the specimen. Necking begins after the ultimate strength is reached. During necking, the material can no longer withstand the maximum stress and the strain in the specimen rapidly increases. Plastic deformation ends with the fracture of the material. Soft thermoplastics have a rather large plastic deformation range while hard plastics have minimal plastic deformation range.

2.2 Materials

Pullulan: Hayashibara Biochemical Laboratory Inc. Japan.

Hydroxypropyl methylcellulose (HPMC): viscosity (2wt% in H_2O, 20℃) 6cps, Mn: 10000, D.S. (Methoxy) 1.80-2.00, M.S. (Propylene oxide) 0.20-0.30, Sigma, Chemical Co, Germany.

Propylene glycol (PG): Sigma, Chemical Co, Germany.

Glycerol: Sigma, Chemical Co, Germany.

D-Sorbitol: 97%, Fisher Scientific, United Kingdom.

D-Mannitol: reagent ASC, Fisher Scientific, United Kingdom.

Polyethylene Glycol 200, 400, 600, 1000, 1500, 2000: Aldrich Organics, New Jersey, USA

DL-Tartaric Acid: Alfa Aesar, Lancs

DL-Malic Acid: Alfa Aesar, Lancs

DL-Lactic Acid: Aldrich Organics, New Jersey, USA.

Citric Acid: Fiaons pls., Loughborough, England.

Yonkenafil: a gift from Tasly Group, China.

2.3 Methods

2.3.1 Preparation of drug-free polymeric films

2.3.1.1 Drug-free Pullulan films

Drug-free pullulan films were prepared using a solvent evaporation method. Plasticiser was dissolved in 9 ml distilled water, then stirred for 30 minutes and heated to 40 °C. Different ratios of pullulan were slowly added into the solution and stirring continued for one hour to ensure dissolution. The solution was cooled to room temperature and stirred again for 30 minutes after 1 ml ethanol was added. The solution was left to stand overnight to remove all the entrapped bubbles, poured into an aluminum petri dish and dried at room temperature for 2 days.

2.3.1.2 Drug-free HMPC films

Drug-free HMPC films were prepared using a solvent evaporation method. Plasticiser was dissolved in 9 ml distilled water, then stirred and heated to 80 °C. Different ratios of HPMC were slowly added into the solution to make all the polymer molecules dispersed (1 to 2 min). The dispersion was stirred in an ice bath for one hour to dissolve the HPMC. The solution was continually stirred at room temperature for 30 minutes after 1 ml ethanol was added. The solution was left to stand overnight to

remove all the entrapped bubbles, poured into an aluminum petri dish and dried at room temperature for 2 days.

2.3.1.3 Pullulan film containing Yonkenafil

Drug contained films were prepared by using a solvent evaporation method. Drug and water-soluble additives (propylene glycol, glycerin, acids etc.) were dissolved in 9 ml distilled water, then stirred for 30 minutes and heated to 40 °C. Different ratios of pullulan were slowly added into the solution when all the drug and additives dissolved and stirring continued for 1 hour. The solution was cooled to room temperature and stirred again for 30 minutes after 1 ml ethanol was added. The solution was left to stand overnight to remove all the entrapped bubbles, poured into a aluminum Petri dish and dried at room temperature for 2 days.

2.3.2 Tensile testing

Tensile tests were conducted at room temperature with a Hounsfield machine. All the films were stored in a desiccator with a saturated sodium nitrite solution (25°C, RH 60±5%) for two days before the test. Film strips were cut (60×10mm) and were visually free of air bubbles or physical imperfections. They were held between two clamps positioned at a distance of 3.5 cm. During the measurement, the strips were pulled by the top clamp at a speed of 4 mm/min until the strips were broken. The pulling force of the whole procedure was recorded by the computer with the interval of the clamp moving 0.001mm. Each measurement was conducted five times and three (excluding the maximum and minimum) were used for analysis.

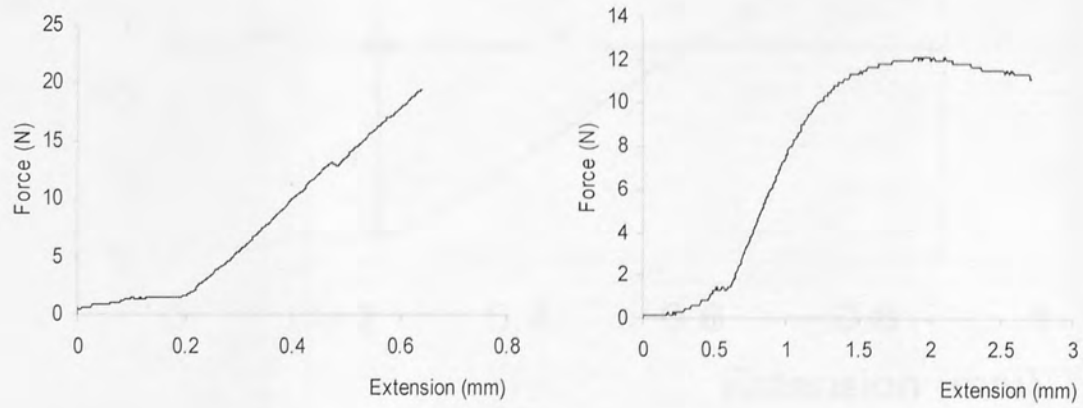
The tensile testing indicated the films' strength and elasticity by three parameters, tensile strength, elongation at break, and elastic modulus (see Figures 2.1 and 2.4).

2.4 Results and discussion

2.4.1 Data analysis

Before discussing the results, an interpretation of the relationship between the mechanical properties and the parameters is necessary. Tensile testing of the pullulan films always resulted in one of two curve-types (see Figure 2.5)

Figure 2.5 Examples of force-extension curves output by the computer connected to the Hounsfield machine



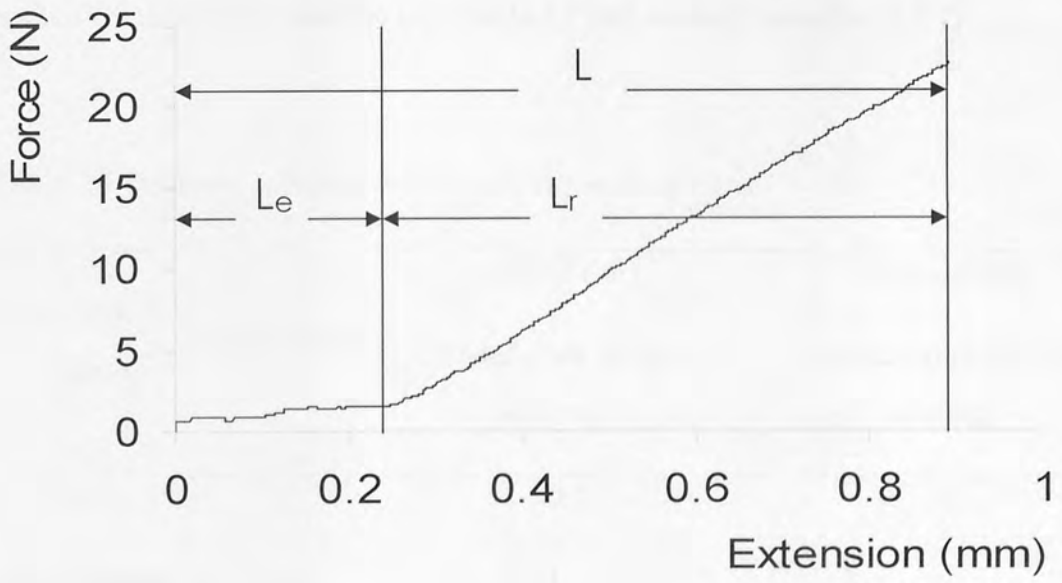
A B

In curve A (Figure 2.5-A), the films have only undergone elastic deformation, so there is a linear relationship between extension and the force, whereas in curve B (Figure 2.5-B), the force increases with the extension at early stages then plastic deformation occurs after elastic deformation. Thus, in situation A, EM = TS / Elongation, and the properties of the films can be discussed only by Elongation and EM, because TS is fixed when EM and Elongation are confirmed, while in situation B, we cannot describe

the film characteristics by only two parameters because EM is not equal to TS/Elongation. Therefore, a ductile and tough film should be characterized by low EM and high Elongation, in contrast, a rigid and brittle film is defined by high EM and low Elongation.

After each film strip is broken, the software outputs the recorded data to the computer which can be transferred to Excel. A typical result is shown in Figure 2.6.

Figure 2.6 Force-extension curve showing modifications required due to clamping procedure



Due to variability in the clamping procedure, extension does not begin at the same time for each film. The total length of extension (L) on the output curve must be sub-divided into two sections, Le, the extra extension and Lr, the real extension. However, the distance shown as Le, is still recognized as the film extension by the apparatus. As a result, the real elongation should be modified by excluding the Le. The original distance between the two clamps is 35 mm, thus, the calculation for the real elongation should be as the following equation.

Real Elongation = ((L - Le) / (35 + Le)) × 100% (Eq. 2.5)

2.4.2 Drug-free polymeric films

2.4.2.1 Effect of solvents on the mechanical properties of the drug free polymeric films

- *Pullulan*

To study the effects of the solvents on the mechanical properties of the pullulan films, water, water and ethanol, and water and acetone were used as the solvent system to make the pullulan gel solution (see Table 2.1 and method in section 2.3.1).

Table 2.1 Different solvent systems used for making films

Solvent systems	Water (ml)	Ethanol (ml) (Added after 30 min stirring)	Acetone (ml) (Added after 30 min stirring)
Water	10	0	0
10% Ethanol	9	1	0
20% Ethanol	8	2	0
10% Acetone	9	0	1
20% Acetone	8	0	2

Figure 2.7 The tensile strength (N/mm^2) of pullulan films made by five solvent systems (mean±s.d., n=3)

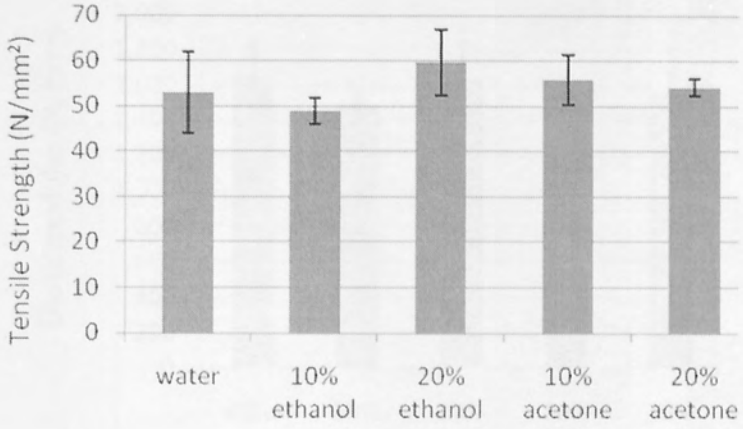


Figure 2.8 The elongation of pullulan films made by five solvent systems (mean±s.d., n=3)

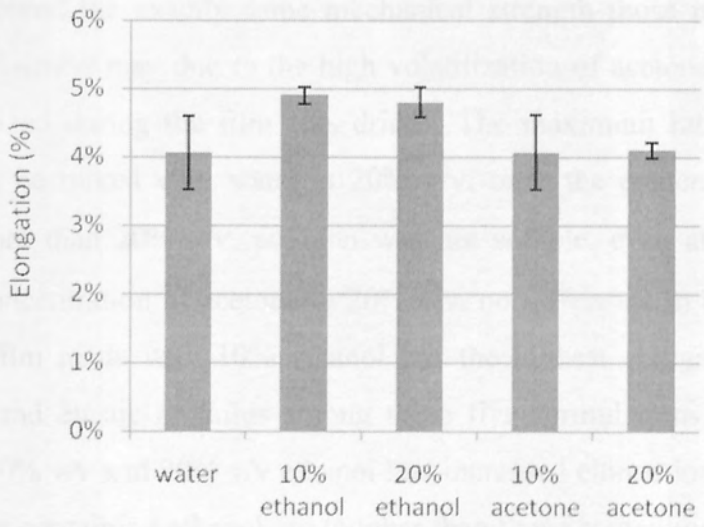
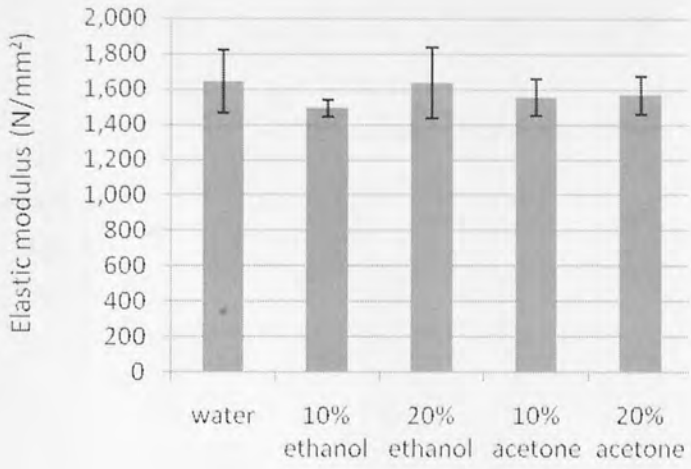
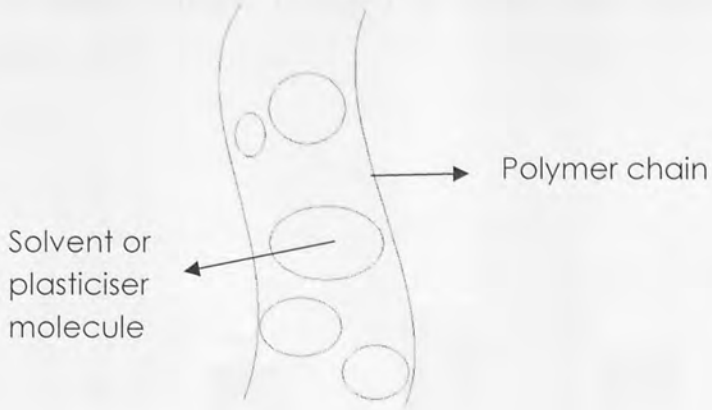


Figure 2.9 The elastic modulus (N/mm^2) of pullulan films made by five solvent systems (mean±s.d., n=3)



From figures 2.7, 2.8, and 2.9, there is no significant effect of acetone on the mechanical properties of the drug-free pullulan films (all $P>0.05$). The films made with acetone showed the exactly same mechanical strength those made with water only. This phenomenon may due to the high volatilization of acetone, most of which might had volatized during the film was dried. The maximum ratio of ethanol or acetone that can be mixed with water is 20% v/v; once the content of the organic solvents was more than 20% v/v, pullulan was not soluble, even at 40℃. Even on increasing the concentration of acetone to 20% v/v, no difference in the film strength was seen. The film made with 10% ethanol has the highest elongation and lowest tensile strength and elastic modulus among these five formulations. Both the films prepared using 10% v/v and 20% v/v ethanol had increased elongation, indicating that the pullulan films containing ethanol are tougher than those containing water only and or water and acetone. This may be due to the ability of ethanol to improve linkage strength of the polymer chains through hydrogen bonds. It is proposed that the ethanol molecules can exist in the free space among the polymer chains, shown in fig 2.10.

Figure 2.10 The arrangement of molecules in the free space between the polymer chains



According to the lubrication and free volume theories (see section 2.1.2), the ethanol molecules could act as a lubricant to reduce frictional forces between polymer chains; therefore, the tensile strength and elastic modulus would reduce, which was observed in 90% water - 10% ethanol. However, when the concentration was increased to 20%, the tensile strength (10%: 48.91±2.88, 20%: 59.69±7.20 N/mm^2) and elastic modulus (10%: 1495.7±47.1, 20% 1637.0±197.1 N/mm^2) increased as well and elongation had a slight decrease (10%: 4.90±0.12, 20%: 4.80±0.23 %) . This result can be explained by the free volume theory: the free space among the polymer chains was occupied by more ethanol molecules so that the chains were no longer freely moving. Additionally, water also played an important role on the plasticization of the films. The higher concentration of ethanol can reduce the drying time and may affect the structure of films (*Bajdik et al., 2005; Kawahara et al., 2003*). The concentration of the residual ethanol and the ethanol - polymer interactions (e.g. hydrogen bonds) have to be studied to prove the effect of organic solvents in the plasticization of the polymeric films in future work. From the present results, 90% water + 10% ethanol should be an optimized solvent system for making the pullulan films.

- *HPMC*

Figure 2.11 The tensile strength (N/mm^2) of HPMC films made by three solvent systems (mean±s.d., n=3)

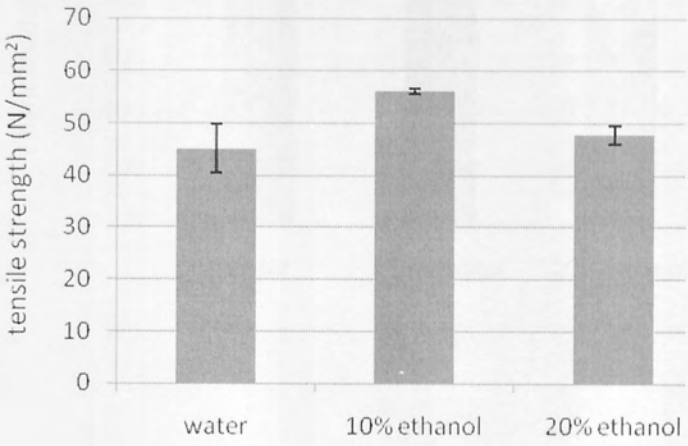


Figure 2.12 The elongation of HPMC films made by three solvent systems (mean±s.d., n=3)

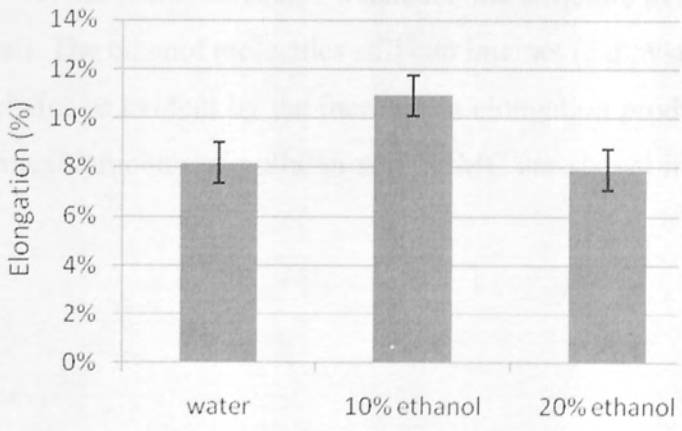
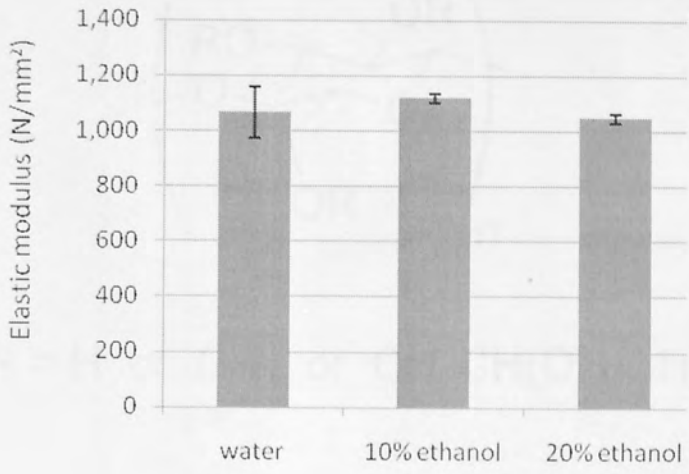
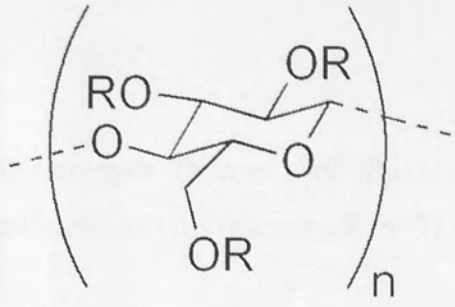


Figure 2.13 The elastic modulus (N/mm^2) of HPMC films made by three solvent systems (mean±s.d., n=3)



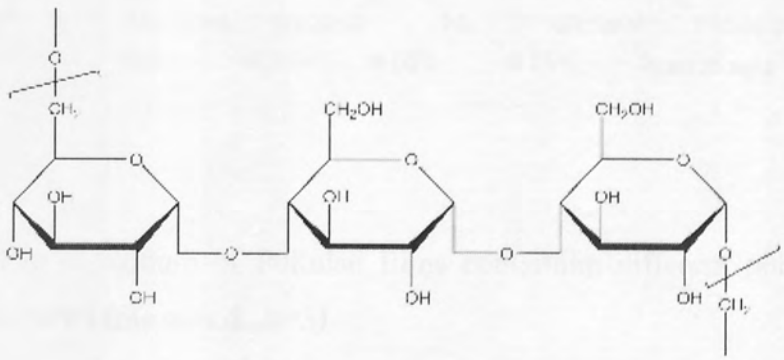
Water, water-ethanol solvent systems were used to make drug free HPMC films. The result shows that 10% ethanol had an effect on the films' elongation, but had no effect on elastic modulus, even increased the tensile strength. This result shows that ethanol did not act as a lubricant in the HPMC films because it did not reduce frictional forces between polymer chains (same value for elastic modulus). When the concentration increased to 20% v/v, the extra molecules weakened the structure of films (resulting in reduced elongation). The ethanol molecules still can interact (e.g., via hydrogen bonds) with the HPMC chains as evident by the increase in elongation produced by 10 % v/v ethanol. The chemical structure of pullulan and HPMC are shown in figures 2.14 and 2.15.

Figure 2.14 Chemical structure of HMPC



$R = H \text{ or } CH_3 \text{ or } CH_2CH(OH)CH_3$

Figure 2.15 Chemical structure of pullulan



The more free hydroxyl groups in pullulan may be the reason for explaining why ethanol could act as a lubricant in the pullulan films but not in the HPMC ones because is it easier for ethanol to form hydrogen bonds with the pullulan molecules rather than HPMC molecules. For acetone, the weaker interactions and high volatilization drove the acetone molecules out of the polymer solution and results were no different from water.

2.4.2.2 Plasticization of polyols

- *Pullulan*

Figure 2.16 The tensile strength (N/mm^2) of Pullulan films containing three concentrations (w/w) of different polyols (mean±s.d., n=3)

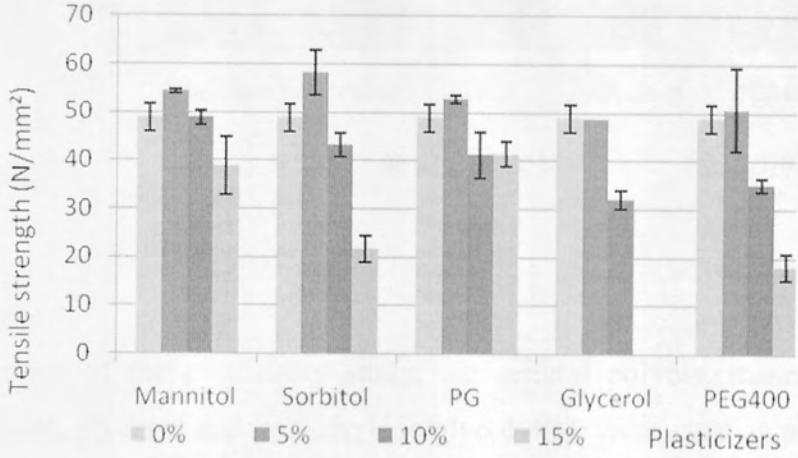


Figure 2.17 The elongation of Pullulan films containing different polyols in three concentrations (w/w) (mean±s.d., n=3)

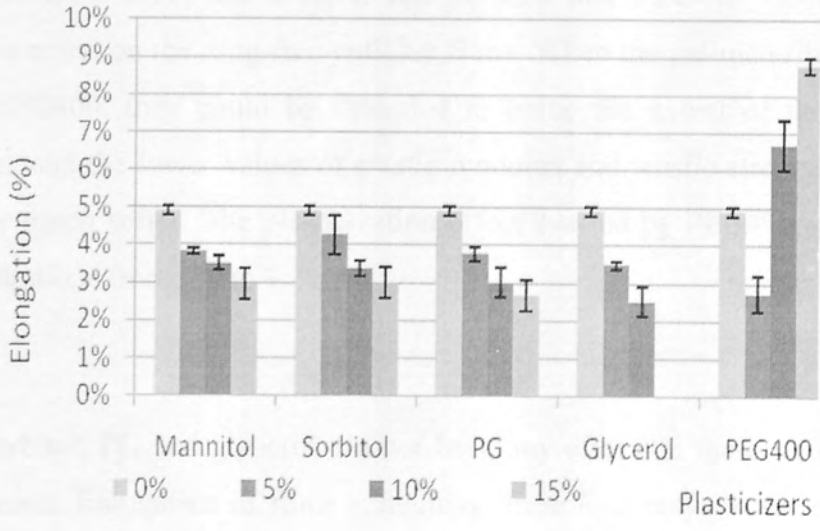
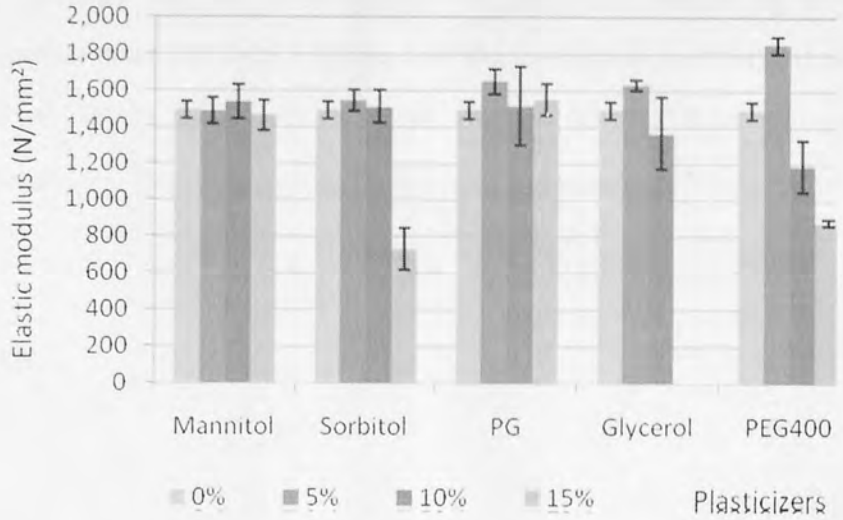


Figure 2.18 The elastic modulus (N/mm^2) of Pullulan films containing different polyols in three concentrations (w/w) (mean±s.d., n=3)



At the beginning of the plasticisers study, the general polyols, mannitol, sorbitol, propylene glycol, glycerol and polyethylene glycol 400, were used as plasticisers for the polymeric films. The concentrations (w/w) of these plasticisers in the formulation ranged from 5% to 15%. The pullulan film containing 15% glycerol was too hygroscopic to be handled and is not included in the results.

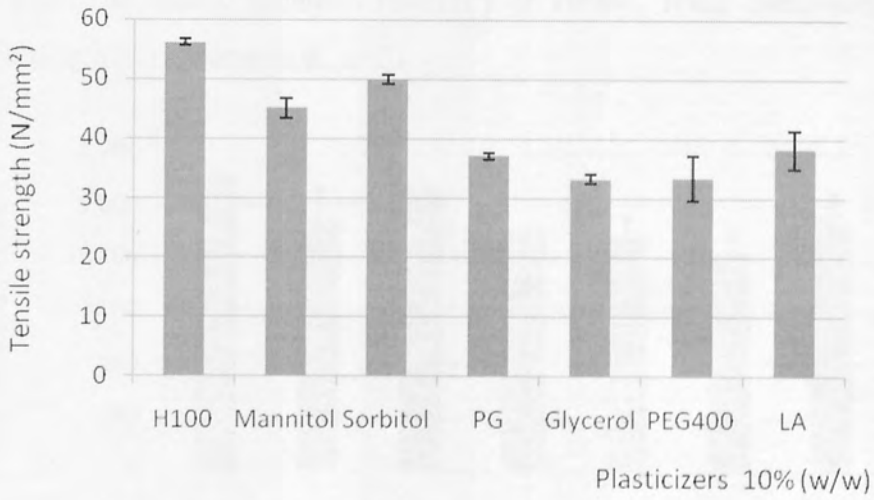
From Figures 2.16, 2.17, and 2.18, it can be seen that PEG400 has a significant plasticization effect on the drug-free pullulan films. When the pullulan films contained 15% w/w PEG400, they could be extended to twice the extent of the rest of the formulations, and the lower values of elastic modulus and tensile strength reflect that the films are much softer. The plasticization effect exerted by PEG400 was intensive over concentrations from 5% to 15%.

Mannitol, sorbitol, PG and glycerol did not have any effect on the drug-free pullulan films' extension. Elongation of films containing these four polyols was reduced with increasing concentrations and this unconventional behavior is called

"antiplasticization". This effect was seen in chitosan-PG (*Nugraha., et al., 2005*), starch-glycerol, and starch-sorbitol systems (*Lourdin ., et al. 1997*). The mechanism of antiplasticization is not yet clearly understood. A strong interaction might be occurring between the polymer and the small quantity of plasticiser, producing a "cross-link" effect, which decreases the free volume and the molecular mobility of the polymer (*Lourdin, et al. 1997*). Moreover, a small amount of plasticiser can increase the polymer crystallinity, thus decreasing the strain of the material (*Nugraha, et al., 2005*).

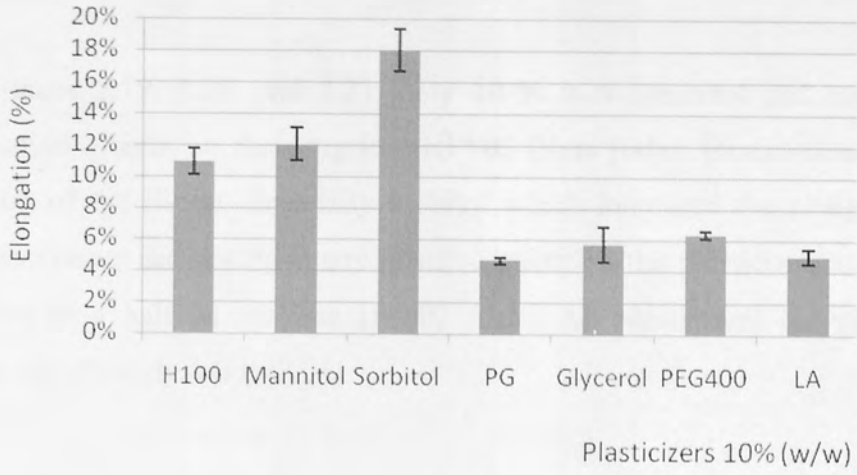
- *HPMC*

Figure 2.19 The tensile strength (N/mm^2) of HPMC films containing 10% (w/w) different plasticisers (mean±s.d., n=3)



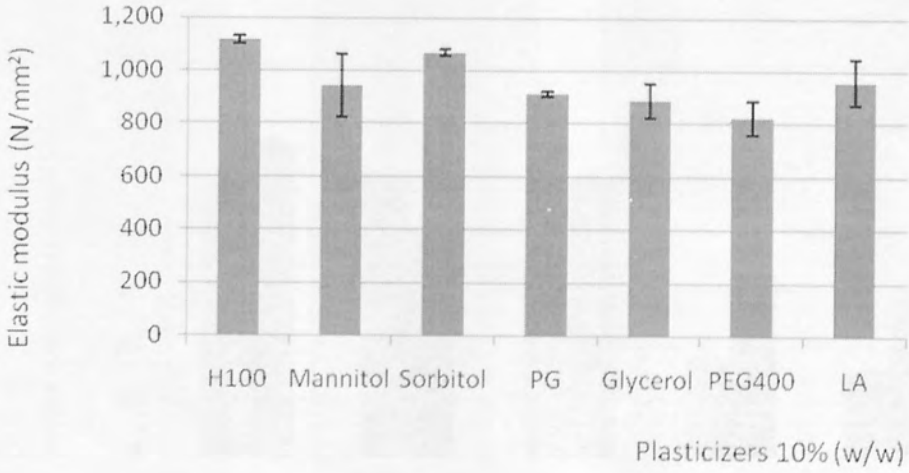
H100: pure drug-free HPMC film

Figure 2.20 The elongation of HPMC films containing 10% (w/w) different plasticisers (mean±s.d., n=3)



H100: pure drug-free HPMC film

Figure 2.21 The elastic modulus (N/mm^2) of HPMC films containing 10% (w/w) different plasticisers (mean±s.d., n=3)



H100: pure drug-free HPMC film

To compare the plasticization effect of the polyols on the pullulan and HPMC films, 10% (w/w) polyols or LA (lactic acid) were added into the films. When lactic acid was added to the pullulan films, the films were too brittle to be peeled off the petri dish;

while the HPMC films containing 10% LA were flexible and tough enough to be peeled off and handled for the tensile experiments.

From Figures 2.19, 2.20, and 2.21, only 10 % w/w mannitol and sorbitol showed plasticization effects on the drug-free HPMC films (other formulations reduced the elongation of the films), especially sorbitol which increased the elongation by 7%. Other plasticisers did not have any positive effect on the plasticization, reducing the elongation to a half as the free HPMC films. All plasticisers reduced the elastic modulus significantly (all $P<0.05$).

2.4.2.3 PEG400 in Pullulan and Sorbitol in HPMC

Figure 2.22 The tensile strength (N/mm^2) of pullulan films containing variable percentages of PEG 400 and HPMC films containing variable percentages of sorbitol (mean±s.d., n=3)

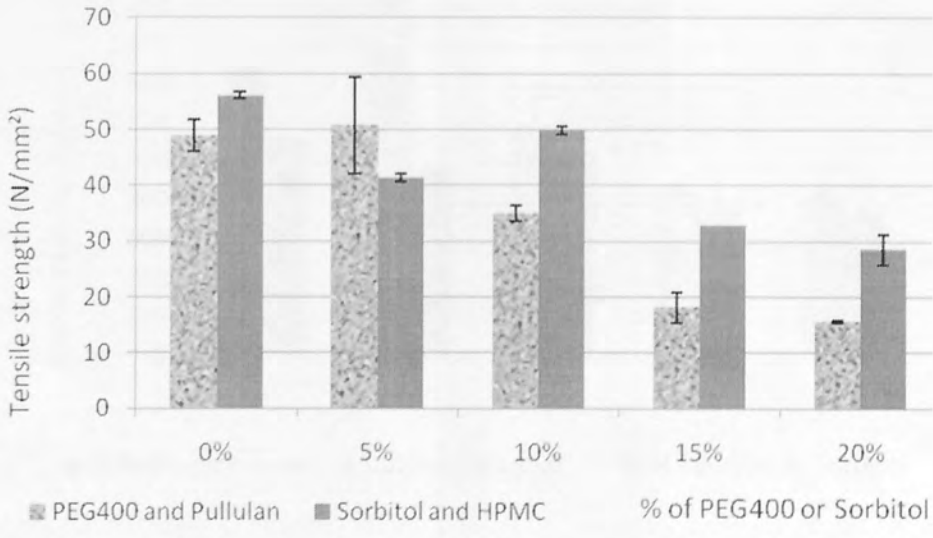


Figure 2.23 The elongation (%) of pullulan films containing variable percentage of PEG 400 and HPMC films containing variable percentage of sorbitol (mean±s.d., n=3)

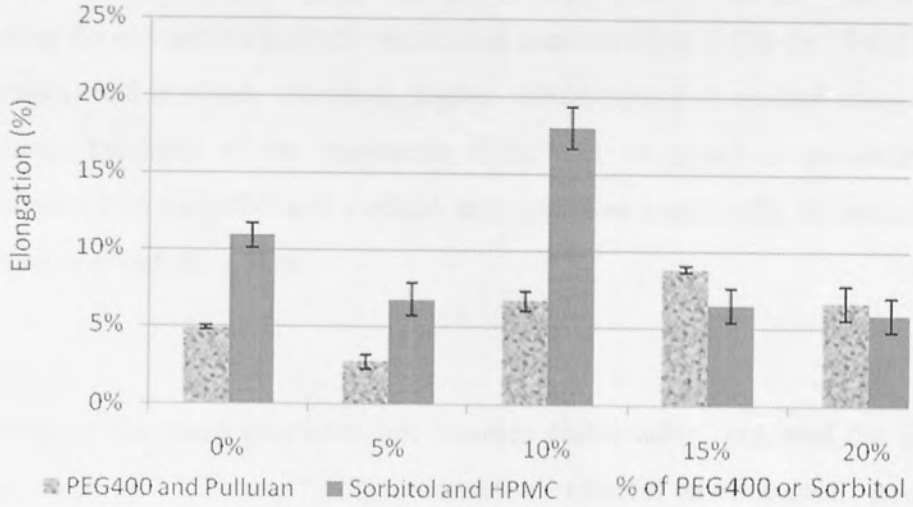
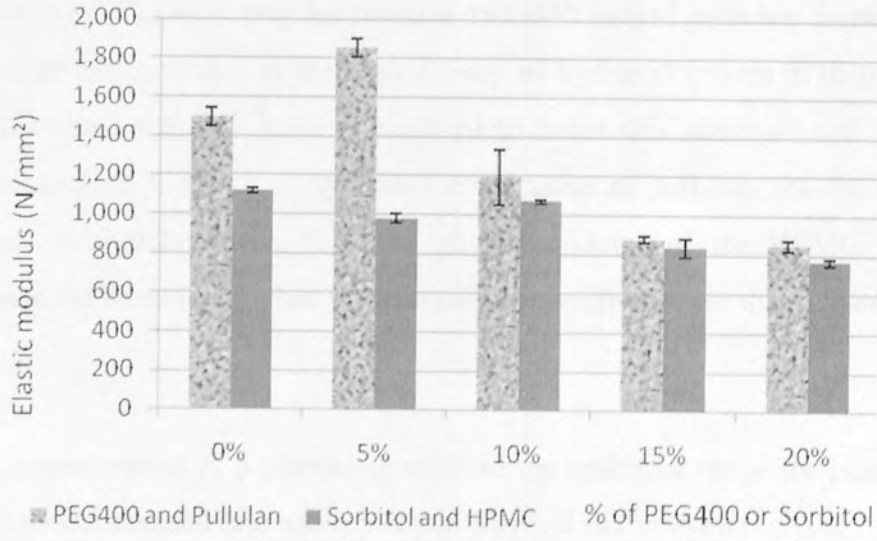


Figure 2.24 The elastic modulus (N/mm^2) of pullulan films containing variable percentage of PEG 400 and HPMC films containing variable percentage of sorbitol (mean±s.d., n=3)



By gradually introducing PEG400 or sorbitol, at concentrations from 0% to 20% (w/w), into the pullulan or HPMC films, I expected to reveal a quantitative relationship between concentrations of plasticisers and their plasticization effect on the films. From

Figures 2.22, 2.23, and 2.24, the greatest elongations in pullulan and HPMC films were found at 15% w/w PEG400 and 10% w/w sorbitol respectively. Both PEG400 and sorbitol reduced the elongation at low concentrations of 5%, followed by increasing the elongation until the optimized concentration (15% for PEG400 and 10% for sorbitol) had reached, after that, higher concentration decreased elongation again. The elastic modulus of the polymeric films was increased or maintained by low concentrations of PEG400 and sorbitol and then was continually decreased while the plasticiser concentration rose.

According to the three plasticization theories (lubrication, gel, and the free volume theories, section 2.1.2) and "antiplasticization" effects, when a small amount of the plasticiser was added into the films, these small molecules interrupted the polymer – polymer network and weakened the interaction of the polymer chains, thus, the elongation of the films reduced when the films contained a small quantity of plasticiser. Meanwhile, the elastic modulus and tensile strength were not affected by the polymer – polymer affinity anymore, and the plasticiser – polymer, and plasticiser – plasticiser interactions all affected film rigidity. The higher elastic modulus at low concentrations of PEG400 may be because PEG400 helped pullulan form a tougher structure as an adhesive due to the high density of hydroxyl groups in their structure. In such situations, a higher force is required to break this structure and extend the films. Because HPMC is not as hydrophilic and polar as pullulan, the the affinity of HPMC and sorbitol is lower, thus, the elastic modulus of the HPMC films was maintained at the same level when sorbitol concentration was less than 15% (w/w).

When the concentration of a plasticiser reached the optimum range for plasticization, the plasticisers increased the movement ability of the polymer chains, no matter whether they acted as a lubricant or increase the free space volume among the polymer chains. The most discriminable mechanical property of the films which were extensively plasticized was that they showed plastic deformation when they were extended to a certain extent (see Figures 2.3 and 2.4). At that moment, the three-dimensional structure of polymer network was the major factor affecting the elastic modulus of the films, rather than the affinity between the plasticiser and polymer,

because the plastic deformation included polymer chain dislocation and reduction in cross-sectional area of the films, and the length of the films can be extended is mainly composed of the length made by plastic deformation.

However, high plasticiser concentrations make the tough structure of the polymer network crack. Once the concentration was beyond the appropriate range, the extra plasticiser molecules can cumulate in the free space among the polymer chains, as a result, removing any polymer – polymer. However, the size of plasticiser molecules are much smaller than the polymer, thus, they are not able to form as strong a linkage to connect the polymer chains as the original network connection, and a potential fracture is created. This may be the reason why the elongation was reduced on incorporation of higher plasticiser concentrations. Additionally, the excessive amounts of plasticiser also reduced the free space volume and interrupted polymer chain movement.

2.4.2.4 PEG series in Pullulan and HPMC

Figure 2.25 The tensile strength (N/mm^2) of drug-free pullulan films containing variable percentage of different PEGs (mean±s.d., n=3)

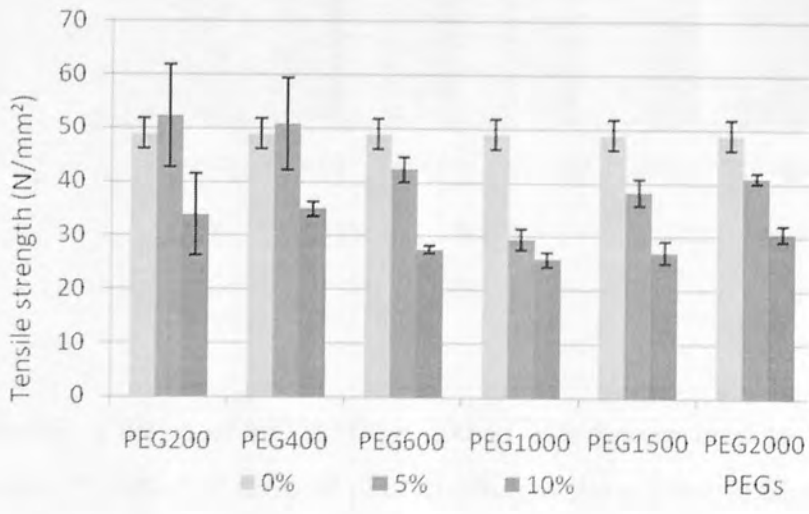


Figure 2.26 The elongation of drug-free pullulan films containing variable percentage of different PEGs (mean±s.d., n=3)

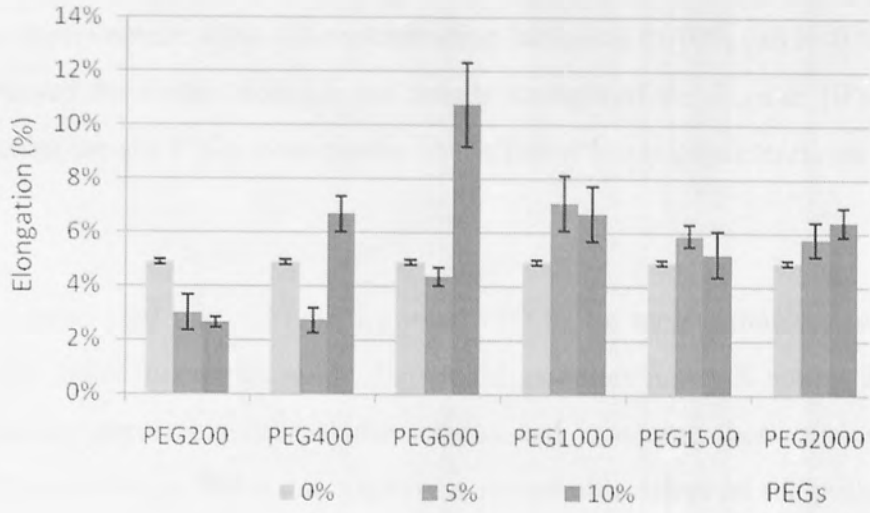
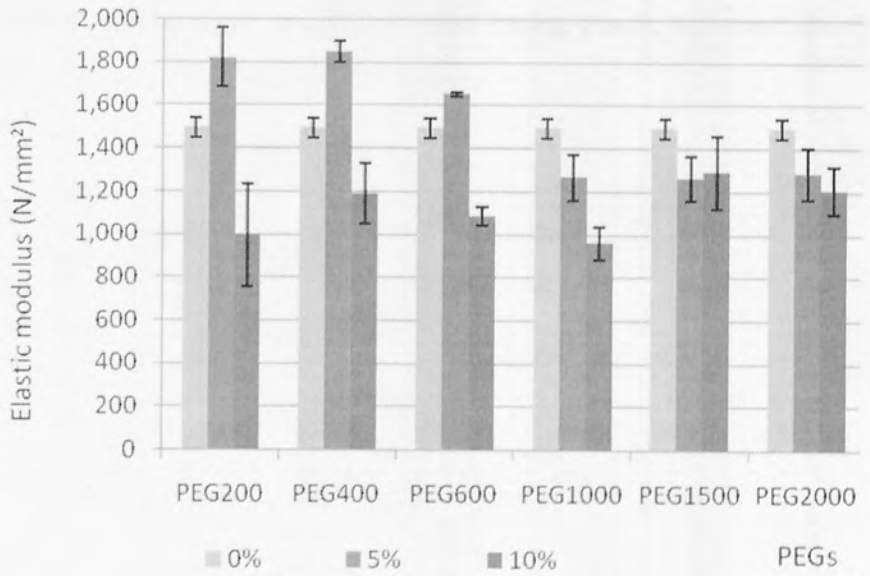


Figure 2.27 The elastic modulus (N/mm^2) of drug-free pullulan films containing variable percentage of different PEGs (mean±s.d., n=3)

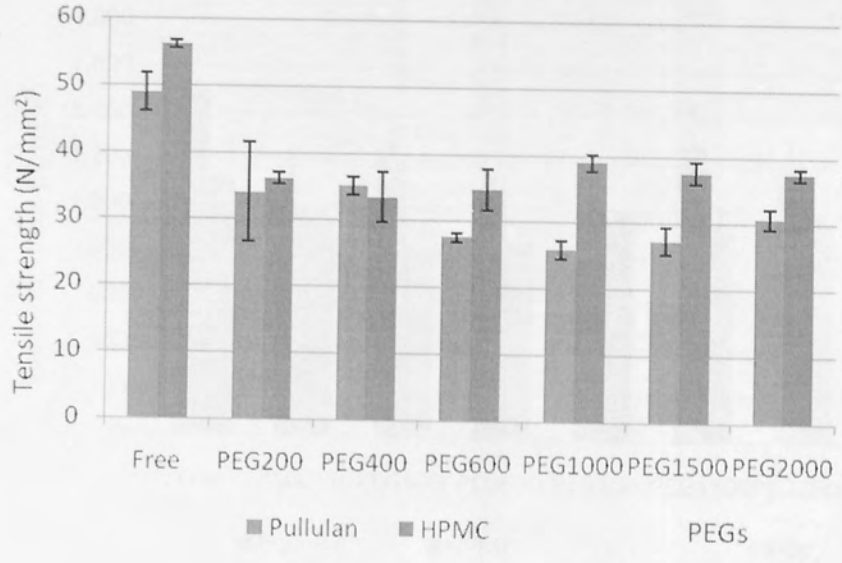


In this experiment, a series of PEGs (from 200 to 2000) were used to study if the molecular weight of plasticiser affected plasticization of the polymeric films. From the results (Figure 2.25, 2.26, and 2.27), PEG 400 and 600 exhibited a superior plasticization effect, reaching the highest elongation at 10% w/w, while weakened at

5% w/w. PEG 200 is the only plasticiser which reduced the elongation at both 5% and 10% (w/w). The higher molecular weight PEGs (1000, 1500 and 2000) increased the elongation even their concentration was 5%, but their plastisization effect did have a significant improvement when the concentration increased to 10% (all $P<0.05$). All the PEGs decreased the elastic modulus and tensile strength of the films at 10% w/w, and high molecular weight PEGs even display this effect at lower concentrations.

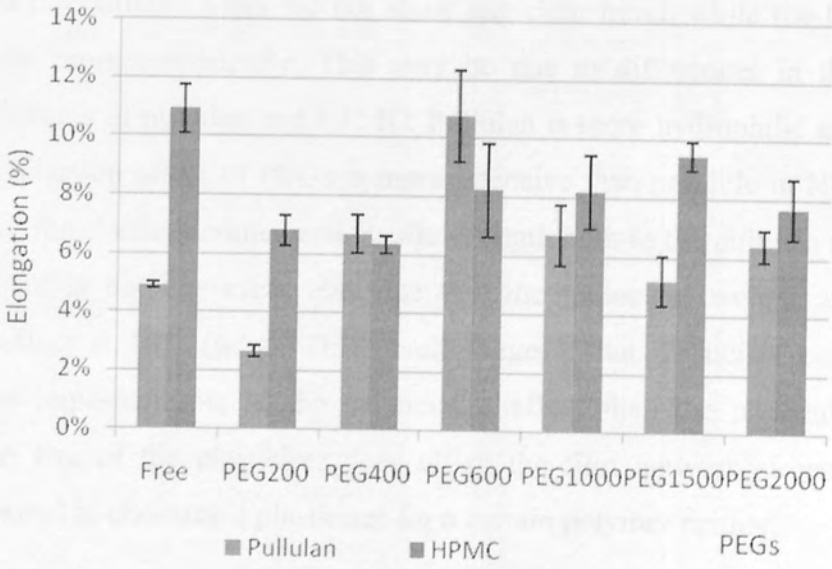
It could be concluded that a PEG of around 600 is the most suitable plasticiser for pullulan. The small molecules might disrupt the polymer network acting like a non-adhesive binder, separating the polymer chains and loosening their connection. The higher molecular weight PEGs did not have a remarkable effect on the pullulan films. A possible explanation is that they existed within the film structure as a polymer rather than a plasticiser. The high molecular weight of PEG 1000, 1500 and 2000 reduced their plasticizing ability, implying the PEGs behave like a relatively small "polymer" rather than a plasticiser, thus, the elongation and elastic modulus are similar to the plasticiser-free pullulan films. The lower tensile strength indicated that these relative small "polymers" can be more easily deformed during plastic deformation compared to pullulan.

Figure 2.28 The tensile strength (N/mm^2) of pullulan and HPMC films containing 10% (w/w) PEGs of different molecular weight (mean±s.d., n=3)



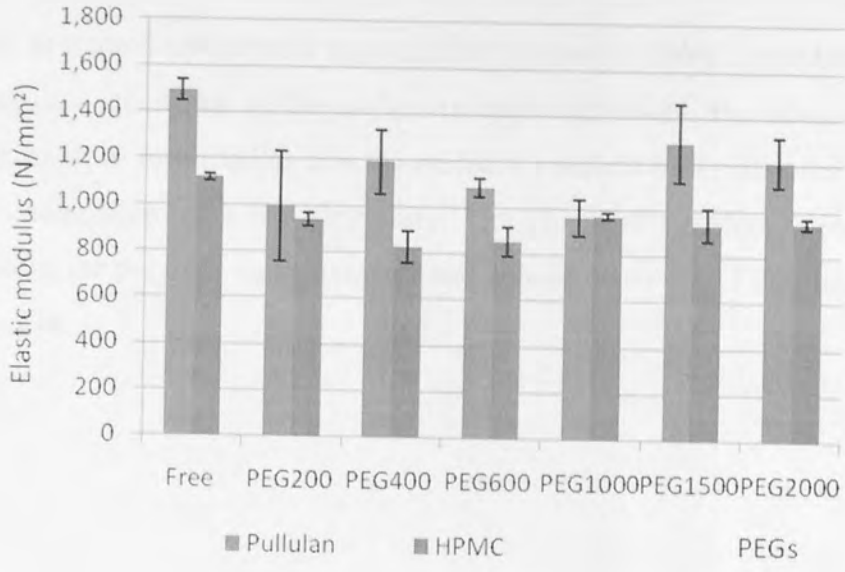
Free: 100% Pullulan or HPMC

Figure 2.29 The elongation of pullulan and HPMC films containing 10% (w/w) PEGs of different molecular weight (mean±s.d., n=3)



Free: 100% Pullulan or HPMC

Figure 2.30 The elastic modulus (N/mm^2) of pullulan and HPMC films containing 10% (w/w) PEGs of different molecular weight (mean±s.d., n=3)



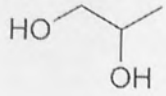
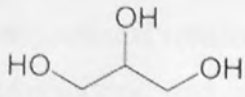
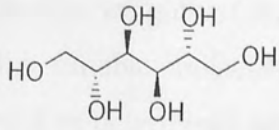
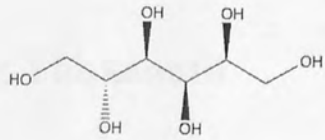
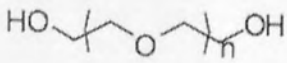
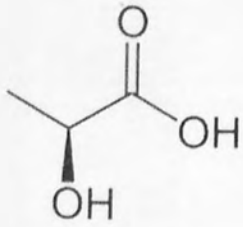
Free: 100% Pullulan or HPMC

I compared effects of the 10% w/w molecular weight PEGs on pullulan and HPMC films and the results are shown in Figure 2.28, 2.29, and 2.30. The changes in elongation of the pullulan films did not show any clear trend; while the HPMC film behaved much more consistently. This may be due to differences in the intrinsic chemical structures of pullulan and HPMC. Pullulan is more hydrophilic and polar so that the plasticization effect of PEGs is more intensive than possible in HPMC. The PEGs reduced the elastic modulus and tensile strength both in the pullulan and HPMC films and there is not any clear evidence that the molecular weight affected the plasticizing effect at 10% (w/w). This result suggests that plasticiser concentration plays a more important role in the plasticizing effect than the molecular weight. However, the size of the plasticiser does affect the film network structure, which cannot be ignored in choosing a plasticiser for a certain polymer neither.

2.4.2.5 Conclusion for the drug-free polymeric films

Based on the presented results with the drug-free polymeric films, it can be concluded that the chemical structures of the polymers and plasticisers, the concentration of plasticiser in the film formulation, and the molecular weight of a plasticiser impact on the film mechanical strength and flexibility. The chemical structures determine the affinity between the polymer and plasticiser and consequently affect the characteristics of the whole film.

Table 2.2 The molecular weight and chemical structure of the polyols and lactic acid

	Molecular Weight	Chemical Structure
Propylene glycol	76.09	
Glycerol	92.09	
Mannitol	182.17	
Sorbitol	182.17	
PEGs	44n+62	
Lactic Acid	90.08	

Lactic acid (the reason why I added an acid into the films is discussed in section 2.4.3.1) is the only agent which has a carboxyl group, and this may be the reason why the drug-free pullulan films containing lactic acid cannot be removed from the petri dish; however, lactic acid acted like a non-carboxylic polyol in HPMC films. Another surprising result came from the HPMC films with mannitol and sorbitol as plasticisers. Mannitol and sorbitol are a pair of stereoisomers with the same molecular formula (see Table 2.2). However, the elongation results of 10% mannitol and sorbitol in HPMC are 12.04±1.06% and 17.99±1.35% respectively. This stresses the importance of

stereochemical structures of plasticisers on the mechanical properties of the HPMC films, because the components of HPMC are chiral polysaccharides. The DSC, TGA, SEM and IR experiments are discussed in Chapter 6, to study the molecular interaction and bond formation of the polymer and plasticisers.

The above results showed that there are no directly proportional relationships between the plasticizing effect and the concentration of plasticisers, and no proportional relationships between the plasticizing effect and molecular weights of the plasticisers either. Therefore, an appropriate concentration and a suitable molecular weight of plasticisers for a polymer to form a desirable film should be determined empirically for each combination.

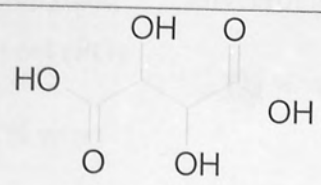
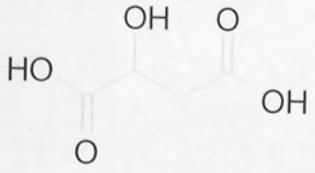
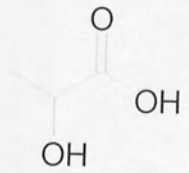
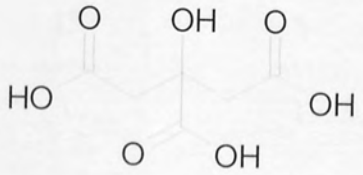
2.4.3 Pullulan films containing Yonkenafil

2.4.3.1 Introduction

- *pH adjustor*

Because the drug has a low solubility at the pH of saliva (see section 4.1.1.2), an acidifier was incorporated into the formulation with the intention of altering the local pH in the vicinity of the film. Tartaric acid, malic acid, lactic acid, and citric acid, which have similar chemical structures (see table 2.3), were selected in order to decrease the pH of the oral cavity, and to study how the different functional groups can affect the film properties. Quantities are expressed as percentage weight per total weight of drug and excipients (excluding solvents) in the formulation.

Table 2.3 The molecular weight and chemical structure of acids incorporated into films

Acid	pK_a (25 °C)	Molecular weight	Chemical structure
Tartaric Acid	pK_1 3.04 pK_2 4.37	150.09	
Malic Acid	pK_1 3.40 pK_2 5.11	134.09	
Lactic Acid	3.86	90.08	
Citric Acid	pK_1 3.14 pK_2 4.77 pK_3 6.39	192.12	

- *Formulations*

I chose propylene glycol and glycerol to be the plasticisers in this study, as the structures are similar while the influence of the presence of hydroxyl groups on the mechanical properties could also be studied. The formulations are shown in table 2.4

Table 2.4 The formulations of polymer, drug and plasticiser used in tensile testing (all have loading of 20% w/w)

	Pullulan (P) (% w/w)	Drug (D) (% w/w)	Propylene glycol (PG) (% w/w)	Glycerol (Gly) (% w/w)
P100	100	0	\	\
PD20	80	20	\	\
PDPG1	79	20	1	\
PDPG2	77	20	3	\
PDPG3	75	20	5	\
PDPG4	70	20	10	\
PDPG5	65	20	15	\
PDGly1	79	20	\	1
PDGly2	77	20	\	3
PDGly3	75	20	\	5
PDGly4	70	20	\	10
PDGly5	65	20	\	15

Group 1: P100, PD20

Group 2: PDPG1-5

Group 3: PDGly1-5

Table 2.5 The formulations of pullulan films containing 20% (w/w) drug, 5% PG (w/w) and different acids used in tensile testing

	Pullulan (% w/w)	Drug (% w/w)	PG (% w/w)	Tartaric acid (T) (% w/w)	Malic acid (M) (% w/w)	Lactic acid (L) (% w/w)	Citric acid (C) (% w/w)
PDPT1	74	20	5	1	\	\	\
PDPT2	72	20	5	3	\	\	\
PDPT3	70	20	5	5	\	\	\
PDPM1	74	20	5	\	1	\	\
PDPM2	72	20	5	\	3	\	\
PDPM3	70	20	5	\	5	\	\
PDPL1	74	20	5	\	\	1	\
PDPL2	72	20	5	\	\	3	\
PDPL3	70	20	5	\	\	5	\
PDPC1	74	20	5	\	\	\	1
PDPC2	72	20	5	\	\	\	3
PDPC3	70	20	5	\	\	\	5

Group 4: PDPT1-3, PDPM1-3, PDPL1-3, PDPC1-3

2.4.3.2 Results and Discussion

Table 2.6 The TS, Elongation and EM of P100 and PD20

Formulation	TS (N/mm^2)	Elongation (%)	EM (N/mm^2)
P100	39.9±2.0	4.63±0.06	1277.2±57.6
PD20	41.7±3.4	3.63±0.08	1300.1±144.9

Figure 2.31 The tensile strength (N/mm^2) of 20% (w/w) drug in pullulan films containing propylene glycol or glycerol (mean±s.d., n=3)

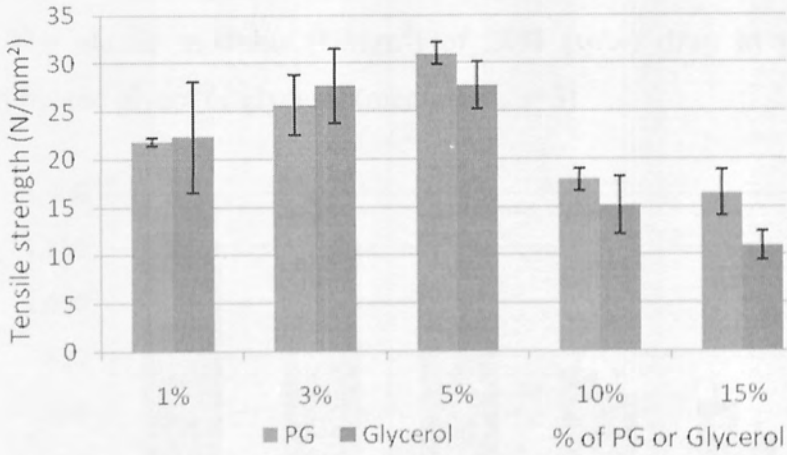


Figure 2.32 The elongation of 20% (w/w) drug in pullulan films containing propylene glycol or glycerol (mean±s.d., n=3)

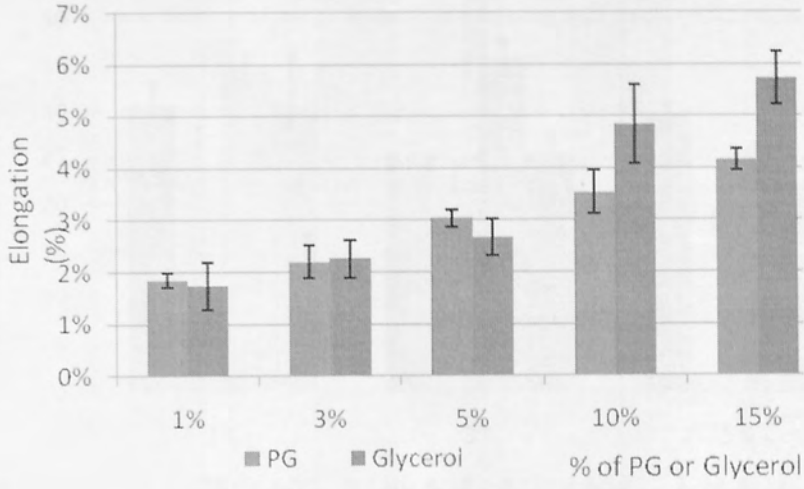


Figure 2.33 The elastic modulus (N/mm^2) of 20% (w/w) drug in pullulan films containing propylene glycol or glycerol (mean±s.d., n=3)

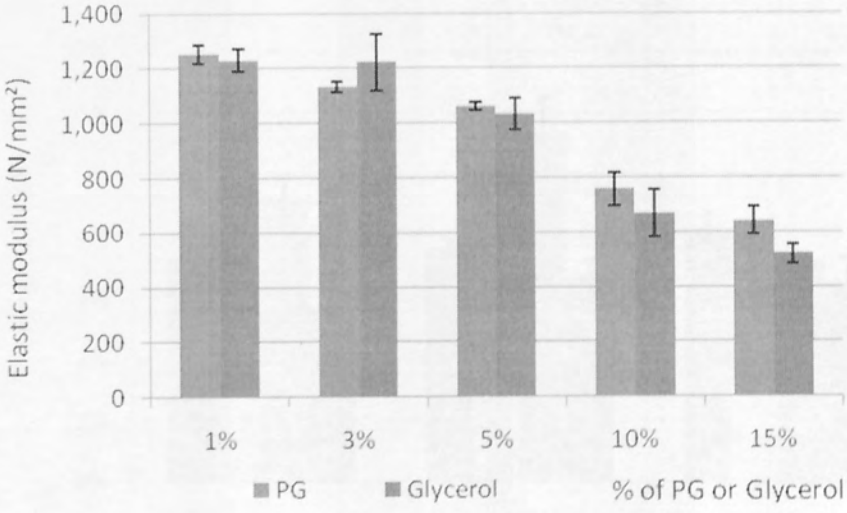


Figure 2.34 The tensile strength (N/mm^2) of 20% (w/w) drug in pullulan films containing different acids (mean±s.d., n=3)

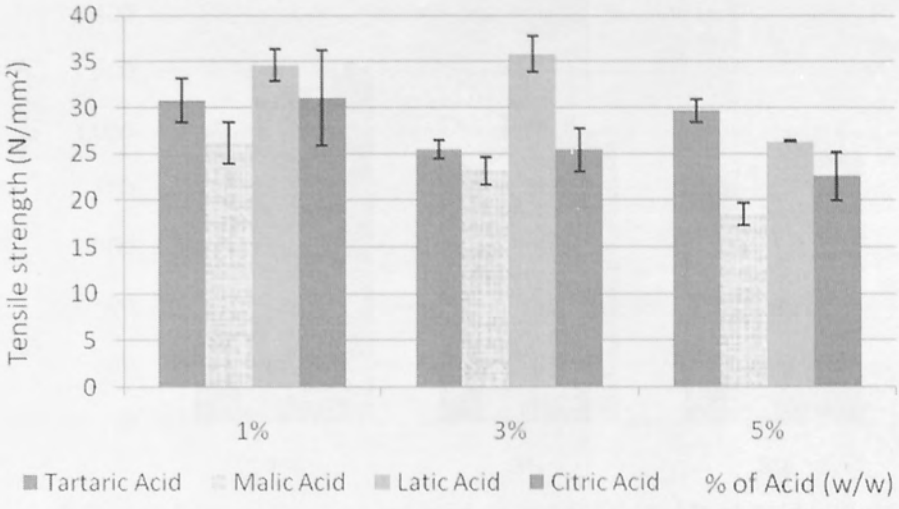


Figure 2.35 The elongation of 20% (w/w) drug in pullulan films containing different acids (mean±s.d., n=3)

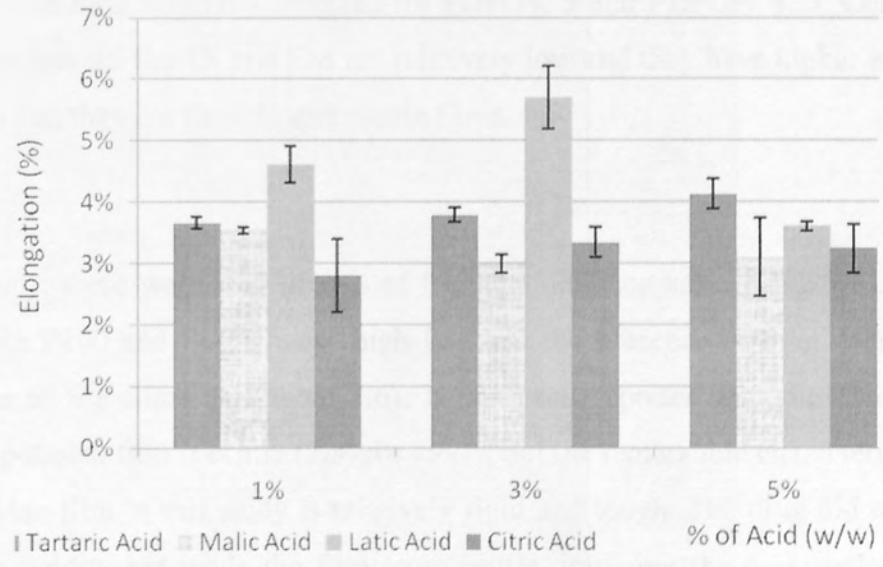
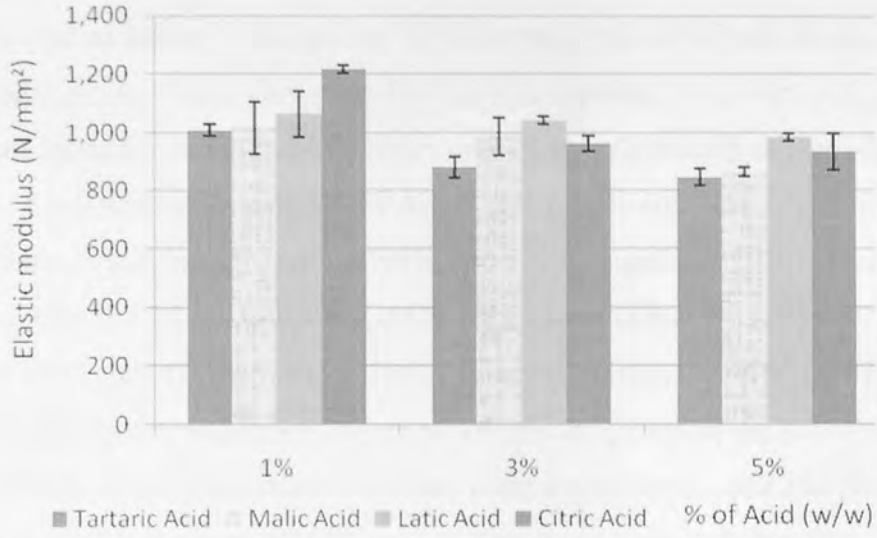


Figure 2.36 The elastic modulus (N/mm^2) of 20% (w/w) drug in pullulan films containing different acids (mean±s.d., n=3)



As discussed in section 2.1.4.3, two types of deformation could be used to describe the mechanical results. Among all the results of pullulan films containing 20% w/w drug, plastic deformation was only detected for PDPG4, 5 and PDPGly 4, 5. Compared to other formulations, the TS and EM are relatively low and they have higher elongation, indicating that they are flexible and elastic films.

In this study, there were four groups of formulations (see table 2.4, 2.5). In the first group, both P100 and PD20 had a high EM and the presence of drug decreased the elongation of the films (see table 2.6). It has been reported that the commercially available pullulan film is brittle (*Tanaka 1991*), but the remarkable characteristic of the pure pullulan film in this study is relatively rigid and tough. The drug did not change the film's rigidity but made the film more brittle, implying the drug molecules take effect by breaking polymer-polymer interactions (e.g., hydrogen bonds, and van der Waals or ionic forces) (*Nugraha et al., 2005*).

Group 2 and group 3 were designed to study how the two plasticisers (propylene glycol and glycerol) affect mechanical properties of the films. It is obvious that the

inclusion of a small amount (≤5% v/v) of these plasticisers weakened the films resulting in decreased elongation even at levels as low as 1% plasticiser (see figure 2.32). Effects are similar to the drug-free pullulan films discussed in section 2.4.2.2, i.e., the "antiplasticization" also applies to low concentrations of polyols applied in the drug-loaded pullulan films. However, for films containing 10 or 15% v/v plasticiser, low EM and high elongation indicate softer and tougher formulations (see Figures 2.33 and 2.34). Statistical analysis (ANOVA) of elongation and EM between the films containing same ratio but different polyols, showed no significant differences between the two polyols for TS, elongation and EM when their content ≤ 10% v/v. There is a significant difference between the polyols for all parameters at loading of 15 % v/v ($P<0.05$). Looking at the chemical structures of PG and glycerol, the latter has an extra hydroxyl group, which may make the films more hygroscopic, and the plasticization effect of moisture may be significant. It has been reported that the humectant properties of glycerol are greater than PG in pure chitosan films (*Nugraha et al., 2005*).

At high levels of PG or glycerol, it was found that the films became sticky and difficult to handle. A moderate amount of PG (5% w/w), was chosen to be a constant plasticiser in the films. Although glycerol had more favorable plasticization as PG is reported to be used as a penetration enhancer (*Aungst & Rogers, 1989*), it may prove to have other more beneficial effects. Figures 2.34, 2.35 and 2.36 show the TS, elongation and EM of the films containing different ratios and different kinds of acids. In these three figures, the most noticeable effect is for films containing 1 and 3% w/w lactic acid (LA) which have a much higher elongation than the other three formulations in the corresponding group. PDPL2 has the highest elongation (5.70±0.51%) of all formulations in the acid-containing group, as high as PDGly5 (5.73±0.51%), which is the highest in plasticiser-group. The EM of PDPL2 is 1040.7±76.7 N/mm^2, not different from that of PDPG3 (1061.5±15.7 N/mm^2) ($P=0.6695$). Thus LA does not significantly change the elasticity of the films but it can markedly increase the film elongation.

In Figure 2.36, no apparent fluctuation can be observed and the lowest EM (846.7±29.6 N/mm^2) results from PDPT3, higher than the EM of PDPG4 (759.2±59.9

N/mm^2) and PDGly4 (666.7±86.3 N/mm2) ($P<0.5$). These three formulations are all composed of 70% polymer, 20% drug and 10% polyol or acid. From this result, it can be concluded that acids do not attribute to the elasticity as much as the glycerol and PG. LA formulations have the highest EM among the acids when the concentration of the acids were 3% and 5%, as PDPT3 and PDPM3 are both significantly lower than PDPL3 ($P=0.0018$, 0.006) (see Figure 2.36). This phenomenon may be attributed to the low molecular weight of LA. *Mangavel et al. 2003* reported that the plasticiser efficiency depends on its molecular weight; i.e., plasticiser efficiency increases with its molecular weight. For LA, this theory is valid for it has the lowest molecular weight of the four acids; however, citric acid has the highest molecular weight, and the EM of PDPC3 is not significantly different from that of PDPL3, even higher than PDPT3 and PDPM3. Thus, the molecular weight is not the only factor affecting the plasticiser efficiency of the acids. Tartaric acid, malic acid, and citric acid have close molecular weight and chemical structure shown in Table 2.7

Table 2.7 The molecular weight, number of hydroxyl group and carboxyl group, EM of PDPx3 of Tartaric acid, malic acid, and citric acid

	Molecular weight	Hydroxyl group	Carboxyl group	EM of PDPx3 (N/mm^2)
Tartaric acid	150.09	2	2	846.7±29.6
Malic acid	134.09	1	2	863.0±15.9
Citric acid	192.12	1	3	935.0±61.4

As discussed in this section, the extra hydroxyl group may increase the plasticiser efficiency for the polyols. In the case of these three acids (except lactic acid), tartaric acid possesses the most hydroxyl groups and least carboxyl groups, and it results in formulations with the lowest EM, in contrast, citric acid has the least hydroxyl groups but most carboxyl groups, and results in formulations with the highest EM. Comparing tartaric acid with malic acid, a reduction in the number of hydroxyl groups results in no significant increase in EM ($p<0.05$), when comparing malic acid and citric acid, an

extra carboxyl group also increases EM. As a whole, one more carboxyl group or less hydroxyl group in the chemical structure of the acids will increase EM of the films. It has been reported that the plasticisers can be more effective if their chemical structures are similar to the polymer and molecular features other than molecular weight may also influence the plasticising efficiency (*Mangavel et al. 2003*). Pullulan is a polysaccharide and each of its monomers have several hydroxyl groups, so a hydroxyl group could play an important role in modifying the elasticity of the pullulan film.

Figure 2.37 The tensile strength (N/mm^2) of drug-loaded pullulan films containing 5% w/w of a polyol or lactic acid (mean±s.d., n=3)

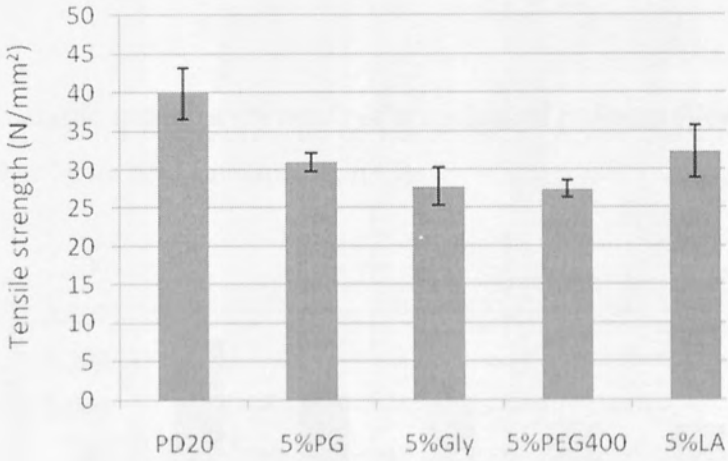


Figure 2.38 The elongation of drug-loaded pullulan films containing 5% w/w of a polyol or lactic acid (mean±s.d., n=3)

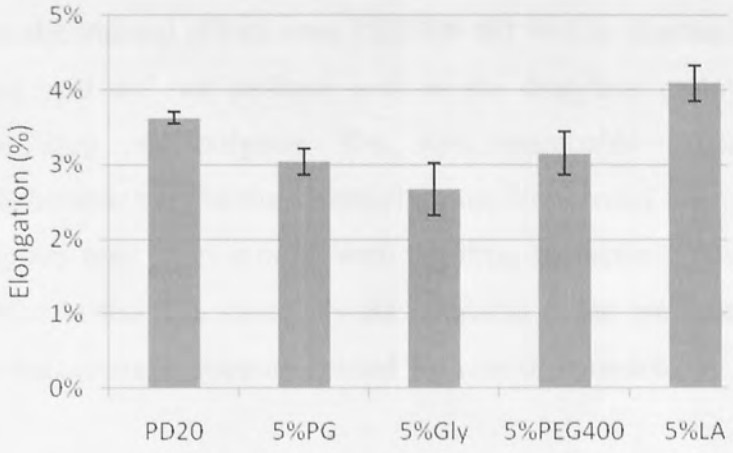
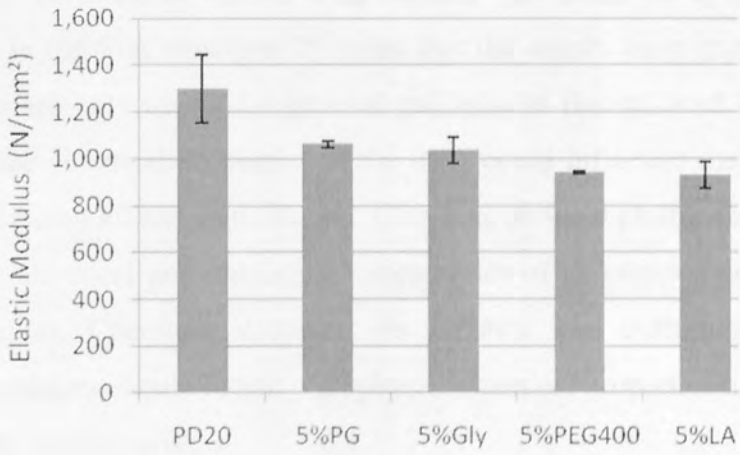


Figure 2.39 The elastic modulus (N/mm^2) of drug-loaded pullulan films containing 5% w/w of a polyol or lactic acid (mean±s.d., n=3)



From Figures 2.37, 2.38 and 2.39, lactic acid is the most effective plasticiser for the pullulan films containing 20% (w/w) Yonkenafil, with the highest elongation and lowest elastic modulus. Compared with the results of drug-free pullulan films, lactic acid damaged the film structure and it was easily broken when it was peeled out from the petri dish. PEG 400 had the most effective plasticization effect on the drug-free films, however, the result was completely reversed when 20% drug (w/w) was added. From Table 2.6, it shows that the drug molecules interrupted the polymer network

because they made the films break more easily (reduced elongation), reflecting that the drug molecules were not incorporated without disruption to the pullulan chains. In this situation, the addition of the polyols (PG, Glycerol and PEG400) was not sufficient to overcome these detrimental effects even PEG400 did well in plasticizing the polymer. Although lactic acid did not perform well in the drug-free pullulan films, it did coordinate the drug and polymer. The most reasonable explanation for this contradictory behaviour may be the carboxyl group. Yonkenafil is a weak base so that the carboxyl group may form a bond with the drug molecule. Lactic acid probably acted as a binder in the film structure; the hydroxyl group was connected with the polymer while the carboxyl group associated with the drug molecule.

2.4.3.3 Conclusion for the pullulan films containing Yonkenafil

It is clear that the mechanical properties of the drug loaded film and the drug-free films have significant differences. As the drug loading increased, the drug plays a more important role in the film structure. It seems that the results from experiments of the drug-free films are only useful for general guidance in the study of the drug-loaded film, because the chemical properties of the drug could influence the polymeric film structure, plasticizing effects significantly. Choosing an ideal plasticiser for these films has to take the chemical and physical characteristics of all compositions of the films into consideration. Chemical structure, its polarity and molecular weight of a satisfactory plasticiser should match with physicochemical properties of a film forming polymer and the loading drug.

2.4.4 Pullulan films loading hydrophobic drugs

As a high water soluble hydrophilic polymer, pullulan is incompatible with a hydrophobic drug in the film system. To challenge the difficulty of loading a hydrophobic drug into the pullulan films, we proposed a complex system composing of pullulan and hydroxypropyl-β-cyclodextrin (HP- β -CD).

Hydroxypropyl-b-cyclodextrin (HP- β -CD) is a hydroxyalkyl derivative of cyclodetrxin, with improved water solubility (*Uekama et. al., 1998*). Cappello reported that the HP- β -CD-containing poly(ethyleneoxide) tablets loading poorly soluble drug carvedilol increased the drug penetration rate through pig buccal mucosa (*Caooello et. al., 2006*).

The maximum amount of HP- β -CD can be added into a free pullulan film is 30% (w/w), when the concentration was over 30%, the films were broken into pieces after drying. The ratio of drug and cyclodextrin in a CD complex is generally 1:1 to 1:2 (mol/mol) (*Uekama, 2004*). The mol weight of HP- β -CD is 1380, thus, if the mol weight of a drug is 1380, the drug loading in the pullulan film is 23.1-37.5% (w/w), theoretically. As the mol weight of the drug is 138, the drug loading in the films will reduce to 2.9-5.7% (w/w). High molecular weight drug usually have low permeability through the buccal mucosa (*Giannola et. al., 2008*), the drug applied in buccal delivery should be a relative small molecule. As a result, the drug loading is quite low the films combined with pullulan and HP- β -CD complex, which is the limitation of this polymeric system.

Loratadine was chosen as a hydrophobic model drug in this research. However, I met the problems of making the loratadine and HP- β -CD complex following the methods introduced in patent WO 2004/082589 (*Sehgal et al., 2004)* and a research literature (*Omar et al., 2007*). Loratadine did not form the complex with HP- β –CD under both experimental conditions introduced in the two papers. As a result, I did not continue the research of using pullulan film to load hydrophobic drug.

Chapter Three

In vitro drug release and polymer dissolution

3.1 Introduction

3.1.1 Polymer dissolution controlled drug release system

Polymer dissolution controlled drug release systems are made of a drug molecularly dispersed or dissolved in a polymeric matrix. As water penetrates the polymer, swelling occurs and a thin layer of polymer in the rubbery state (gel layer) is formed. Drug diffusion through this gel layer is relatively fast. During this diffusion process, as swelling and dissolution are competing, at the beginning the gel layer thickness increases due to swelling, and then remains constant due to synchronization of swelling, resulting in drug diffusion, dissolution, and finally decreases as dissolution takes over (*Narasimhan et al., 1997*).

During the dissolution process, the polymer carrier passes through three distinct regimes of macromolecular configuration, as indicated in Figure 3.1.

Figure 3.1 The procedure of polymer chains disentangling in a solution

a b c

Thus, the swollen rubbery layer is the concentrated regime, indicated in Figure 3.1a, where the chains are completely entangled. The semi-dilute regime (Figure 3.1b) is a diffusion boundary layer just outside the rubber/solvent interfaces in the solvent. In the boundary layer, the chains are just disentangling. When the polymer is fully dissolved, the completely disentangled chains move freely in the solvent, this is the dilute regime indicated in Figure 3.1c.

3.1.2 Empirical and semi-empirical mathematical models

Diffusion, swelling and erosion are the most important mechanisms to describe a drug release from the polymeric system and polymer dissolution process (*Langer et al., 1983*). Diffusion can be described using Fick's first and second law (equations 3.1 and 3.2).

Fick's First Law:

$$J = -D\frac{dC}{dx}$$

(Eq. 3.1)

where J is the diffusion flux, D is the diffusion coefficient, dC/dx = the incremental change in concentration with distance.

$$\frac{\partial C_x}{\partial_t} = D \frac{\partial^2 C_x}{\partial_{x^2}}$$

(Eq. 3.2)

where Cx is the concentration at a distance x from the reference point, D is the diffusion coefficient and t is time.

Case II transport (*Enscore et al., 1977*) reflects how movements of the polymer chain in the matrix affect the drug release. There are several mathematical equations that can be applied to describe the drug release rate from a polymeric system, detailed in sections 3.1.2.2.

3.1.2.1 Higuchi equation

The equation of the Higuchi model (*Higuchi, 1961*) is:

for $c_0 > c_s$

$$\frac{M_t}{A} = \sqrt{D(2c_0 - c_s)c_s t}$$

(Eq. 3.3)

where M_t is the cumulative absolute amount of drug, released at time t, A is the surface area of the controlled release device exposed to the release medium, D is the drug diffusivity in the polymer carrier, and c_0 and c_s are the initial drug concentration, and the solubility of the drug in the polymer, respectively. And the basic equation can be expressed as:

$$\frac{M_t}{M_\infty} = K\sqrt{t}$$

(Eq. 3.4)

where M_∞ is the absolute cumulative amount of drug released at infinite time (which should be equal to the absolute amount of drug incorporated within system at time t=0), and K is a constant reflecting the design variables of the system.

From the Higuchi equations, the drug release rate is proportional to the root of time. The model makes a number of assumptions:

1. The initial drug concentration is much higher than the solubility of the drug ($c_0 > c_s$).
2. Mathematical analyses are based one-dimensional, and the edge effect must be negligible.
3. The suspended drug is in a fine state such that the particles are much smaller in diameter than the thickness of the system.
4. Swelling or dissolution of the polymer carrier is negligible.
5. The diffusivity of the drug is constant.

6 Perfect sink conditions are maintained.

Because these assumptions are not valid for most controlled systems, considering the polymer swelling, transition of the polymer molecules from glassy to rubbery state, polymer dissolution, concentration-dependent water and drug diffusion etc., the fractional amount of drug released and the square root of time was derived from an exact solution of Fick's second law of diffusion for thin films of thickness δ under perfect sink conditions, uniform initial drug concentration with $c_0 < c_s$ (monolithic solutions) and assuming constant diffusivities (*Crank, 1975; Carslaw et al., 1959; Baker et al., 1974*).

$$\frac{M_t}{M_\infty} = 4\left(\frac{Dt}{\delta^2}\right)^{1/2}\left\{\pi^{-1/2} + 2\sum_{n=1}^{\infty}(-1)^n ierfc\frac{n\delta}{2\sqrt{Dt}}\right\}$$

(Eq. 3.5)

M_t and M_∞ are the absolute cumulative amount of drug released at time t and infinite time, respectively; D represents the diffusivity of the drug from polymeric systems. As the second term in the second brackets vanishes at early times, a sufficiently accurate approximation of Eq. 3.5 for $M_t/M_\infty<0.60$ can be written as follows:

$$\frac{M_t}{M_\infty} = 4\left(\frac{Dt}{\pi\delta^2}\right)^{1/2} = k'\sqrt{t}$$

(Eq. 3.6)

where k' is a constant.

Therefore, the fractional amount of drug released is still proportional to the square root of time, based on more complicated circumstances which are different from those studied by Higuchi for the derivation of his classical equation (monolithic solutions versus monolithic dispersions). Diffusion is the critical mechanism in both cases, therefore, a proportionality between the cumulative drug released and the square root of time is usually regarded as a sign for diffusion-controlled drug release (*Siepmann, 2001*).

3.1.2.2 Power law

Compared with the Higuchi equation, Power law is more comprehensive and still simple, and its equation is:

$$\frac{M_t}{M_\infty} = kt^n$$

(Eq. 3.7)

Here, M_t and M_∞ are the absolute cumulative amount of drug released at time t and infinite time, respectively; k is a constant incorporating structural and geometric characteristics of the device, and n is the release exponent, indicative of the mechanism of drug release.

From equation 3.7, when n=1.0, M_t/ M_∞=kt, the drug release rate is independent of time, which means zero-order release kinetics. For slabs, the mechanism that creates the zero-order release is known as case-II transport. In this case, water is imbibed into the polymer system and the macromolecules are disentangled to control the drug release, termed swelling-controlled drug release. When n=0.5, indicating that the polymer system fits the Higuchi model, and is called diffusion-controlled release. When n between 0.5 and 1.0, it can be regarded as anomalous transport. It has to be paid attention that 0.5 and 1.0 are only valid for slab geometry. For spheres and cylinders the values are different, shown in Table 3.1 (*Ritger et al., 1987-a; Ritger et al., 1987-b*).

Table 3.1 Exponent n of the power law and drug release mechanism from polymeric delivery systems of different geometry

Exponent, n			Drug release mechanism
Thin film	Cylinder	Sphere	
0.5	0.45	0.43	Fickian diffusion
0.5<n<1.0	0.45<n<0.89	0.43<n<0.85	Anomalous transport
1.0	0.89	0.85	Case-II transport

3.1.2.3 Other empirical and semi-empirical models

Peppas and Sahlin (*Peppas, et al., 1989*) developed another model:

$$\frac{M_t}{M_\infty} = k_1 t^m + k_2 t^{2m}$$

(Eq. 3.8)

where k_1 , k_2 and m are constants. The first term on the right hand side represents the Fickian diffusional contribution, F, whereas the second term the case-II relaxational contribution, R. The ratio of both contributions can be calculated as follows:

$$\frac{R}{F} = \frac{k_2 t^m}{k_1}$$

(Eq. 3.9)

3.2 Materials

Pullulan: Hayashibara Biochemical Laboratory Inc. Japan.

Propylene glycol (PG): Sigma, Chenmical Co, Germany.

Polyethylene Glycol 400: Aldrich Organics, New Jersey, USA

DL-Lactic Acid: Aldrich Organics, New Jersey, USA.

Yonkenafil: a gift from Tasly Group, China.

Acetonitrile: HPLC grade, Fisher Scientific, United Kingdam

Triethylamine: HPLC grade, Fisher Scientific, United Kingdam

3.3 Methods

3.3.1 *In vitro* drug release from the drug loaded films

Film strips, visually free of air bubbles or physical imperfections, were cut (10×10mm) and placed in a 25 ml conical flask. Two ml of simulated saliva fluid (2.38 g Na_2PO_4, 0.19 g KH_2PO_4 and 8.00 g NaCl per liter of distilled water with phosphoric acid to pH 5.5) (*Kok et.al., 1999*) was added and fixed in a 37 °C shaking bath at speed 5 (about 40 repetitions per minute and the amplitude of vibration was 2.5 cm). One ml samples

were removed at 0.5, 1, 2, 3, 4, 5, 6 and 8 minutes and replaced with 1 ml of fresh simulated saliva fluid. The film remaining in the vessel was then used to determine the expected total amount of drug in the sample. These were continued being shaken for at least two hours to ensure samples had fully dissolved. Each time point measurement was repeated five times separately and used the three middle values used for analysis. Each removed sample was diluted to 2 ml with simulated saliva fluid. The concentration of the drug dissolved in the medium was assayed by high-performance liquid chromatography (HPLC, 10Avp system, Shimadzu corp., Japan), under following conditions, ODS Hypersil column (150 × 4.6 mm, Supelco corp.), 20 mM citric acid solution (adjusted to pH 5.4 with triethylamine) – acetonitrile (40:60) eluent, 1.0 ml/min flow rate, detection, UV at 324 nm. A calibration curve using known concentrations of the drug was performed prior to each analysis. A typical example is shown in Figure 3.2. The drug released (%) was calculated using the following formula.

Drug released (%) = (Amount of drug released / Total amount of drug) × 100% (Eq. 3.10)

Figure 3.2 The concentration-HPLC peak area calibration curve for the drug (mean±s.d., n=3)

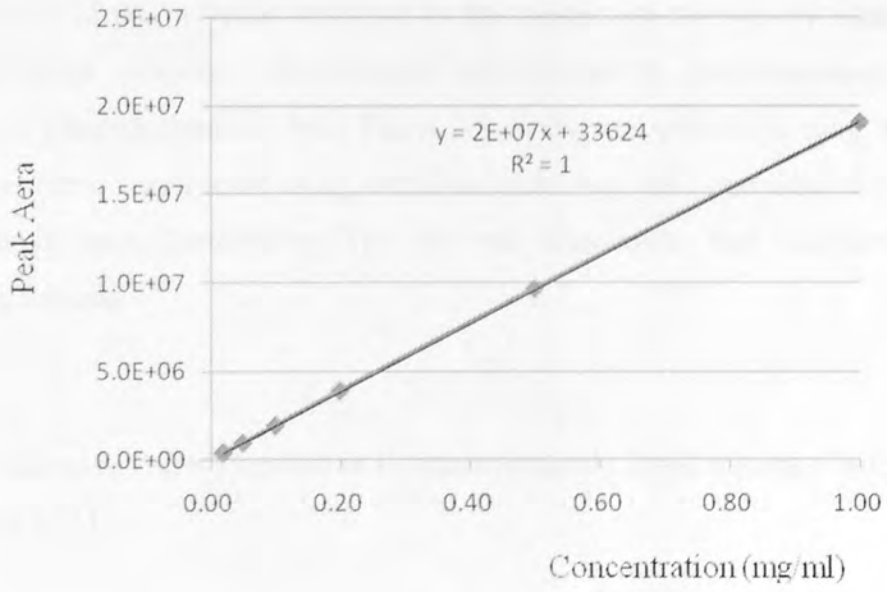


Table 3.2 The formulations used in *in vitro* drug release studies

Formulation	Polymer (% w/w)	Yonkenafil (% w/w)	Propylene glycol (% w/w)	Latic acid (% w/w)
PD20	80	20	0	0
5% PG	75	20	5	0
5% LA	75	20	0	5
5% PEG400	75	20	0	0

3.3.2 *In vitro* polymer dissolution from the drug loaded or drug free films

Film strips, visually free of air bubbles or physical imperfections, were cut (10×10mm) and were placed in the 25 ml conical flask, 2 ml of stimulated saliva fluid shaking bath was added and fixed in a 37 °C shaking bath at speed 5 (about 40 repetitions per minute and the amplitude of vibration was 2.5 cm). One ml samples were removed at 0.5, 1, 2, 3, 4, 5, 6 and 8 minutes and 2 ml stimulated saliva fluid replaced. Each time point measurement was repeated five times separately and used the three without maximum and minimum for analysis. Each of the samples was diluted to 3 ml, and the concentration of the polymer dissolved in the media was assayed by measuring the viscosity of the samples. The viscosity was assayed by microviscometer (AMVn Automated Microviscometer, Anto Paar.). Viscosity was calculated using a standard calibration curve constructed using solutions containing the same ratio of pullulan to excipients in each formulation. The polymer dissolution was calculated by the following formula.

Polymer dissolved (%) = (Amount of Polymer released / Total amount of polymer) × 100% (Eq 3.11)

Figure 3.3 The concentration-viscosity calibration curve for P100 formulation (mean±s.d., n=3)

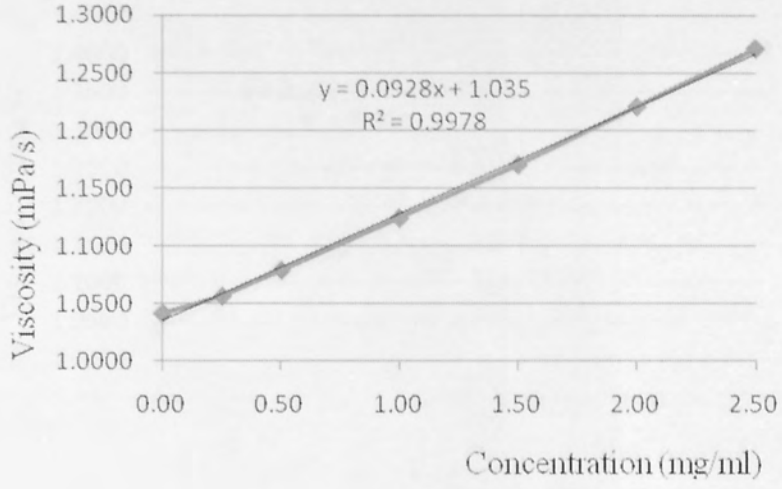


Figure 3.4 The concentration-viscosity calibration curve for pullulan containing 5% propylene glycol (mean±s.d., n=3)

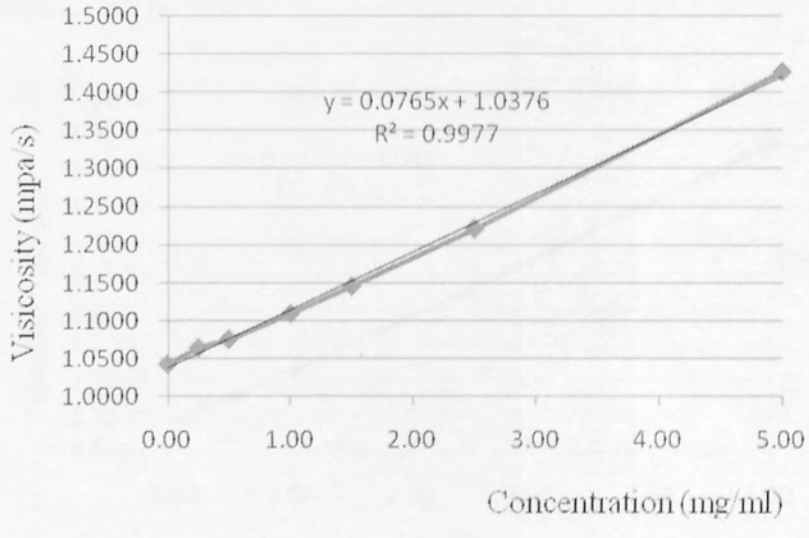


Figure 3.5 The concentration-viscosity calibration curve of pullulan containing 5% lactic acid (mean±s.d., n=3)

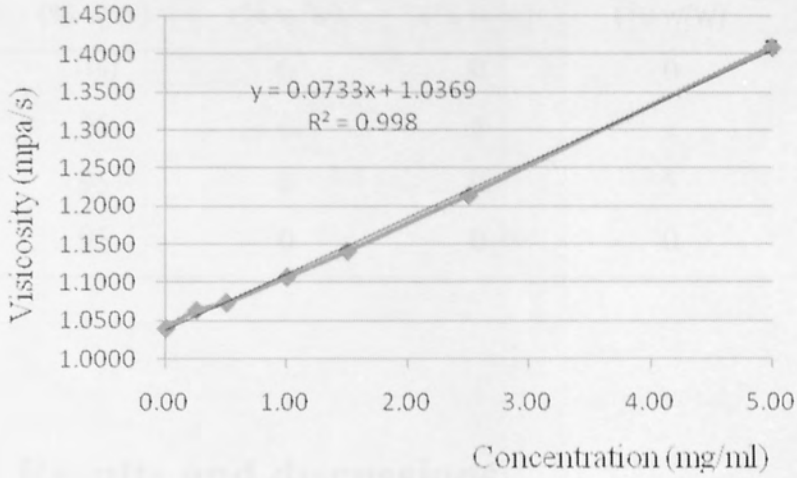


Figure 3.6 The concentration-viscosity calibration curve of pullulan containing 5% PEG400 (mean±s.d., n=3)

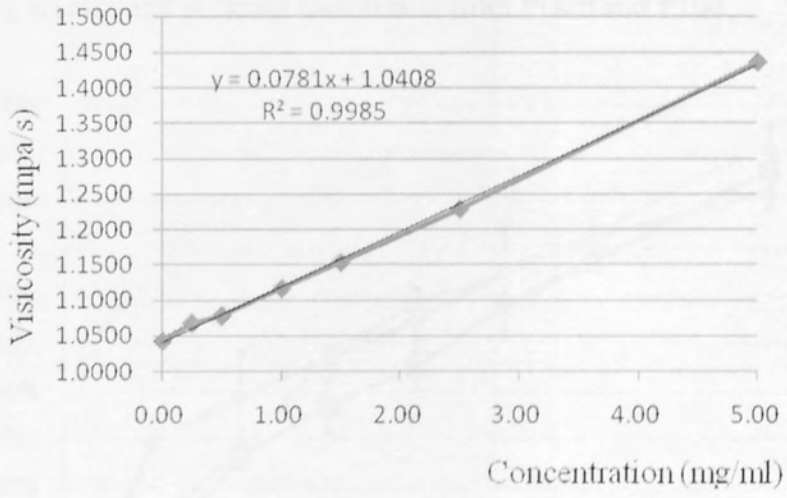


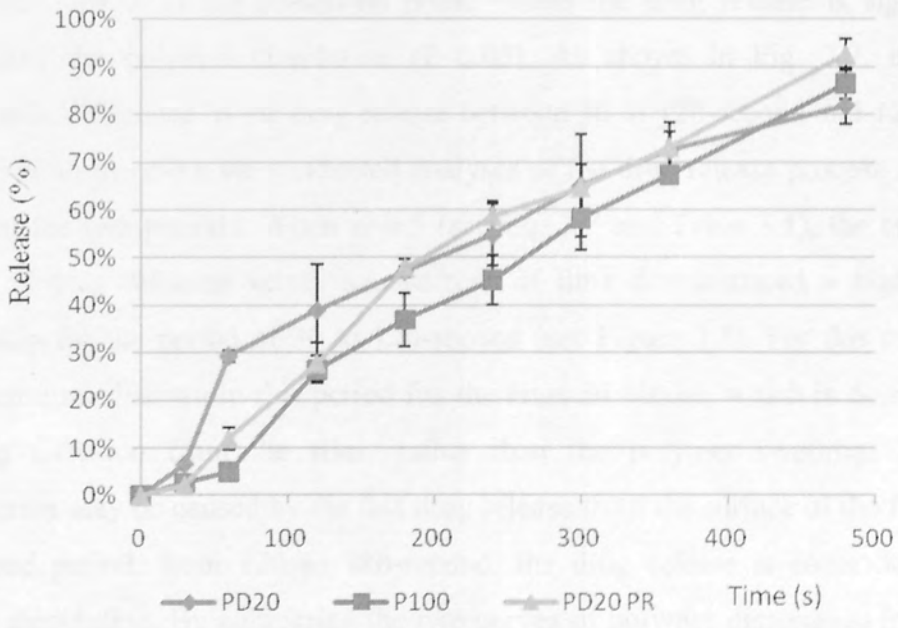
Table 3.3 The formulations used in polymer dissolution from the drug-free films

Formulation	Polymer (% w/w)	Drug (% w/w)	PG (% w/w)	LA (% w/w)	PEG400 (% w/w)
P100	100	0	0	0	0
5%PG	95	0	5	0	0
5%LA	95	0	0	5	0
5% PEG400	95	0	0	0	5

3.4 Results and discussions

3.4.1 P100 and PD20

Figure 3.7 Drug release and polymer dissolution from PD20 and P100



PD20: drug release from PD20

P100: polymer dissolution from P100

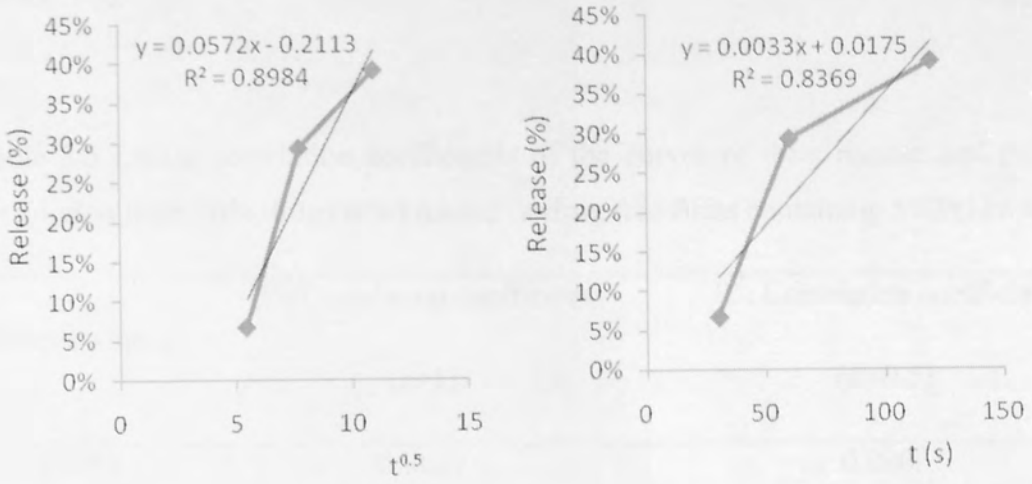
PD20 PR: polymer dissolution from PD20

Table 3.4 Linear correlation coefficients of the curves of drug release and polymer dissolution from PD20 and P100

Release Curve	R^2: Correlation coefficient (release (%) vs t (s))
PD20	0.9227
P100	0.9715
PD20 PR	0.9883

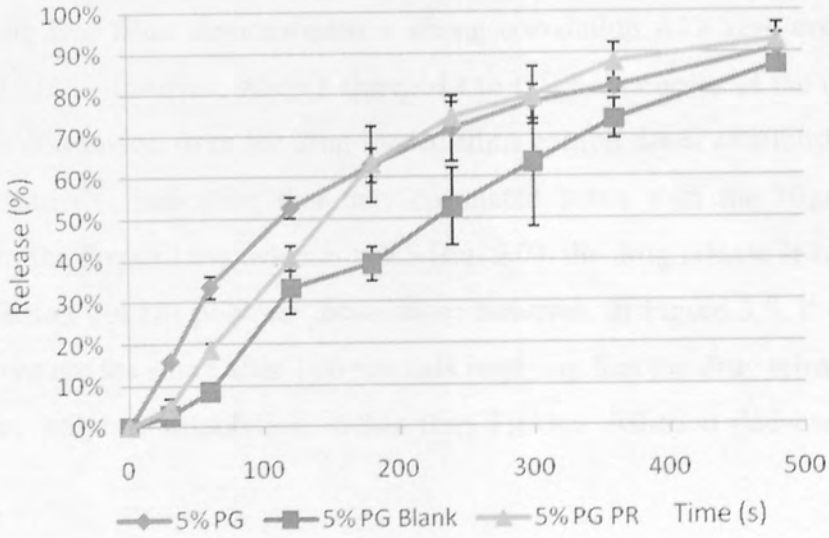
Figure 3.7 shows the drug release from PD20, and polymer dissolution from P100 and PD20. Table 3.4 showed the correlation coefficients of the curves. From these results, it seems that the drug release and polymer dissolution were controlled by the erosion of polymers (see Table 3.1 when n=1, drug release mechanism is dominated by Case-II transport). In the Fig. 3.7, it was clear that the most noticeable difference of the release and dissolutions is at the 60-second point, where the drug release is significantly higher than the polymer dissolution ($P<0.05$). As shown in Fig. 3.7, there is a considerable difference in the drug release between 30 to 120-second and 120 to 480-second period, therefore we conducted analyses of the drug release process separately according the two periods. When n=0.5 (see Eq. 3.7 and Table 3.1), the cumulative amount of drug released verses square root of time demonstrated a higher linear relationship for the period of 30 to 120-second (see Figure 3.8). For this reason, the drug release mechanism in this period fits the Higuchi Model, which is dominated by the drug diffusion from the films rather than the polymer swelling. This sink phenomenon may be caused by the fast drug release from the surface of the films.. For the second period, from 120 to 480-second, the drug release is controlled by the polymer dissolution. By comparing the two curves of polymer dissolution from PD20 and P100 (Fig. 3.7), it can be seen that introduction of the drug into the films of PD20 and P100 has little effect on the polymer dissolution, because the two curves of polymer dissolution from PD20 and P100 (Fig. 3.7) showed the similar release rate.

Figure 3.8 Drug release from PD20 from 30-120 seconds (%) as a function of $t^{0.5}$ and t^{1}



3.4.2 5% PG

Figure 3.9 Drug release and polymer dissolution from 20% drug (w/w) loaded or drug-free films containing 5%PG (w/w)



5%PG: drug release from 20% drug (w/w) loaded films containing 5% PG (w/w)

5%PG blank: polymer dissolution from drug-free films containing 5% PG (w/w)

5%PG PR: polymer dissolution from 20% drug (w/w) loaded films containing 5% PG (w/w)

Table 3.5 Linear correlation coefficients of the curves of drug release and polymer dissolution from 20% drug (w/w) loaded or drug free films containing 5%PG (w/w)

Release Curve	R^2: Correlation coefficient (n=1)	R^2: Correlation coefficient (n=0.5)
5%PG	0.8657	0.9805
5%PG blank	0.8970	0.9493
5%PG PR	0.9716	N/A

Figure 3.9 shows the drug release and polymer dissolution from the drug-loaded films containing 5% (w/w) PG, and polymer dissolution from the drug-free films containing 5% (w/w) PG. From Table 3.5, it can be seen only the curve of polymer dissolution from the drug free films demonstrated a strong correlation with zero order kinetics, with $R^2 = 0.9716$. However, when I changed t to $t^{0.5}$, both curves of the drug release and polymer dissolution from the drug loaded films exhibit linear relationship between the M_t/M_∞ and $t^{0.5}$, indicating that they correlated better with the Higuchi model. According to the Power Law, when $n = 0.5$ (Eq. 3.7), the drug release is controlled by Fickian diffusion but not polymer dissolution; however, in Figure 3.9, it can be seen that the curves are the same after 180-seconds implying that the drug release might be controlled by polymer dissolution, rather than Fickian diffusion deduced by Power law.

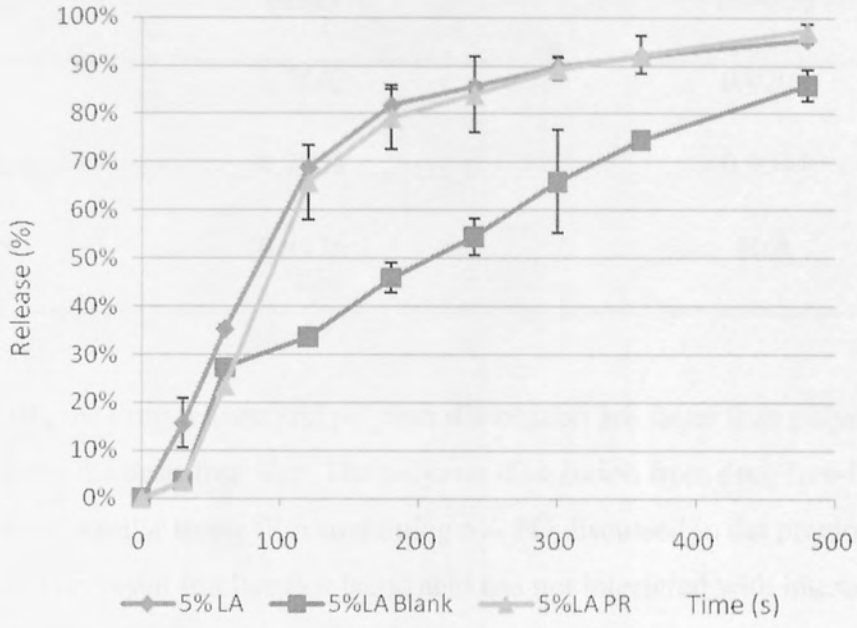
Rana (*Rana et al., 2007*) described that adding 30% (w/w) of glycerol as a plasticiser decreased the release of lidocaine hydrochloride from medicated carbopol 971P films, because glycerol is capable of forming hydrogen bonds with the polymer through its three hydroxyl groups, and decreasing the capacity for water uptake. Thirty% (w/w)

propylene glycol (PG) had no effect on the release of lidocaine hydrochloride supporting the theory that hydrogen bond formation between the polymer and plasticiser restricts water uptake, thus hindering drug release. In my case, according to Rana's hypothesis, the PG should not affect polymer dissolution as shown in Figure 3.15 (see section 3.5.1.2) because PG could not form hydrogen bonds with pullulan. This result supports those of the tensile study discussed in chapter two, where I found the drug-free film containing 5% PG (w/w) is more easily broken than the pure pullulan films (see Figures 2.16, 2.17 and 2.18), caused by a weaker intrinsic integrating force in the film. The above results from the different experiments demonstrated that by introducing 5% PG (w/w) into the pullulan, the affinity between the pullulan molecules was decreased and as a result the film 5% PG (w/w) became less tensile. However, when the films were loaded with 20% (w/w) drug, the polymer dissolution was accelerated and this is probably due to PG and the drug increasing the water uptake and promoting polymer swelling. Accordingly, both the drug and PG affected dissolution of the film; therefore, polymer dissolution and the drug release from the films could follow the same process, and the drug release was not restricted by the polymer dissolution. Therefore, it is more reasonable to use Power Law to explain the drug release mechanism of my results. In my case, both drug release and polymer dissolution followed a diffusion process, which is the reason why these two curves almost overlapped from 180-second. And the overlap of the two curves cannot be considered as the evident for that the drug release was controlled by the polymer dissolution.

Between 30 and 120-seconds (Fig 3.9), there was a "burst release" of the drug, which was not proportional to the polymer dissolution. This may be due to a rapid diffusion of the drug from the surface of the film into the media, as discussed in the section 3.4.1 of "PD20 and P100" and the Power Law is not applicable.

3.4.3 5% Lactic Acid

Figure 3.10 Drug release and polymer dissolution from 20% drug (w/w) loaded or drug-free films containing 5% lactic acid (w/w)



5%LA: drug release from 20% drug (w/w) loaded films containing 5% LA (w/w)

5%LA blank: polymer dissolution from drug free films containing 5% LA (w/w)

5%LA PR: polymer dissolution from 20% drug (w/w) loaded films containing 5% LA (w/w)

Table 3.6 Linear correlation coefficient of the curves of drug release and polymer dissolution from 20% drug (w/w) loaded or drug free films containing 5% lactic acid (w/w)

Release Curve	R^2: Correlation coefficient (n=1)	R^2: Correlation coefficient (n=0.5)
5%LA	0.7602	0.9202
5%LA blank	0.7868	0.9044
5%LA PR	0.9476	N/A

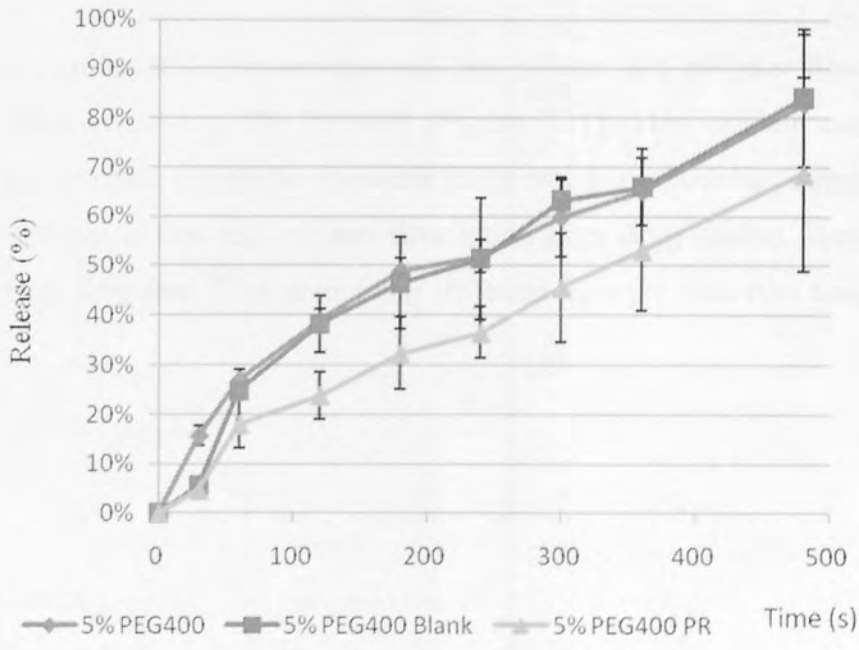
In Figure 3.10., the drug release and polymer dissolution are faster than polymer dissolution from the drug-free film. The polymer dissolution from drug free-films is linear with time, similar to the film containing 5% PG discussed in the previous section 3.4.2. This result implies that lactic acid has not interfered with interactions of pullulan molecules and not weakened their affinity. There was a noticeable increase of the polymer dissolution in the first minute caused by introducing 5% lactic acid into the film (Figure 3.10), and this increase was obviously more intensive than that caused by 5% PG (Figure 3.9 and Figure 3.10). This phenomenon may be due to lactic acid being more water-soluble than PG resulting from its carboxylic acid group. The correlation coefficients in Table 3.6 indicate that the drug release followed Fickian diffusion. In comparison with the results for films containing5% PG, it may be concluded that lactic acid could increase the water uptake into the drug-loaded film more rapidly and to a greater extent than PG.

However, according to *Rana et al., (2007)* and my studies of drug-loaded pullulan film containing 5% PG, it is expected that LA should not form hydrogen bonds with the polymer and therefore produce films with poorer mechanical properties such as low elongation, high elastic modulus and tensile strength. However this was not the case (Chapter two, Figures 2.38, 2.39 and 2.30), wherein the films containing 5% LA (w/w) demonstrated the most suitable mechanical properties. It was assumed that lactic acid

acted as a binder in the film structure by connecting the polymer and the drug with its carboxylic acid group and hydroxyl group (see the last paragraph of section 2.4.3.2). Previous reports discussed the hydrogen bond formed by hydroxyl groups with the polymer, which would not include a compound with a hydroxyl and a carboxylic group, such as LA (*Rana et al., 2007*); therefore, these results from the film containing 5% LA are beyond the scope of previous reports. It can be concluded that the carboxyl group in LA plays an important role in the drug release and polymer dissolution from the drug-loaded films. For the pullulan films containing Yonkenafil, lactic acid is an effective plasticiser that also facilitates drug release.

3.4.4 5% PEG400

Figure 3.11 Drug release and polymer dissolution from 20% drug (w/w) loaded or drug-free films containing 5% PEG400 (w/w)



5% PEG400: drug release from 20% drug (w/w) loaded films containing 5% PEG400 (w/w)

5% PEG400 blank: polymer dissolution from drug free films containing 5% PEG400 (w/w)

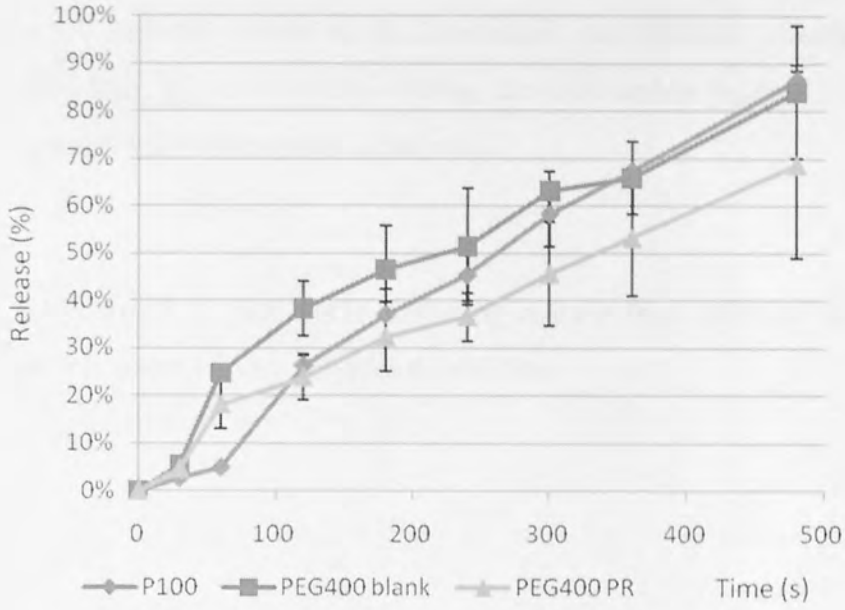
5% PEG400 PR: polymer dissolution from 20% drug (w/w) loaded films containing 5% PEG400 (w/w)

Table 3.7 Linear correlation coefficients of the curves of drug release and polymer dissolution from 20% drug (w/w) loaded or drug free films containing 5% PEG400 (w/w)

Release Curve	R^2: Correlation coefficient (release (%) vs t (s))
5% PEG400	0.9341
5%PEG400 blank	0.9391
5% PEG400 PR	0.9784

There is no significant difference between drug release and polymer dissolution for drug free films containing 5% PEG400 (Figure 3.11). This incident could not be reflecting any intrinsic correlation between these two films. Another surprising result in this experiment is that the polymer dissolution from drug–loaded films is slower than those from drug free films, completely different from previous film studies.

Figure 3.12 Polymer dissolution from P100, drug free and 20% drug loaded (w/w) films containing 5% PEG400



P100: polymer dissolution from P100

5% PEG400 blank: polymer dissolution from drug free films containing 5% PEG400 (w/w)

5% PEG400 PR: polymer dissolution from 20% drug (w/w) loaded films containing 5% PEG400 (w/w)

Firstly, in Figure 3.12, it can be seen that 5% PEG 400 (w/w) in the drug free films did not have a significant effect on polymer dissolution from the films. The mechanical properties results from chapter two (see Figure 2.16, 2.17 and 2.18) also proved the 5% PEG400 (w/w) did not plasticize the drug-free pullulan film. Even for the drug-loaded films, the inclusion of 5% PEG400 did not have a major impact on the mechanical properties, yet it inhibits dissolution

PEG400 was an effective plasticiser for drug-free pullulan films as concentrations of 15% w/w (see sections 2.4.2.2 and 2.4.2.3 and Figures 2.22, 2.23 and 2.24). This indicated it did have an effect on the polymer network, however, at levels of 5% w/w, this was not effective..Comparing the ratio and proportion of components of the films,

when 20% drug (w/w) was loaded into the films, the amount of PEG400 relative to polymer was actually increased because the amount of pullulan was reduced by 20%. (5% PEG400 : 75% pullulan = 6.67% PEG400 : 100% pullulan). It has been shown that 15% PEG400 (w/w) is the maximum concentration effective for plasticizing pullulan, thus, by incorporation of drug, the relationship between the PEG and pullulan is disrupted and water uptake is reduced.

From Figures 3.11 and Table 3.7, drug release from pullulan films containing 5% PEG400 is controlled by polymer dissolution.

3.5 Conclusion

3.5.1 The relationship between drug release and polymer dissolution

3.5.1.1 Drug release and polymer dissolution from drug-loaded films

Figure 3.13 Drug release from PD20 and 20% drug-loaded films containing 5% (w/w) different plasticisers

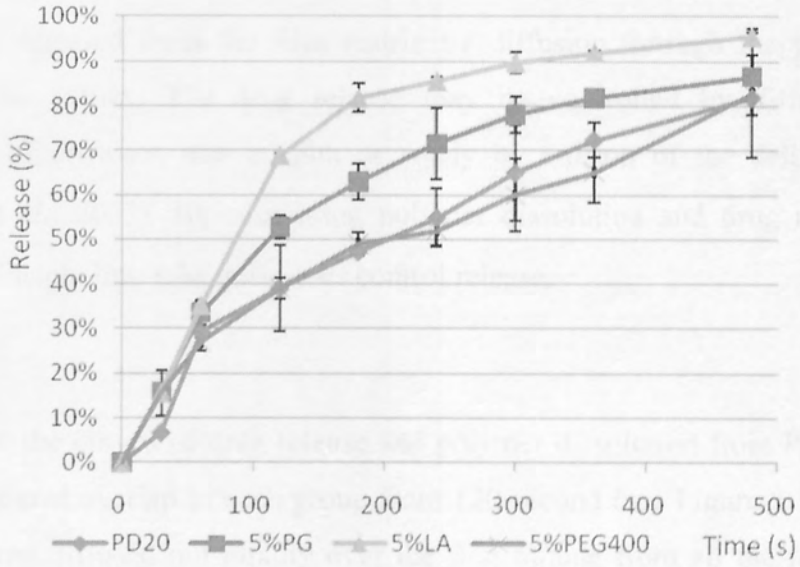
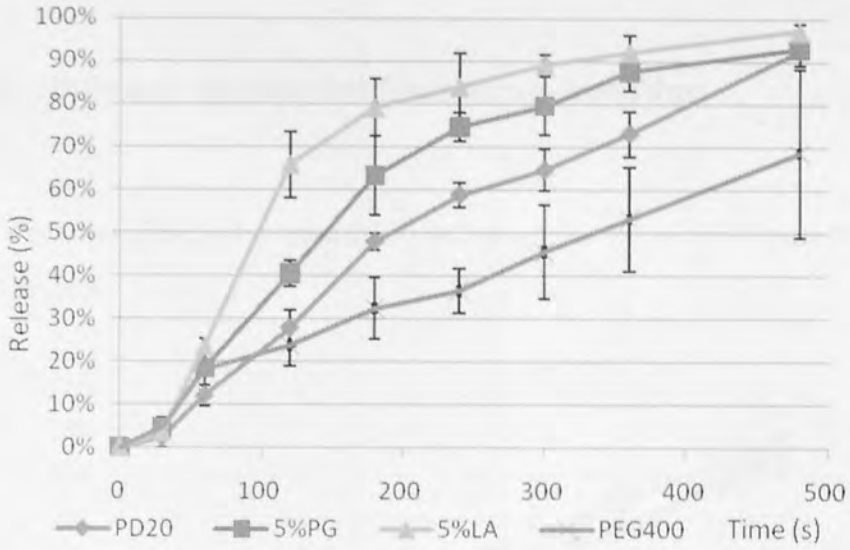


Figure 3.14 Polymer dissolution from PD20 and 20% drug-loaded films containing 5% (w/w) different plasticiser



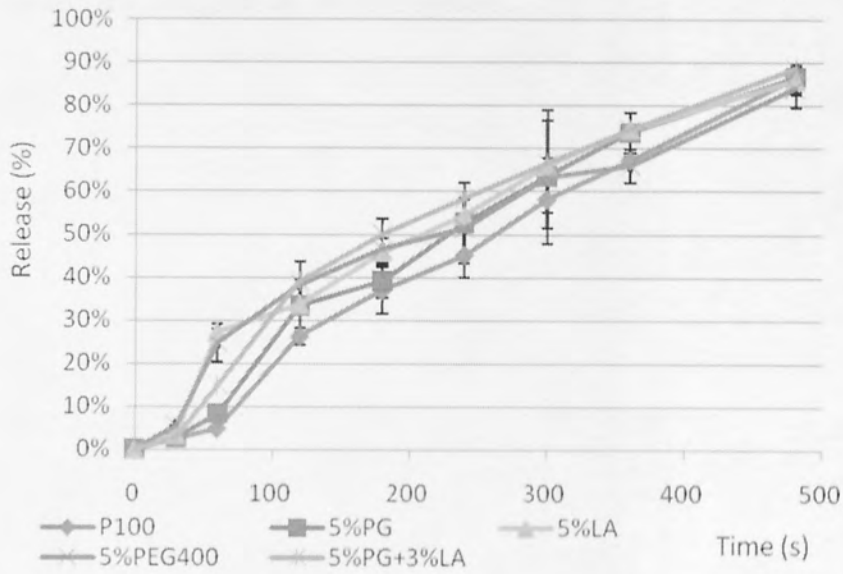
Drug can be released from the film matrix by diffusion through the polymer and erosion of the matrix. The drug release may be controlled by diffusion, by a combination of diffusion and erosion or solely by erosion of the delivery system (*Tuovinen et al., 2003*). By comparing polymer dissolution and drug release, it is hoped to get insight into what processes control release.

In my results, the curves of drug release and polymer dissolution from PD20, 5%PG and 5%LA almost overlap in each group from 120-second (see Figure 3.13, 3.14 and 3.15). The drug diffused out rapidly over the first minute from all the formulations, (Figure 3.17). The inclusion of both 5% LA and 5% PG in the formulation increased the drug release rate by increasing the polymer dissolution, and the mechanism for drug release from these two formulations is Fickian diffusion. While drug release from the formulations PD20 and 5% PEG400 is controlled by the polymer dissolution. We cannot judge the drug release mechanisms to be a polymer control release from the drug release and polymer dissolution at a same release rate, by showing the two release and dissolution curves overlapping each other. When the polymer dissolution is

affected by the drug and plasticiser, it just is a simple diffusion process and similar to the drug release.

3.5.1.2 Polymer dissolution from drug-free films

Figure 3.15 Polymer dissolution from P100 and drug-free films containing 5% (w/w) different plasticiser



All the curves of polymer dissolution from drug free films are linear dissolution, and the plasticisers do not have any major impact on dissolution at these levels. Additionally, as there is no consistent relationship to drug release mechanism it is not possible to predict the effect of plasticiser on drug release from drug-free films.

3.5.2 The application of Power Law

From my results, it is clear that Power Law is applicable for the most situations, except the drug "burst effect" in the early stages. I designed three series experiments (drug

release from films, polymer dissolution from drug loaded films, polymer dissolution from drug free films) to study the relationship between the drug release and polymer dissolution and reveal the drug release mechanism. However, when I compared the results from the experiments and used Power Law to analyze drug release models, I found that there was a conflict between these two methods. After studying the polymer, drug, and plasticiser interactions, the chemical structure of the compositions, and making a comparison of the results from the mechanical properties studies from Chapter two, it can be concluded that the Power Law is more reasonable for explaining the drug release mechanism, and should be considered when designing a polymeric vehicle to control the drug release for this system.

Chapter Four

In Vitro penetration through porcine esophagus

4.1 Introduction

4.1.1 Buccal mucosa as an potential site for drug delivery

4.1.1.1 Oral mucosa

- *Anatomy of oral mucosa*

The oral cavity contains four anatomically distinct sites with unique tissue types, which include the palatal, gingival, sublingual, and buccal mucosa. As the barrier is relatively thin and not keratinised (Table 4.1), the sublingual and buccal areas are the most commonly used for oral mucosal drug delivery.

Table 4.1 Characteristics of some human epithelia in the oral cavity

Location	Thickness (μm)	Keratinisation
Buccal	500-600	No
Sublingual	100-200	No
Gingival	200	Yes
Palatal	250	Yes

Modified from. *Stablein et al. (1984)*

The primary role of the buccal mucosa is to protect the underlying structures from foreign subjects. The surface of the buccal mucosa consists of a stratified squamous epithelium which is separated from the underlying connective tissue (lamina propria and submucosa) by an undulating basement membrane (a continuous layer of extracellular material approximately 1-2 μm in thickness) (*Rathbone et al. 1991*).

The rich arterial blood supply to the oral mucosa is derived from the external artery. The buccal artery, some terminal branches of the facial artery, the posterior alveolar artery, and the intraorbital artery are the major sources of blood supply to the lining of the cheek in the buccal cavity (*Stablein et al. 1984*).

The surface area of the mucosal tissue of the human oral cavity is approximately 100 cm^2, which is relatively small compared with that of the gastro-intestinal tract (GIT) (101 m^2). However, due to the lower enzymatic activity, less hostile environment, better stability of drugs including proteins and peptides, and the high vascular supply of the oral cavity, it is an attractive route for local absorption and an ideal environment for the initial dissolution of the dosage form. However, the high constant production and turnover of saliva and mouth activities can be variable and may have an impact on the performance of drug delivery systems, including film formulations.. In situations when salivary glands are stimulated, a more substantial amount of drug will be 'washed' to the GIT and the drug will be subject to the same restrictions as conventional systems, for example, it will be subject to first-pass metabolism.

- *The barrier nature of the buccal mucosa*

Membrane-coating granules (MCGs)

It has been demonstrated that the barrier properties of the buccal mucosa have been attributed to the upper one-third to one-quarter of the buccal epithelium and the permeability barrier may be attributed to the materials extruded from the MCGs (*Nicolazzo et al. 2005 a*). MCGs are spherical or oval organelles that are 100-300 nm in diameter, found near the upper, distal, or superficial border of the cells, and a few occur near the opposite border (*Nazila et al. 2005 a*). They discharge their contents, which are neutral lipids (mainly ceramides and acylceramides) (*Elias et al. 1979; Elias et al. 1981*), into the intercellular space and forms a barrier to the permeability of various compounds. This barrier exists in the outermost 200 μm of the superficial layer (*Amir et al. 1998*). MCGs are found in almost all stratified squamous epithelia, regardless of whether the epithelium is keratinised or not. (*Niicolazzo et al. 2005 a*).

Keratinisation

The mucosae of areas subject to mechanical stress (the gingivae and hard palate) are keratinised similar to the epidermis. The mucosae of the soft palate, the sublingual, and the buccal regions, however, are not keratinised. The keratinised epithelia are relatively impermeable to water because they contain neutral lipids like ceramides and acylceramides. In contrast, non-keratinised epithelia only have small amounts of ceremide and neutral but polar lipids (mainly cholesterol sulfate and glucosyl ceramides). The non-keratinised epithelia have been found to be considerably more permeable to water than keratinised epithelia (*Amir et al. 1998*).

The limit of penetration coincides with the level where MCGs were observed by researchers, and the same pattern was observed in both keratinised and non-keratinised epithelia, which indicated that keratinisation of the epithelia, in and of itself, is not expected to play a major role as a barrier to penetration (*Nazila et al. 2005*). However, the components of the MCGs of keratinised and non-keratinised epithelia are different. The MCGs of keratinised mucosae are composed of lameller lipids stacks, including sphingomyelin, glucosylceramides, ceramides, and other nonpolar lipids. For the non-keratinised epithelia, the major MCG lipid components are cholesterol esters, cholesterol, and glycosphingolipids (*Amir et al. 1998*) and a small number of them also contain lipid lamellae. The absence of organised lipid lamellae in the intercellular spaces of the buccal mucosa results in a greater permeability to exogenous compounds, compared to keratinised epithelia (*Nicolazzo et al. 2005 a*).

Mucus

A gel-like secretion known as mucus covers the entire oral cavity. Mucus is bound to the apical cell surface and acts as a protective layer to the cells below. It is also a visco-elastic hydrogel, and primarily consists of 1-5% of water-insoluble glycoproteins, 95-99% water, and several other compounds in small quantities, such as proteins, enzymes, electrolytes, and nucleic acids (*Nazila et al.* 2005). In performing its functions, mucus may adversely affect the absorption or action of drugs administered by the oral route (*Kavita et al. 2001*).

4.1.1.2 Pig esophageal mucosa as a permeability barrier model for buccal tissue

For the in vitro drug permeation through buccal mucosa, tissues from several animals have been used, including rabbit (*Nair & Chien, 1993; Dowty et al., 1992*), hamster (*Tsutsumi et al., 1999; Eggerth et al., 1987*), dog (*Galey et al., 1976*) and pig (*Chen et al., 1999; Artusi et al., 2003; Sandri et al., 2004*), however the epithelium of the rodent tissue is thick and keratinised, and the surface area is small (*Shojaei 1998*). Dog buccal mucosa is nonkeratinised and similar to human, but it is expensive for the routine use for in vitro penetration experiments (*Shojaei 1998*). Pig buccal mucosa is nonkeratinised and is closet to human tissue in terms of structure and permeability (*Shojaei 1998*), in addition, its low cost makes it a ideal model for drug penetration studies. The disadvantages of using the porcine buccal mucosa are: small cheek surface, damaged by mastication, firmly attached to the underlying muscular tissue. To overcome these drawbacks, I used pig esophageal mucosa to replace the buccal mucosa.

The porcine esophageal mucosa offers a large surface area and the tissue is smooth and intact. It consists of a stratified, squamous, nonkeratinised epithelium supported on a connective-tissue layer (*Squier & Kremer, 2001*). Furthermore, membrane coating granules (MCGs), which extrude lipids to form the permeability barrier in the buccal mucosa (*Shojaei 1998*), have also been found in esophageal epithelium (Hopwood et al., 1978). The appearance and localization of the MCGs in esophageal mucosa are similar to those in the buccal mucosa, and it has been proved that the secreted material has been correlated to the performance of the permeability barrier (*Hill et al., 1982; Hopwood et al., 1991*). Buccal and esophageal mocusae have very similar structures. Both of them comprise a stratified non-keratinised epithelium, supported on a loose connective tissue layer, which contains the microcirculation (lamina propria). The basal lamina separates the epithelium from the lamina propria. One difference between the mucosae is the presence of a smooth muscle cell layer arranged longitudinally in the esophagus, the muscularis mucosa (*Squier & Kremer, 2001*).
Del Consuelo and his colleagues compared the permeability of pig esophageal mucosa

and buccal tissue and fentanyl citrate was chosen as the model drug. The results from their studies showed that the permeability of pig esophageal mucosa and buccal mucosa was very similar to fentanyl. The permeability barrier of esophageal mucosa and buccal mucosa are both located in the epithelium. Separation of epithelia did not disturb the integrity of the barrier function. Moreover, freezing the tissues did not affect fentanyl transport through the mocusae (*del Consuelo et al., 2005*). According to the results showed from the experiments performed by del Consuelo, I used pig esophageal mucosa to replace buccal mucosa as the a penetration barrier model for Yonkenafil.

4.1.1.3 Saliva

Saliva is an adverse, unpredictable factor in intraoral drug delivery due to its complicated composition and great variability between individuals. Therefore, it has a great effect on polymer erosion, drug dissolution, release and absorption which cannot be ignored. For fast-dissolving films, their physical and chemical properties in saliva are critical in controlling the quality of the films, however, the properties are usually quite different from those in water, and it is important but difficult for researchers to build a mimic test system *in vitro* which is reliable and facile.

- *saliva Composition*

Saliva is a very dilute fluid, composed of more than 99% water and a variety of electrolytes, including sodium, potassium, calcium, magnesium, bicarbonate, and phosphates. Also found in saliva are immunoglobulins, proteins, enzymes, mucins, and nitrogenous products, such as urea and ammonia. The normal pH of saliva is 6 to 7, and can range from 5.3 (low flow) to 7.8 (peak flow) in the salivary flow (*Sue et al. 2000*).

- *Salivry Flow*

The individual salivary flow rate is quite variable (Table 4.2).

Table 4.2 The salivary flow rate in different situations (Modified from *Humphrey et al. 2001)*

Situation	Salivary flow rate
Unstimulated saliva normal flow	≥0.1 ml/min
Stimulated saliva normal flow	≥0.2 ml/min
Hypofunctional unstimulated saliva flow	<0.1 ml/min or 50% reduction in individualized base rate
Average unstimulated flow rate during waking hours (16 hours)	0.3 ml/min
Sleep	Nearly zero
Maximum stimulated flow rate	7 ml/min
Total daily flow of saliva	500 ml – 1500 ml
Summer	Circannian (yearly) low flow
Winter	Circannian (yearly) peak flow

The secretion of saliva is controlled by a salivary centre composed of nuclei in the medulla (*Grant et al. 1988*), but there are specific triggers or stimuli for this secretion, including mechanical, gustatory, olfactory, psychic factors, certain types of medication, and various local or systemic diseases affecting the glands themselves (*Humphrey et al. 2001*).

- *Salivary pH*

Buffering action is an important function of saliva through its components: bicarbonate, phosphate, urea, and amphoteric proteins and enzymes, which work much more efficiently during stimulated high flow rates but are almost ineffective during periods of low flow with unstimulated saliva (*Roth & Calmes, 1981; Edgar, 1990*).

Bicarbonate: the most important buffering system
Protein: >90% of the nonbicarbonate buffering ability of saliva is attributed to low-molecular-weight, histidine-rich peptides
Urea: release and increase plaque pH
Phosphate: likely to be important as a buffer during unstimulated flow

(Modified from *Humphrey et al. 2001*)

- *Salivary Secretion*

The secretion of saliva is affected by disease and stimuli. An acidic excipient can simulate the secretion of saliva, which is an important consideration in selecting formulation excipients (*Hao et. al., 2003*).

4.1.1.4 Routes of drug transport through buccal mucosa

The existence of hydrophilic and lipophilic regions in the oral mucosa has led to the proposition of the existence of two routes of drug transport through the buccal mucosa, paracellular (between the cells) and transcellular (across the cells). However, evidence in the literature suggests that most compounds actually traverse the buccal mucosa via the intercellular lipid domain and the existence of a transcellular route is questionable (*Nicolazzo et. al., 2005 a*). Therefore, another classification was presented comprising polar and nonpolar routes, which is more appropriate. The nonpolar route involves lipid elements of the mucosa by the partitioning of the drug into the lipid bilayer of the plasma membrane or into the lipid of the intercellular matrix, whereas the polar route involves the passage of hydrophilic compounds through ion channels in the intercellular spaces of the epithelium (*Harris & Robinson, 1992; Gandhi & Robinson, 1994*).

4.1.2 Penetration enhancement strategies

Considering the buccal mucosa as a subsequent potential site for transdermal drug delivery to the skin, most research and application of penetration strategies are developed from the techniques which have been employed on the skin. As the structure and composition are different from the skin, such as keratinisation, and lipids constitution, the researchers are seeking and modifying the most optimized penetration pathway for the buccal mucosa.

4.1.2.1 Supersaturation

Generally, the flux of a drug from a delivery device across a membrane is predicted by Fick's first law of diffusion (*Higuchi, 1960*):

$$J = \frac{C_v}{C_{s,v}} \times \frac{D \times C_{s,m}}{L}$$

(Eq. 4.1)

where J is the permeation rate of the drug through the barrier, C_v is the drug concentration dissolved in the vehicle; $C_{s,v}$ and $C_{s,m}$ are the solubility of the drug in the vehicle and in the barrier, respectively; the ratio $C_v/C_{s,v}$ represents the degree of saturation of the drug in the formulation; D represents the diffusion coefficient of the drug in the membrane; and L is the diffusion pathlength across the barrier. In this equation, J is proportional to C_v, which means that the degree of saturation directly effects the penetration flux, consequently the more drug dissolved in the vehicle, the higher the permeation rate that can be achieved.

Although the supersaturated system enhances the driving force for drug delivery and increases the skin permeation, the thermodynamic instability restricts its application. Varieties of cosolvents and polymeric vehicles (*Davis & Hadgraft, 1991; Iervolino et al. 2000; Kondo et al. 1987; Megrab et al. 1995.*) were added to overcome this drawback, or the supersaturated system can be made immediately prior to application if the long-term storage is not feasible for those supersaturated dosages.

Generally, there are three ways to gain a supersaturated system (*Moser et al. 2001*).

- By the solvents permeating through the skin
- The volatilization of the solvent during administration
- By creating a cosolvent system wherein vehicle changes are produced immediately prior to application of the formulation.

4.1.2.2 Bioadhesion

Bioadhesion refers to a synthetic or natural macromolecule that forms any bond with a

biological surface so that they are attached each other (*Longer & Robinson, 1986*). In generally, it is used to describe the adhesion between polymers and the mucosal tissues (i.e., buccal mucosa, gastrointestinal mucosa) or other biological surface.

There are three steps involved in the process of bioadhesion (*Duchene et al. 1988; Ponchel et al. 1991*):

- The polymer is hydrated and swells once it contacts the tissue
- The polymer chains start to interpenetrate into the epithelium or entangle with the mucin chains
- Chemical or physical bonds are formed between the entangled chains

Mechanisms of polymer attachment to mucosal surfaces are not yet fully understood. However, certain theories of bioadhesion have suggested that it might occur via physical entanglement and/or chemical interactions, such as electrostatic, hydrophobic, hydrogen bonding, and van der Waals' interactions. A variety of factors affect the mucoadhesive properties of polymers, such as molecular weight, flexibility, hydrogen bonding capacity, cross-linking density, charge, concentration, hydration (swelling) of a polymer, and the environmental factors. Mucoadhesive dosage forms for buccal administration include buccal tablets, buccal patches, buccal films, buccal gels and ointments (*Nazila et al. 2005*). Apart from the function of increasing the retention time of the drug on the mucosal surface to enhance the bioavailability, some polymers can be used as enzyme inhibitors and penetration enhancers (*Lueßen et al. 1994; Lueßen et al. 1995*). It has been proven that the presence of numerous polymers absorb the water from the epithelial cells to widen the tight junction (*Artursson et al. 1994*).

4.1.2.3 Penetration enhancers

Penetration enhancers are the agents designed to enhance drug permeability through the skin or mucosa (*Ganem-Quintanar et al. 1997*). Basically, a classic penetration enhancer should be nonirritant, nontoxic, and non-allergenic. Moreover, it should work rapidly, be compatible with the drug and vehicle, and have no reactions within the tissue (*Barry, 1993*). For the purposes of buccal delivery, an odorless and tasteless penetration enhancer would be more acceptable by the patients.

- *Ethanol*

As a solvent or co-solvent, ethanol usually can increase the drug solubility in the vehicle to maximize drug loading. Secondly, evaporation from the vehicle is likely to lead supersaturation during the drug release, thereby increase the drug permeation rate. Lastly, it has been suggested that ethanol disrupts the lipid molecules arrangement. This has been demonstrated with the transport of tritiated water across the oral mucosa (*Howie et al. 2001*), but the mechanism is unclear. Ethanol has been used as a absorption enhancer in skin and is claimed to induce modification at the polar head group region of the lipid bilayers (*Ghanem et al. 1992*), however, it is doubtful that the same reaction would happen in buccal mucosa because the lipid composition is different from skin (*Nicolazzo et al. 2005 a*).

- *Surfactants*

There are several mechanisms and hypotheses to explain the effect of enhanced permeability through the buccal mucosa produced by surfactant, and the main conclusion is that surfactants extract the intercellular lipids (*Nicolazzo et al. 2005 b*). It is known that surfactants can form different structural micelles in different concentration. As a result, the effect on lipid extraction is affective by the micelles forming. Moreover, the presence of drug and other pharmaceutical excipients may also contribute to the formation of micelles, therefore, drug transportation through the mucosa and the solubility in the tissue is complicated. Additionally, the nature of the drug is critical in determining the enhancing power of surfactants. For example, SDS enhanced the permeability of chemicals with logP < 3 but had no effect on those with logP >3 in an *in vitro* experiment using rat skin (*Borra's-Blasco et al. 1997*). It is difficult to comment on the ability of a surfactant to enhance the permeability of compounds generally, because there are too many variables involved in the mechanism of action as a penetration enhancer to a specific drug, as a result, it is important to study the micelles forming process of the applied drug and surfactant.

- *Fatty acids*

The precise mechanism of fatty acid enhancement of buccal permeation is unclear because there is no direct evidence to fully support any hypothesis (*Nicolazzo et al. 2005 a*). The probable mechanism is based on increasing the fluidity of intercellular lipid, shown by using DSC and FTIR (*Golden et al. 1990; Francoeur et al. 1990; Mak et al. 1990*). From fluorescence anisotropy, it has been proven that oleic acid reduces the lipid packing order in buccal epithelium cell membranes (*Turunen et al. 1994*)

- *Azone*

Azone has been widely studied and applied as a skin penetration enhancer. However, the only effect of azone on enhancing permeability through buccal mucosa that has been demonstrated is increasing the buccal mucosal uptake and retention of compounds (*Nicolazzo et al. 2004 a; Nicolazzo et al. 2005 b)* . According to Fick's first law of diffusion (Eq. 4.1), the higher drug concentration in the buccal mucosa may increase the diffusion rate of transport from mucosa to the body system, but when the diffusion rate is constant and not affected by the drug concentration in the mucosa, the drug will accumulate in the mucosa and probably cause irritation, allergic reaction or toxicity to the buccal mucosa. From the present research, azone has been used to promote local rather than systemic delivery. Moreover, the azone used in the previous experiments is 5% w/v in EtOH 95% v/v, and ethanol may provide a degree of enhancement itself that should be considered. It has been shown using fluorescence anisotropy that azone reduces the lipid packing order of buccal epithelium cell membranes (*Turunen et al. 1994*), but there is little detail evidence to identify the mechanism of action of azone on buccal mucosa.

- *Sunscreen skin penetration enhancers*

Sunscreen agents, such as octisalate and padimate O, have been shown to improve transdermal permeability of a number of compounds *in vivo* and *in vitro* (*Morgan et al. 1998a; Morgan et al. 1998b; Morgan et al., 1998c*). Little work has been done on the action of these agents on buccal mucosa, but it has been reported that both octisalate

and padimate O have no effect on caffeine and triamcinolone permeability, and even reduce the permeability of estradiol through porcine buccal epithelium. Padimate O had a similar ability with azone, increasing the reservoir capability of buccal mucosa for estradiol (*Nicolazzo et al. 2004 b*).

- *Terpenes*

Terpenes are a large and varied class of hydrocarbons, which are primarily produced by plants and have been used as medicines, flavorings and fragrance for a long time. Menthol is a monocyclic terpene with a pleasant taste and odor. It has been shown to exert an effect on transdermal absorption of drugs (*Okabe et al., 1989; Williams and Barry, 1991; Kobayashi et al., 1994; Kaplun-Frischoff and Touitou, 1997; Gao and Singh, 1998; Kommuru et al., 1998*). The most significant advantage of menthol as a penetration enhancer is its relatively low toxicity (*Yamaguchi et al., 1994*). Since the taste and odour emitted from menthol will stimulate the patient's smell and taste, there may cause some relative physiological or psychological reaction in the oral cavity (i.e., increasing or reducing of saliva flow or discomfort if the patient is repelled by the taste and odor etc.). But there has been not any systematic research based on these effects so far. However, such side effects should be considered in product development because they will have an impact on the drug delivery (affected by physiological reactions) and drug efficacy (affected by psychological factors). To date, only one report has described the permeation enhancement ability of menthol used in buccal drug delivery. That experiment evaluated menthol for improving the permeability of dideoxycytidine across porcine buccal epithelium *in vitro*. The result showed that the presence of low concentrations (0.1 - 0.3mg/ml) of l-menthol elevated dideoxycytidine permeation through the membrane from the solution in the donor chamber, due to increased partitioning into the tissue (*Amir et al., 1999*). However, this transbuccal permeation experiment lasted 1440 minutes, and there is no significant difference over early stages (from 0 to 240), which means menthol may not be an effective permeation enhancer for buccal drug delivery because it acts too slowly and it is difficult to maintain a device in the mouth for such a long time, considering the saliva flow, mechanical movement of the mouth and tongue, and patient acceptability.

- *Other physical strategies*

There are other penetration enhancement strategies for the drug delivery through skin, such as microneedles (*Mark 2004*), phonophoresis (*Hideo et al., 1995*) and laser treatment (*Gómez et al., 2008*). However, these methods would not be suitable for buccal drug delivery because most of them have an effect on the stratum corneum which does not exist in buccal mucosa and the apparatus would not lend itself to adaptation for buccal delivery.

4.2 Materials and Methods

4.2.1 Materials

Pullulan: Hayashibara Biochemical Laboratory Inc. Japan.

Propylene glycol (PG): Sigma, Chenmical Co, Germany.

Polyethylene Glycol 400: Aldrich Organics, New Jersey, USA

DL-Lactic Acid: Aldrich Organics, New Jersey, USA.

Yonkenafil: a gift from Tasly Group, China.

Ethanol: Laboratory grade, Fisher Scientific, United Kingdom.

Sodium dodecyl sulfate (SDS): 98%, Fisher Scientific, United Kingdom.

Menthol: natural, BDH Chemical Ltd, England.

Lauric acid: Aldrich Organics, New Jersey, USA

1-Octanol: anhydrous, Aldrich Organics, New Jersey, USA

Acetonitrile: HPLC grade, Fisher Scientific, United Kingdam

Triethylamine: HPLC grade, Fisher Scientific, United Kingdam

4.2.2 Tissue preparation

Porcine oesophagus tissue from domestic pigs was purchased from an abattoir (J F Longman LTD, Stoke-on-Trent, UK) after slaughter and was transported in a sealed foam box filled with biological ice cubes. The oesophagus was cut longitudinally and rinsed with isolated saline water (0.9% w/v sodium chloride solution), the mucosa was separated from the muscular layer by cutting the loose connective fibers with a scalpel, then dried on filter paper and stored in a standard freezer at -20°C wrapped in aluminium paper. Storage was no longer than 3 weeks. For the experiments using isolated epithelium, the excised mucosae were defrosted in 37°C saline, then immersed in saline at 65 °C for 1 min, following which the epithelia were carefully peeled away from the connective tissue (*del Consuelo et al., 2005).*

4.2.3 Permeation methodology

The prepared oesophagus mucosa membranes were mounted between the donor and receiver chamber of vertical Franz type diffusion cells (Telesystem, Variomag) with diffusion area of 1.77 cm^2. The receiver chambers were filled with 7 ml of pH 5.5 PBS (in order to ensure sink conditions of Yonkenafil) and the donor chambers were filled with 1ml pH 6.75 PBS to stabilize for 30 min. The temperature was maintained at 37°C using a water jacket surrounding the chambers and the media in receiver chambers was stirred by a magnetic stirring bar at 400 rpm. Prior to the experiment, the PBS in the donor chamber was removed and replaced with 0.5 ml of the relevant penetration enhancer (95% ethanol (v/v), saturated SDS, menthol, oleic acid, lauric acid pH 6.75 buffer solutions) separately and left to equilibrate for 1 min. The enhancer solution was then removed and 1 ml of a saturated yonkenafil pH 5.5 buffer solution (4.5 mg/ml) was added to the donor chamber.

To study drug penetration from the films (PD20, 5%LA and 5p%PEG400 see Table 4.3), they were pre-wetted with one drop of PBS then laid on the membrane following equilibration with enhancers or PBS, according to the protocols outlined in table 4.3. One ml PBS (pH 5.5, 6.75 7.4) was added to the donor chamber and the experiment was initiated. 0.5 ml samples were withdrawn from the receptor chamber at

predetermined time intervals (30, 60, 120, 180, 240 and 300 minutes), replaced with the same volume of fresh medium and subsequently assayed by HPLC (see section 4.2.4).

Table 4.3 The protocol of drug penetration experiments

Reference Num.	Protocol
PS1	1 ml 4.5 mg/ml YKN in pH 5.5 PBS
PS2	Pretreated with 0.5ml ethanol, removed, then add 1ml 4.5mg/ml YKN in pH5.5 PBS
PS3	PD20* in 1ml pH7.4 PBS
PS4	PD20 in 1ml pH6.75 PBS
PS5	PD20 in 1ml pH5.5 PBS
PS6	5%LA* in 1ml pH6.75 PBS
PS7	5%PEG400* in 1ml Ph6.75 PBS
PS8	Pretreated 0.5ml lauric acid saturated pH 6.75 PBS, removed, then PD20 in pH6.75 buffer
PS9	Pretreated 0.5ml oleic acid saturated pH 6.75 PBS, removed, then PD20 in pH6.75 buffer
PS10	Pretreated 0.5ml SDS saturated pH 6.75 PBS, removed, then PD20 in pH6.75 buffer
PS11	Pretreated 0.5ml 95% ethanol (v/v), removed then PD20 in pH6.75 PBS
PS12	Pretreated 0.5ml menthol, removed, then PD20 in pH6.75 PBS
PS13	Pretreated 0.5ml lauric acid, removed, then add 1ml 4.5mg/ml YKN in pH5.5 PBS
PS14	Pretreated 0.5ml menthol, removed, then add 1ml 4.5mg/ml YKN in pH5.5 PBS
PS15	Pretreated 0.5ml SDS, removed, then add 1ml 4.5mg/ml YKN in pH5.5 PBS

PD20*: 20% drug, 80% pullulan (w/w)

5%LA*: 20% drug, 75% pullulan, 5% lactic acid (w/w)

5%PEG400*: 20% drug, 75% pullulan, 5% PEG400 (w/w)

4.2.4 HPLC Method

As prescribed before, the concentration of the drug penetrated through the membrane into the receptor chamber was assayed by high-performance liquid chromatography (HPLC, 10Avp system, Shimadzu Corp., Japan), under following conditions, ODS Hypersil column (150 × 4.6 mm, Supelco corp.), 20 mM citric acid solution (adjusted to pH 5.4 with triethylamine) – acetonitrile (40:60) eluent, 1.0 ml/min flow rate, detection, UV at 324 nm. A calibration curve using known concentrations of the drug was performed prior to each analysis.

4.2.5 1-Octanol/buffer partition coefficient determination

Octanol/buffer coefficients for Yonkenafil were determined between 1-octanol and saturated solutions of three penetration enhancers (menthol, oleic acid and lauric acid) in phosphate buffer (pH 6.75) and at three different pHs (5.5, 6.75, 7.4). The partition coefficients of Yonkenafil between 1-octanol and buffer solutions were determined by a shake-flask method. Buffer solutions were previously saturated with 1-octanol. Different but precisely weighed quantities of Yonkenafil (see table 4.4) were dissolved in the buffer solutions separately (because Yonkenafil has significant different solubility in different buffer solutions).

Table 4.4 The amount of Yonkenafil added in different saturated penetration enhancer pH 6.75 buffer solutions

Buffer	Menthol	Oleic Acid	Lauric Acid
Amount of Yonkenafil added (mg/ml)	1.00	2.00	1.00
Solubility of Yonkenafil (mg/ml)	1.13	2.23	1.39

Equal volumes (5ml) of Yonkenafil-buffer solution and 1-octanol were mixed in the screw-capped glass scintillation vials. The two phases were equilibrated under constant shaking at 37°C for 24 hours. The aqueous phase was separated using separatory

funnels and diluted prior to analysis using HPLC. The partition coefficient (Ko/w) was calculated as follows:

$$K_{o/w} = \frac{C_{aq} - C_{eq}}{C_{eq}}$$

(Eq. 4.2)

C_{aq} and C_{eq} denote the initial and equilibrium concentration of Yonkenafil in the buffer solution respectively.

4.3 Result and Discussion

4.3.1 The solubility and partition coefficient of Yonkenafil in different buffer solutions

The result of the solubility and partition coefficients (Ko/w) of Yonkenafil in pH 5.5, 6.75 and 7.4 phosphate buffer, menthol saturated pH 6.75 buffer and lauric acid saturated pH 6.75 buffer are shown in table 4.5 below:

Table 4.5 The solubility and partition coefficient of Yonkenafil in different buffer solutions (n=3; mean ±s.d.)

	Solubility (mg/ml)	Partition Coefficient (Ko/w)
pH 5.5	4.52±0.13	10.22±1.23
pH 6.75	1.23±0.09	25.67±1.25
pH 7.4	0.16±0.02	0.88±0.21
Menthol saturated	1.13±0.10	21.00±1.65
Lauric acid saturated	1.39±0.08	40.54±3.87

Yonkenafil is soluble in pH 5.5 buffer but is almost insoluble in pH 7.4 buffer. Menthol and lauric acid had no significant effect on the drug solubility in pH 6.75 buffer; but, lauric acid increased the partition coefficient by 15 compared with the pH 6.75 buffer. The effect of solubility and partition coefficient of Yonkenafil to the permeability will

be discussed in the following sections separately.

4.3.2 Drug penetration through oesophageal mucosa from drug saturated pH 5.5 PBS

4.3.2.1 Pretreated with 95% Ethanol

Figure 4.1 Penetration of Yonkenafil through normal pig oesophagus (standard) and those pretreated with ethanol over 5 hours (n=5; mean±s.d)

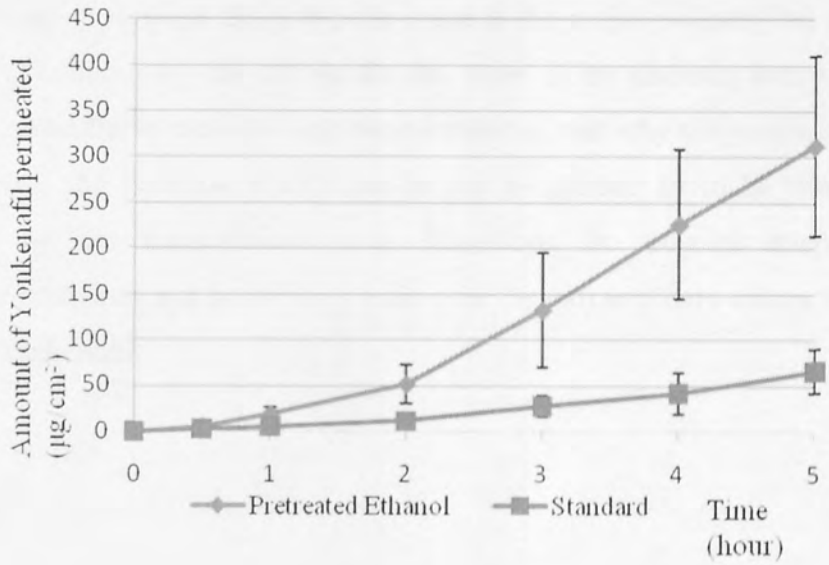


Table 4.6 The lag time and steady state drug flux through the oesophageal mucosa pretreated with ethanol

Protocol	t_{lag} (h)	J * 10^{-3} (μg/cm^2/s)
Standard	1.24±0.14	5.61±0.91
Ethanol pre-treatment	1.46±0.21	24.28±7.19

The permeation profile of Yonkenafil in pH 5.5 buffer with or without pretreated with ethanol are shown in Figure 4.1. The t_{lag} and steady state drug flux (J, from 2 to 5 hour, μg/cm^2/s) were calculated from the penetration graph and are listed in Table 4.6.

From Figure 4.1, it can be seen that ethanol pretreatment significantly increased the

permeability of Yonkenafil through the pig oesoghageal mucosa and reached a steady state after two hours.

As outlined in section 4.1.2.3, the mechanism of the enhancing effect of ethanol on buccal mucosa has not been identified yet. Only a few reports have demonstrated that ethanol can improve hydrophilic molecules permeability through buccal mucosa by affecting the intercellular domains of the epithelium. Yonkenafil, an amphiphilic compound, has a partition coefficient ($K_{o/w}$) between pH 5.5 buffer and 1-octanol of 1.01. Therefore, both the transcellular and intercellular routes are viable for this drug. According to the present research, the significant enhancement effect produced by ethanol may result from its improvement of the paracellular transport of the drug. However, it has been reported that passive diffusion of the un-ionized form of the drug across the mucosa through the non-polar route is the major pathway for drug buccal absorption (*Nicolazzo et. al., 2008*). So far, there is no research into how ethanol affects the transcellular route through buccal mucosa, and why the presence of ethanol could increase the partition coefficient in the membrane to make more drug be transported by the transcellular route. Therefore, the ethanol interrupting the intercellular route may not be the only reason for the extraordinary enhancing effect of ethanol on Yonkenafil.

4.3.2.2 Pretreated with Menthol or Fatty acid saturated pH 6.75 buffer

Figure 4.2 Penetration of Yonkenafil through normal pig oesophagus (standard) and those pretreated with menthol or lauric acid over 5 hours (n=5; mean±s.d)

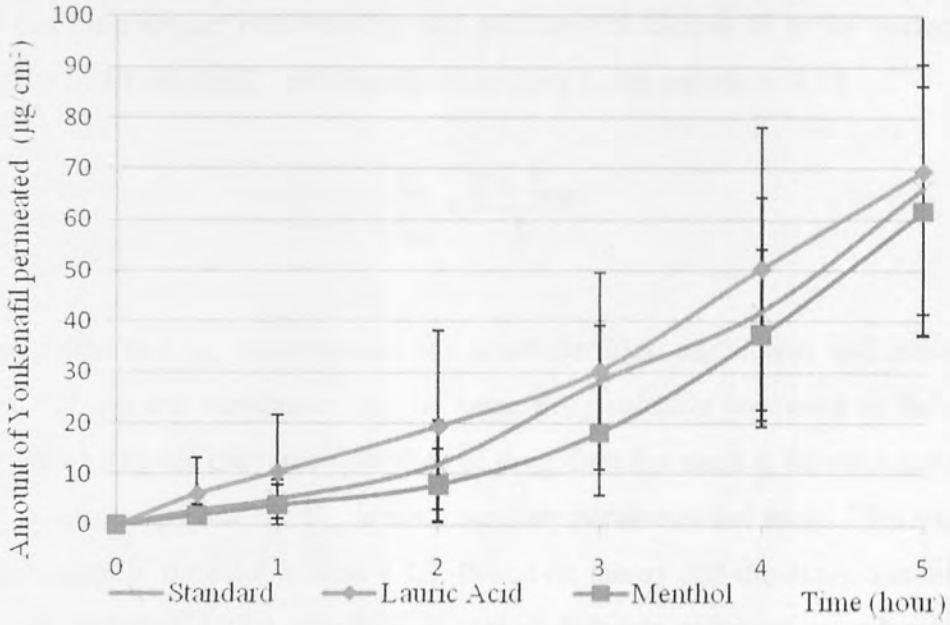


Table 4.7 The lag time and steady state drug flux through the oesophageal mucosa pretreated with saturated menthol or lauric acid pH 6.75 buffer solution

Protocol	t_{lag} (h)	J * 10^{-3} (µg/cm^2/s)
Standard (PS1)	1.24±0.14	5.61± 0.91
PS 13	1.82±0.13	5.07±1.35
PS 14	1.85±0.53	4.68±1.25

Table 4.8 The solubility and partition coefficients of Yonkenafil in saturated menthol or lauric acid pH 6.75 buffer solution

Buffer	Solubility (mg/ml)	Partition Coefficient (Ko/w)
pH 6.75	1.23±0.09	25.67±1.25
Menthol saturated	1.13±0.10	21.00±1.65
Lauric acid saturated	1.39±0.08	40.54±3.87

The drug permeation rate through the mucosa pretreated with menthol and lauric acid

saturated pH 6.75 buffer solution are shown in Figure 4.2. From the statistics study, it could not be seen a significant difference among the three curves ($P_{1\text{-}13}$=0.5294, $P_{1\text{-}14}$=0.3017, $P_{13\text{-}14}$=0.7150). As penetration enhancers, menthol and lauric acid, neither of them has an effect on the Yonkenafil permeability through the oesophageal mucosa.

The drug solubility and partition coefficients are shown in Table 4.8.The presence of menthol has little impact on solubility and partition coefficient as in the buffer, only reducing the partition coefficient slightly. According to the equation (4.1)

$$J = \frac{C_v}{C_{s,v}} \times \frac{D \times C_{s,m}}{L}$$

J is proportional to $C_{s,m}$, which means the lower partition coefficient will reduce the drug flux through the membrane. As the same drug solution was used in the donor chamber, that being the case, the solubility of drug does not need to be considered, but when the films are applied, the $C_{s,v}$ is not a constant parameter any more. This situation will be discussed in the later section 4.3.3. Based on theory and the experimental data, it could be concluded that menthol is not a suitable penetration enhancer for Yonkenafil. Amir et al. (1999) reported that menthol was seen to increase the partitioning of dideoxycytidine into the tissue thus increasing the drug permeability. The methodology is different as Amir et al. (1999) added menthol into the donor solution, while, in the current study a buffer solution saturated with menthol was used to pretreat the tissue. My data was gathered over 5 hours rather than 24 h (Amir et. al,, 1999) and Amir reported that there was no significant difference in the first 4 hours from his Data. Therefore, menthol was not seen to exert any effect on the tissue within the time course of this study and although this may be due to a long lag time, it would not prove useful in practice.

As for lauric acid, which has little solvating effect on the drug, however, it increased the partition coefficient to 1.58 times compared with the pH 6.75 buffer. It is reported that the fatty acid is likely to increase the fluidity of intercellular lipid (*Golden et al. 1990; Francoeur et al. 1990; Mak et al. 1990*), but this hypothesis is not supported in these results. There are no significant differences ($P_{1\text{-}13}$=0.5294) between the two curves over the time course of this experiment, although it does not rule out changes at

extended times.

4.3.2.3 Pretreated with Saturated Sodium Dodecyl Sulfate (SDS) pH 6.75 buffer

Figure 4.3 Penetration of Yonkenafil through normal pig oesophagus (standard) and those pretreated SDS over 5 hours (n=5; mean±s.d)

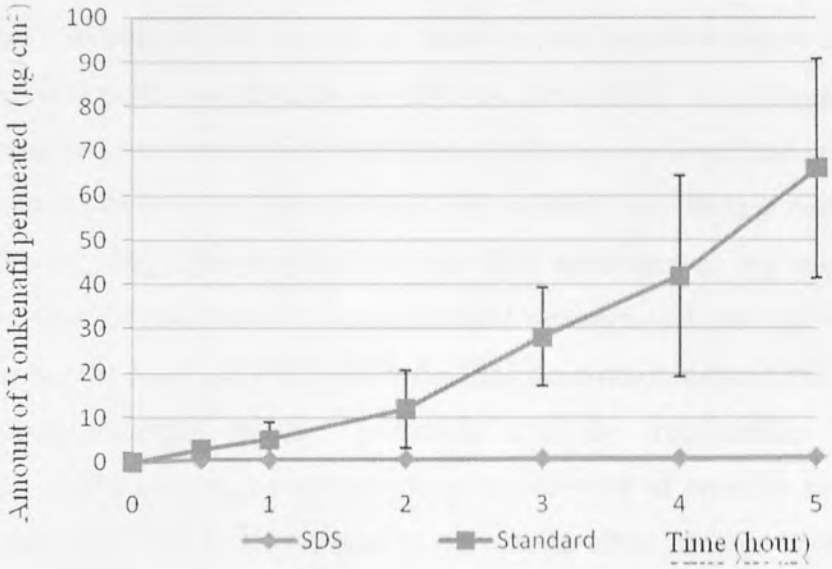


Table 4.9 The lag time and steady state drug flux through the oesophageal mucosa pretreated with saturated SDS pH 6.75 buffer solution

Method	t_{lag} (h)	J * 10^{-3} (μg/cm^2/s)
Standard	1.24±0.14	5.61±0.91
PS 15	N/A	0.05±0.02

Drug penetration through the mucosa pretreated with SDS saturated pH 6.75 buffer is shown in Figure 4.3. The result indicates that pretreatment with SDS reduced the drug flux to nearly zero within 5 hours. It is reported that SDS can affect the lipid bilayers in the skin epithelium intercellular spaces, which may also happen in buccal mucosa, to increase the hydrophilic compound's permeability. (*Nicolazzo et al. 2004 b*). Otherwise, as a surfactant, SDS and the drug can form micellar-bound complex which does not permeate biological membranes (*Ganem-Quintanar et al. 1997*). For these two reasons, micelle formation should be the most probable reason attributed to the

significant reduction of drug permeability. Yonkenafil, as an amphiphilic compound, is able to pass through the mucosa by either the paracellular route or intercellular route. Therefore, as SDS is likely to increase the drug permeability by affecting the lipid bilayers, it was expected that permeation would increase. Previous studies using 0.05, 0.1 and 1% SDS in Krebs bicarbonate Ringer solution to pretreat the mucosa for 2 hours caused a marked loss of superficial cell layers (*Nicolazzo et al. 2004 b*). In the current the concentration of the saturated SDS buffer solution is much higher than 1%, and there is no morphological research investigating the effect of such high concentration SDS solution on epithelium, therefore, we cannot conclude that whether the saturated SDS buffer solution has an effect on the mucosa, even though the tissue was only pretreated for 1 minute and the SDS solution was removed and replaced after pretreated. On the other hand, any residual SDS solution may form a micllear-bound complex with the drug. The level of residual SDS solution was not calculated and measured because the solution for tissue pretreated was removed and replaced with the drug solution or the fresh pH 6.75 buffer (in film penetration experiment). For these reasons, Yonkenafil-SDS micelle formation and the relationship with SDS concentration would need to be understood and controlled in order to avoid micelle formation, and SDS may have penetration enhancing effect at lower concentration. However, due to the unpredicatability of this relationship and because it is hard to estimate and to control the surfactant concentration in the environment after it releases from the films, it as decided that SDS would not be a suitable permeation enhancer for this formulation.

4.3.3 Drug penetration through oesophageal mucosa from drug loaded pullulan films

4.3.3.1 Effect of pH

The pH effect on ionisable drug absorption through buccal mucosa has been widely studied (*del Consuelo et al., 2005-b; Mashru et al., 2005; Kokate et al., 2007*). The flux of drug through paracellular and transcellular route can be expressed by equations 4.3 and 4.4.

$$J_h = \frac{D_h \times e \times C_d}{h_H}$$

(Eq. 4.3)

$$J_L = \frac{(1-e) \times D_l \times K_p \times C_d}{h_L}$$

(Eq. 4.4)

J_h: flux of drug in paracellular route

e: faction of surface area of the paracellular route

D_h: diffusion coefficient in the intercellular spaces

h_H: pathlength of paracellular route

C_d: donor side drug concentration

J_L: flux of drug in transcellular route

h_L: pathlength of transcellular route

In equation 4.4, Kp is the partition coefficient between the lipophilic region (cell membrane) and hydrophilic region (delivery solution, intercellular space and cytoplasm). Because the partition coefficient of an ionisable drug is pH dependent, drug absorption is also pH dependent when the drug is transported via the transcellular route. Otherwise, pH should not affect drug absorption rate if it is transported via the paracellular route where is no partitioning involved, except for influences on solubility. Table 4.10 shows the solubility of Yonkenafil in pH 5.5, 6.75 and 7.4 buffer solutions. The normal pH of saliva is 6 to 7, and can range from 5.3 (low flow) to 7.8 (peak flow) (*Humphrey et al. 2001*). According to the significantly different solubility ($P_{5.5\text{-}6.75}$=0.9985 $P_{6.75\text{-}7.4}$=0.9937) of Yonkenafil in at different pH, the variation of the pH in oral cavity may have an effect on the drug permeability and release from the polymeric films.

Table 4.10 The solubility and partition coefficient of Yonkenafil in different pH buffer solutions

pH	Solubility (mg/ml)	Partition Coefficient (Ko/w)
5.50	4.52±0.13	10.22±1.23
6.75	1.23±0.09	25.67±1.47
7.40	0.16±0.02	0.88±0.21

Figure 4.4 Penetration of Yonkenafil from PD20 through pig oesophagus in different pH media over 5 hours (n=5; mean±s.d)

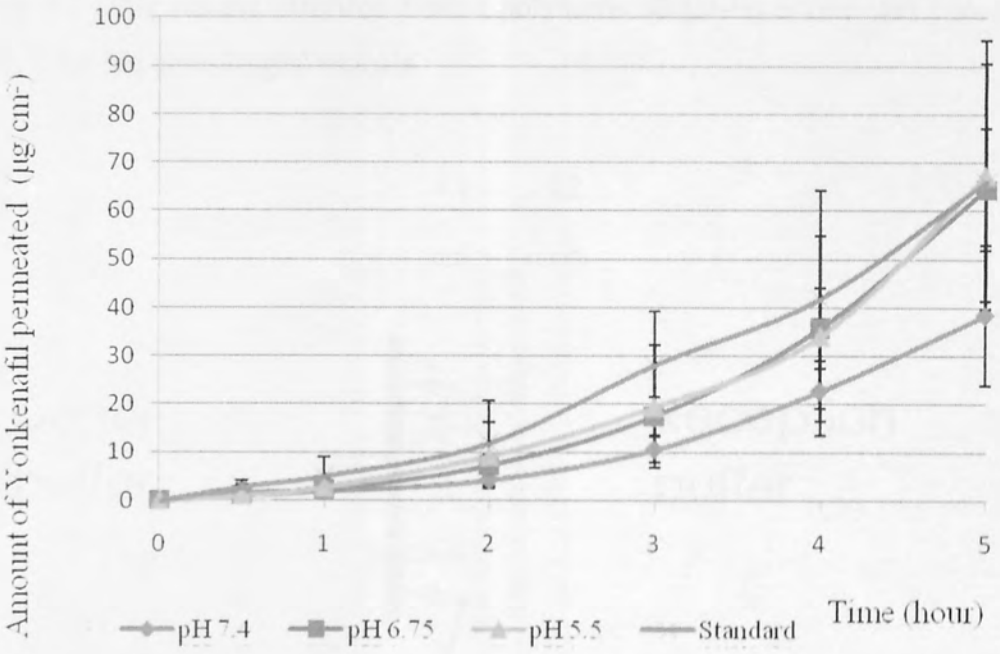
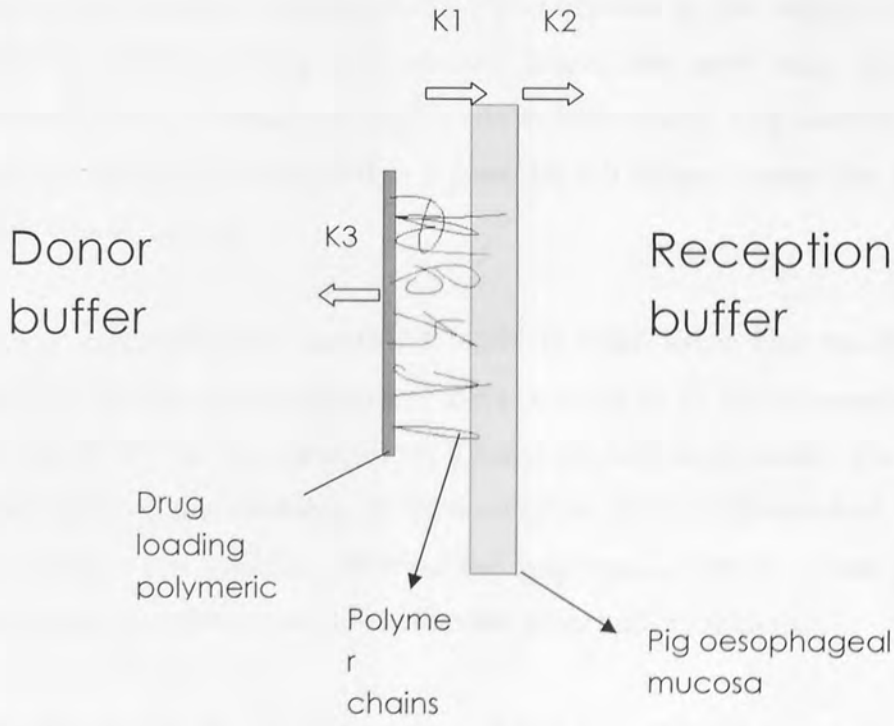


Table 4.11 The lag time of drug release from PD20 and steady state drug flux through the oesophageal mucosa in different pH buffer solutions

pH	t_{lag} (h)	J * 10^{-3} (μg/cm^2/s)
Standard	1.24±0.14	5.61±0.91
5.5	1.53±0.38	5.25±0.43
6.75	1.83±0.19	5.26±1.22
7.4	1.78±0.26	3.19±1.23

The penetration profile of Yonkenafil through pig oesophageal mucosa in different pH buffers is shown in Figure 4.4 above. From the graph, it can be seen Yonkenafil was transported faster in pH 5.5 and 6.75 buffer media than in pH 7.4 from the pullulan films. There are three main parameters that should be considered when the drug is loaded by a film; the first is the drug release rate from the pullulan films, the second is the drug concentration in the donor chamber, and third is the drug ionisation in the media. The relationship between drug release from the films, drug penetration rate through the mucosa are shown in Figure 4.5.

Figure 4.5 Drug release direction from a polymeric single-layer film and penetration through the pig oesophageal mucosa



K1: Drug partition coefficient between the surface of mucosa and cell membrane or intercellular space

K2: Drug partition coefficient between the mucosa and reception buffer. K2 is determined by the coefficient of paracellular transport and intercellular transport.

K3: Drug release from a film into the donor buffer

In comparison with the standard (saturated drug pH 5.5 PBS) curve (Figure 4.4), the lag time of drug transported in pH 6.75 and pH 5.5 buffer from the films (see table

4.10 t_{lag}) is about 0.5 hour higher than from the saturated drug pH 5.5 PBS. This is because it took more time for the drug release to begin from the polymeric films than the drug saturated solution. In the drug release experiments discussed in Chapter Three, Yonkenafil was completely released from the films in about 10 minutes in a shaking water bath experiment. It is apparent that the pullulan film is a fast releasing drug delivery system. From Figure 4.4, there is no significant difference between permeation from the films and the saturated drug solution ($P_{1\text{-}5}$=0.7667), indicated that the drug release from the films will not affect the penetration rate through the membrane in pH 5.5 and 6.75 buffer solution.

Although the solubility of Yonkenafil in pH 5.5 buffer is approximately three times higher than in pH 6.75, about 1.23 mg/ml, there is no difference in permeation ($P>0.05$). It is therefore concluded that J is a constant in this situation, which is not affected by the donor drug concentration. Due to the rapid drug release from the polymeric films and similar penetration rate as the saturated drug solution, the pullulan film as a vehicle for Yonkenafil is a potential fast release system for drug delivery through buccal mucosa.

The drug penetration rate in pH 7.4 buffer is much lower than the other two; the cumulative amount of drug penetrated in 5 hours was 38.52 $\mu g/cm^2$, compared to 66.01, 67.61 and 64.45, for the standard, pH 5.5 and pH 6.75 respectively. The main reason for this result is the solubility of Yonkenafil in pH 7.4 buffer, which is only 0.16 mg/ml. Such a low solubility inhibited the drug release from the films, therefore, the donor drug concentration was lower than the other buffer solutions.

As the pH of salivary can be vary from 5.3 to 7.8, this will have a large impact on solubility of Yonkenafil and the implications of this on release and permeation of Yonkenafil is a quite important issue for the future research.

4.3.3.2 Effect of 5% PRG400 and 5% Lactic Acid

Figure 4.6 Penetration of Yonkenafil from different films through pig oesophagus over 5 hours (n=5; mean±s.d)

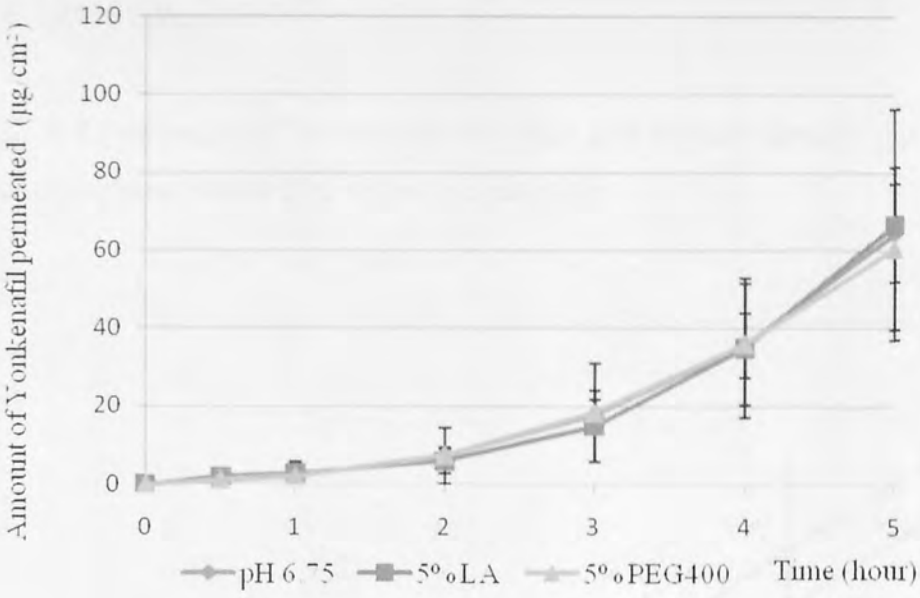


Table 4.12 The lag time and drug release from films containing 5% LA or 5% PEG 400 and steady state drug flux through the oesophageal mucosa in pH 6.75 buffer solutions

Method	t_{lag} (h)	J * 10^{-3} (μg/cm^2/s)
pH 6.75	1.83±0.19	5.26±1.22
PS 6	2.00±0.16	5.21±1.81
PS 7	1.96±0.29	5.45±1.21

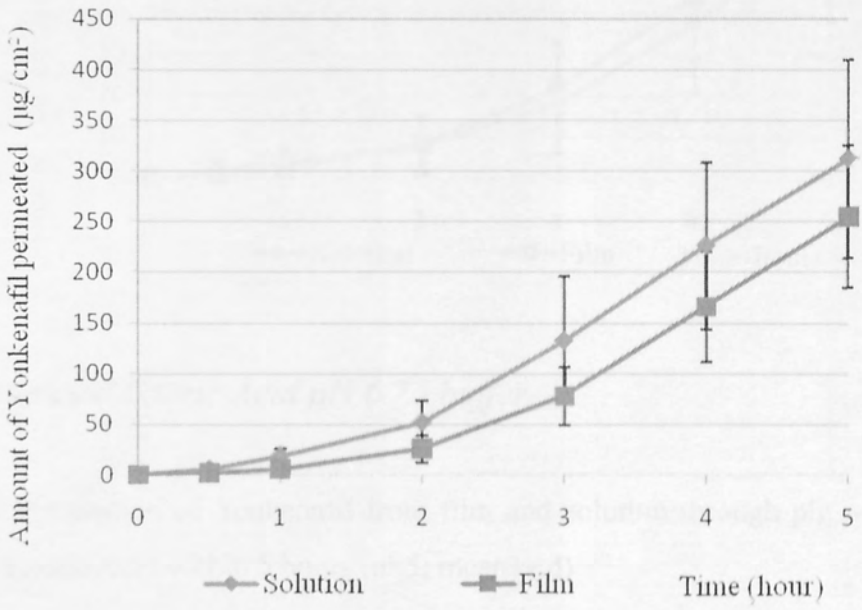
In this group, formulations containing 5% PEG400 and 5% Lactic Acid, which gave the slowest and fastest release in Chapter Three (see section 3.X), were selected for further study . The curves in this graph are overlapped, and the statics studies from Table 4.12 showed all the P values > 0.05, indicating that there is no difference in drug penetration from the three different films. According to this result, it could be concluded that the plasticizers had no effect on the drug penetration due to their low concentration in the film formulations (only 5%w/w). It should be noticed that application of plasticizers in the films may result in faster drug release rates from the films, consequently leading to more drug being washed by the salivary flow into the

stomach, lowering the bioavailability through buccal absorption.

4.3.3.3 Effect of penetration enhancers

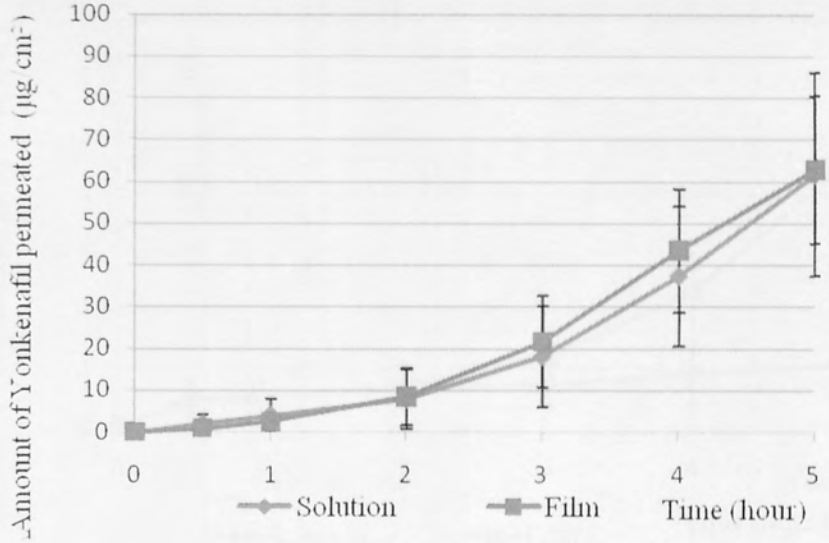
- *95% Ethanol*

Figure 4.7 Penetration of Yonkenafil from film and solution through pig oesophagus pretreated ethanol within 5 hours (n=5; mean±s.d)



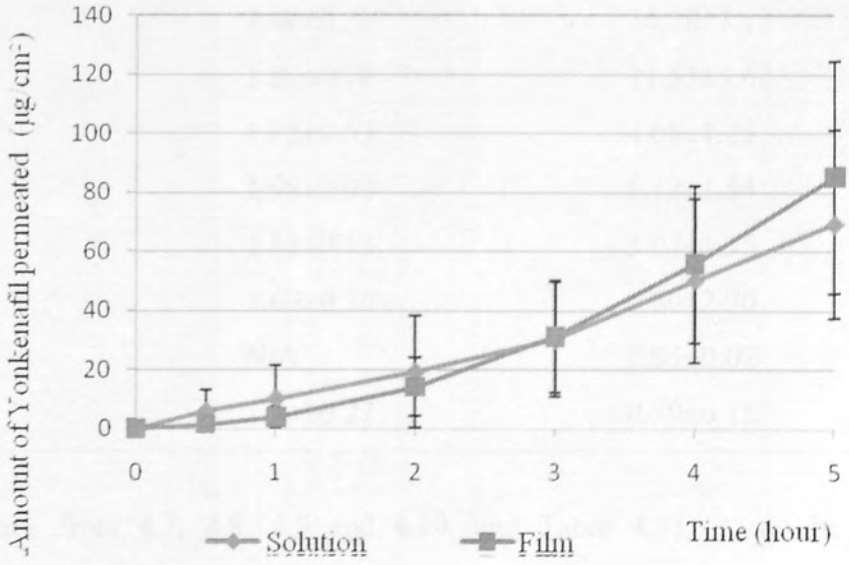
- *Saturated Menthol pH 6.75 Buffer*

Figure 4.8 Penetration of Yonkenafil from film and solution through pig oesophagus pretreated with menthol over 5 hours (n=5; mean±s.d)



- *Saturated Lauric Acid pH 6.75 buffer*

Figure 4.9 Penetration of Yonkenafil from film and solution through pig oesophagus pretreated Lauric Acid within 5 hours (n=5; mean±s.d)



- *Saturated SDS pH 6.75 buffer*

Figure 4.10 Penetration of Yonkenafil from film and solution through pig oesophagus pretreated SDS within 5 hours (n=5; mean±s.d)

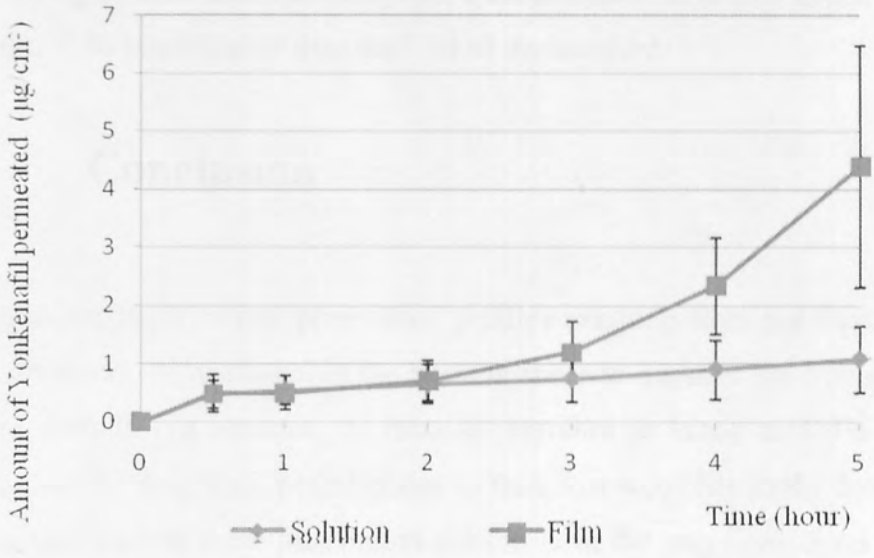


Table 4.13 The lag time of drug release from films containing and steady state drug flux through the oesophageal mucosa in all formulations

Method	t_{lag} (h)	J * 10-3 (µg/cm^2/sec)
Standard (PS1)	1.24±0.14	5.61±0.91
pH 5	1.53±0.38	5.25±0.43
PS 2	1.46±0.21	24.28±7.19
PS 11	1.82±0.18	21.53±5.62
PS 14	1.85±0.53	4.68±1.25
PS 12	1.98±0.07	5.12±1.54
PS 13	1.82±0.13	5.07±1.35
PS 8	1.69±0.30	6.60±2.70
PS 15	N/A	0.05±0.02
PS 10	1.73±0.27	0.39±0.15

From figures from 4.7, 4.8, 4.9 and 4.10, and Table 4.13, it can be concluded pretreatment with penetration enhancers has the same impact on drug flux through

mucosa irrespective of the dosage form used. The enhancing ability of each penetration enhancer is the same on both drug loaded pullulan films and saturated drug solutions ($P_{1\text{-}5}$=0.7667, $P_{2\text{-}11}$=0.5204, $P_{12\text{-}14}$=0.6895, $P_{8\text{-}13}$=0.3395), except PS 10 and PS 15 ($P_{10\text{-}15}$=0.0039). Pretreatment with a penetration enhancer and loading the drug in the films will increase the lag time until the onset of release. Compared with the standard, the t_{lag} of other methods except PS 2 are around 1.70 to 2.00 hours, which are about 0.30 to 0.80 hours higher than the 1.24 of the standard.

4.4 Conclusion

According to the experimental penetration profiles resulting from pre-treatment with different enhancers, 95% ethanol is the best enhancer to improve the permeability of Yonkenafil through pig oesophageal mucosa. Menthol or lauric acid did not show much effect on the drug flux, probably due to their low solubility in the donor media, illustrating that loading these penetration enhancers in the polymeric films would be unlikely to enhance the permeability of the drug. Penetration enhancers had a similar effect on drug permeation from both solutions and pullulan films, but increased the lag time also. It is likely that higher concentrations of surfactant lead to Yonkenafil-SDS micelle formation and prevented drug permeation. The different pH values of media affected the drug release from the polymeric films because the drug solubility is largely influenced by pH. The plasticisers contained in the films did not affect the drug release and penetration in this experiment, suggested that the drug release *in vitro* (Chapter three) can only be used to assess the loss of drug washed by salivary flow but cannot used to estimate the speed of drug penetration through the mucosa. As a result, the major factors influencing drug penetration through the pig oesophageal are pretreatment with high concentrations of penetration enhancers, pH and the drug solubility in the donor media.

Chapter Five

DSC, TGA, FT-IR and SEM studies of the polymeric films

5.1. Introduction

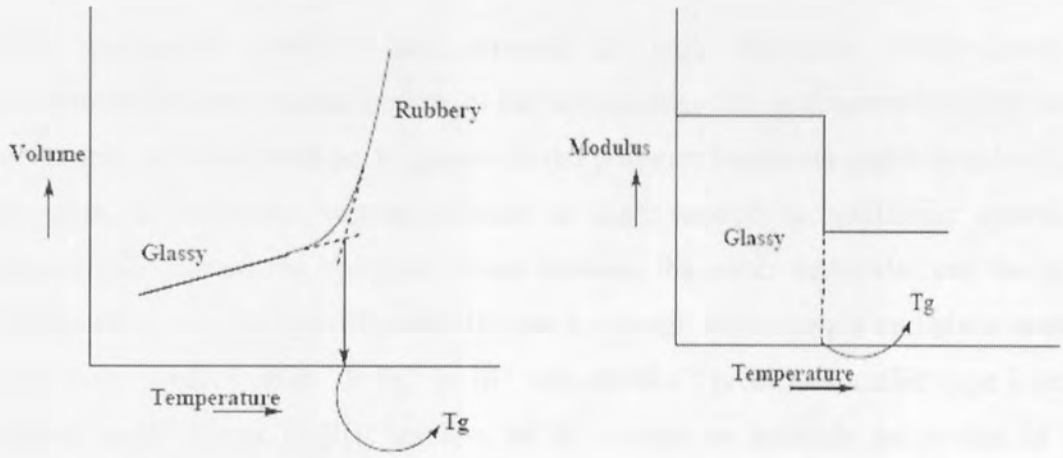
Differential scanning calorimetry (DSC) and Thermogravimetric analysis (TGA) are well established as standard analytical tools for the study of the thermal behaviour of materials. On their own, however, these techniques only provide physical data about a sample, though an experienced researcher can often deduce some chemical properties from these results. Fourier transform-infrared (FT-IR) spectroscopy, on the other hand, is able to obtain chemical information about a thermal process much more directly, and with a higher degree of confidence.

5.1.1. Glass transition temperature

The glass transition temperature (T_g) is an important parameter in describing physicochemical properties of solid materials, such as polymers. The T_g is the temperature at which the mechanical properties of a polymer change from a brittle to a rubber state. As the temperature drops below T_g, a polymer behaves in an increasingly brittle manner. When the temperature is increased above T_g, there is an increase in free volume and the polymer chains which are able to move; as a result, the polymer becomes more rubber-like. Therefore, in line with increasing T_g of polymers, the restriction in mobility within the polymer chains or crystallinity of the polymers is increased. In addition, the physical properties (hardness, volume, modulus, elongation, etc.) undergo a drastic change at the glass transition temperature of a polymer (*Fekton,*

2007). Figure 5.1 shows the relationship between volume, modulus and temperature respectively (*http://plc.cwru.edu/tutorial/enhanced/files/polymers/therm/therm.htm*)

Figure 5.1 The relationship between the physical properties (volume and modulus) of a polymer and temperature



The mechanical properties of polymers are strongly dependant on their intrinsic thermodynamic responds. Furthermore, responses of polymers to the application of a force are generally indicated by two main types of behaviours: elastic and plastic. Elastic materials will return to their original shape once the applied force is removed, while plastic materials will not regain their original shape. Most materials demonstrate a combination of elastic and plastic behaviour, showing plastic behaviour after the elastic limit has been exceeded.

The T_g of glass occurs between 510 and 560 ℃, meaning that it is a brittle solid at room temperature and easily broken. The T_g of a polymer can be lowered by adding a moderate amount of plasticizer, to make it more flexible at a specific temperature (*http://en.wikipedia.org*).

5.1.2. Water content in polymeric films

It is important to understand the role of water as a plasticizer in the polymer matrix system, which has a major effect on the physical properties of the solid dosage form. It has been reported that reduction of Tg by moisture-induced changes is sufficient to induce recrystallization of low molecular weight substances through pharmaceutical processing (*Carstensen et. al., 1990*).

When polymeric materials are exposed to high humidity, water forms a monomolecular layer on the surface of the substances. This is characterized by strong interactions of water with polar groups of the polymer backbone and it is called type III water. If the water vapour pressure is high enough, a multilayer system is subsequently created via hydrogen bonds between the water molecules and the polar groups, and it is called type II water. If there is enough water supply and given enough time, water is taken up in ''bulk'' or in ''solvent like'' processes, called type I water. Bonded water (types II–III) has less of an impact on intrinsic properties of the polymeric materials, while solvent-like water (type I) can change the physical and chemical properties of polymer matrices, for example, their glass transition temperature and water vapour permeability. Type I water can even change the properties of the entire film formulation (*Zografi, 1988; Resio et. al., 1999; Lourdin et. al., 1997; Ford 1999*).

Since water molecules can interact not only with the polymer matrix through polymer polar groups but also with themselves via hydrogen bonding, the state of water in the polymer matrix depends on the polymer structure, water-polymer interaction and water activity. Water is simply distributed among the polymer matrix when its activity is low. At high activities, water will cause a plasticization effect on the polymer matrix by forming hydrogen bonds between the water molecules or between water and hydrophilic groups. (*Chu et. al., 2006*)

There is another description of the water in a polymeric system. Based on the extent of interaction between the water and polymer mainly by hydrogen-bonding between

water and polar groups of the polymer, the water on a hydrophilic polymer system can be proposed for three states:

(1) free water, which may be crystallized at 0℃,

(2) freezing bound water, which is crystallized at 0℃,

(3) non-freezing bound water, which does not crystallize even at lower temperature (*Hietala et. al., 1997; Ping et. al., 2001*).

Pullulan is a hydrophilic polysaccharide which has many hydroxyl groups capable of interacting with water through hydrogen bonding in the polymeric films, thus, it is necessary to study the plasticizing effect of water on physicochemical properties of the pullulan films.

5.2. Materials

Pullulan: Hayashibara Biochemical Laboratory Inc. Japan.

Hydroxypropyl methylcellulose (HPMC): viscosity (2wt% in H_2O, 20℃) 6cps, Mn: 10000, D.S. (Methoxy) 1.80-2.00, M.S. (Propylene oxide) 0.20-0.30, Sigma, Chenmical Co, Germany.

Propylene glycol (PG): Sigma, Chemical Co, Germany.

D-Sorbitol: 97%, Fisher Scientific, United Kingdom.

Polyethylene Glycol 400: Aldrich Organics, New Jersey, USA

DL-Lactic Acid: Aldrich Organics, New Jersey, USA.

Yonkenafil: a gift from Tasly Group, China.

5.3. Methods

5.3.1. Differential scanning calorimetry (DSC)

Glass transition temperature (T_g) of the sample films were determined using differential scanning calorimeter (Pyris Diamond DSC, PerkinElmer Instruments). Approximately 3 mg of film samples were loaded into non-hermetically sealed aluminum pans and tested under a N_2 atmosphere. The samples were heated from 20 to 200 ℃ at a rate of 10 ℃/min. Nitrogen was used as the purge gas at a flow rate of 20 ml/min. The T_g for each film was determined from the midpoint of a endothermic rise of the pre- and post-transition baseline. Each measurement was repeated three times.

5.3.2. Thermogravimetric analysis (TGA)

The water content was measured by thermogravimetric analysis (Pyris 1 TGA, PerkinElmer Instruments). Film samples (2-5 mg) were placed on the microbalance and the surface is heated by 10 ℃ per minute, from 50 to 200 ℃. The test determined mass change as a function of temperature and is used to determine residual solvent in a sample and to evaluate the water content in the polymeric films. Each measurement was repeated three times.

5.3.3. FT-IR analysis

The spectra of films were recorded by Fourier transform infrared (FTIR) spectrometry (Shimadzu, FTIR-8400S, Japan). The light source of transmittance was 650 to 4000 cm^{-1}. The spectra obtained were used to determine possible interaction of functional

groups between the polymer and other additives (drug, plasticizer). Each measurement was repeated three times.

5.3.4. Scanning electron microscopy (SEM)

The morphological structures of the polymeric films were studied by scanning electron microscopy. Films samples were adhered on double-stick tape mounted on a specimen holder, coated with 100 to 200 Å thickness of gold and photographed using SEM apparatus (Stereosacn 90, Cambridge Instruments).

5.3.5. Optical microscopy

The film surfaces were observed with optical microscope (Reichert-Jung, Polyvar) under transmitted light and the images at 200× and 500× magnification were captured using a CDD camera.

5.4. Results and discussion

5.4.1. FT-IR spectra of pullulan films

5.4.1.1. Drug-free pullulan films

The functional groups associated with pullulan are CH, CH_2, C-O-C, C-O, and H-O-H. Sakata reported the assignment of each peak in the film is as follows (*Sakata et. al., 2009*).

1240-1460 cm^{-1}: CH stretching absorption

2850-2980 cm^{-1}: CH_2 stretching absorption

1640 cm^{-1}: absorbed water (H-O-H)

3300 cm^{-1} (broad band): OH stretching vibration and hydrogen bonding

1155, 1107, 1080, 1020, 1000 cm^{-1}: valent vibration of the C-O and C-C bonds and deformational vibrations of the CCH, COH, and HCO bonds (*Firsov et. al., 1999*)

1155 cm^{-1}: C-O-C valent vibrations, and glycosidic bridge (*Kačuráková et. al., 2000*)

1107 cm^{-1} (broad peak): the valent vibration of C-O bond at the C4 position of a glucose residue (*Kačuráková et. al., 1996*)

1080 cm^{-1}: vibrations involving the stretching of the C_6-O_6 bind with participation of the deformational vibrations of the C_4-C_5 bond (*Shingel 2002*)

Figure 5.2 The FT-IR spectrum of P100

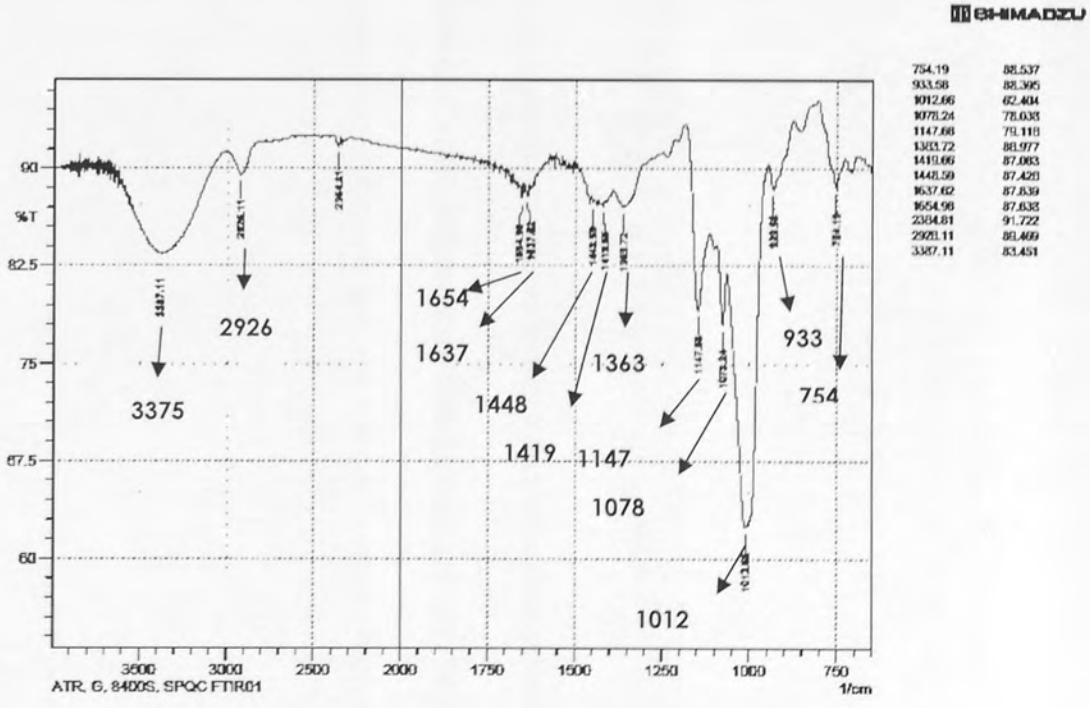


Figure 5.2 shows the FT-IR spectrum of drug-free pullulan film. The major peaks in are 3375 (broad band), 2926, 1654, 1637, 1448, 1419, 1363, 1147, 1078, 1012, 933, 754 cm^{-1}. According to the report from *Sakata et al., 2009*, a reasonable assignment for each peak is as follows.

3375 cm^{-1} (broad band): OH stretching vibration and hydrogen bonding. The frequency shift to a higher degree compared with 3300 cm^{-1} from Sakata's report may due to less hydrogen bonding because of the different film forming procedure and the deviation of the FT-IR detector.

2926 cm^{-1}: CH_2 stretching absorption.

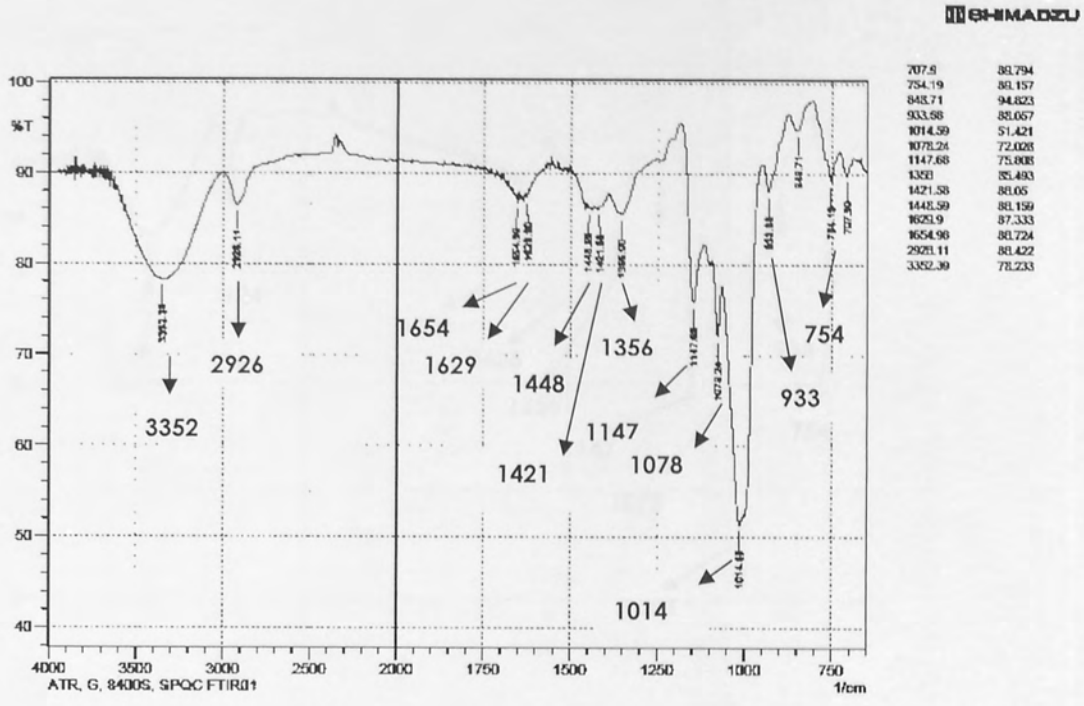
1654, 1637 cm^{-1}: absorbed water (H-O-H)

1448, 1419, 1363 cm^{-1}: CH stretching absorption

1147, 1078, 1012, 933 cm^{-1}: valent vibration of the C-O and C-C bonds and deformational vibrations of the CCH, COH, and HCO bonds

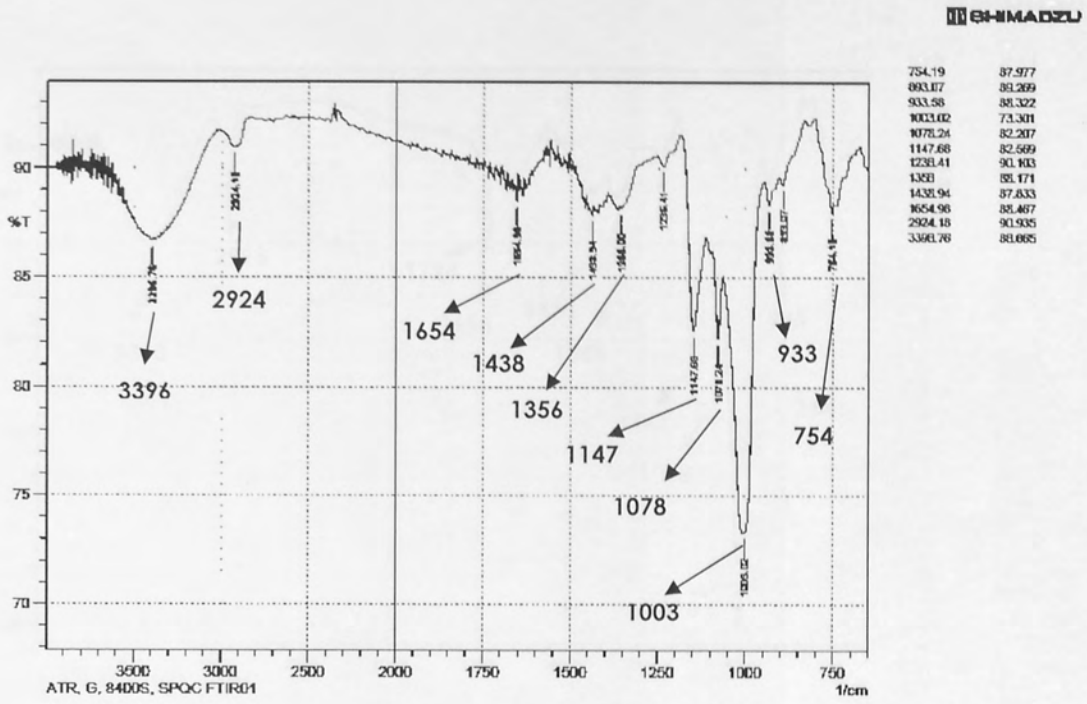
The result is basically coincident with the results reported by Sakata.

Figure 5.3 The FT-IR spectrum of drug-free pullulan film containing 10% (w/w) PEG 400



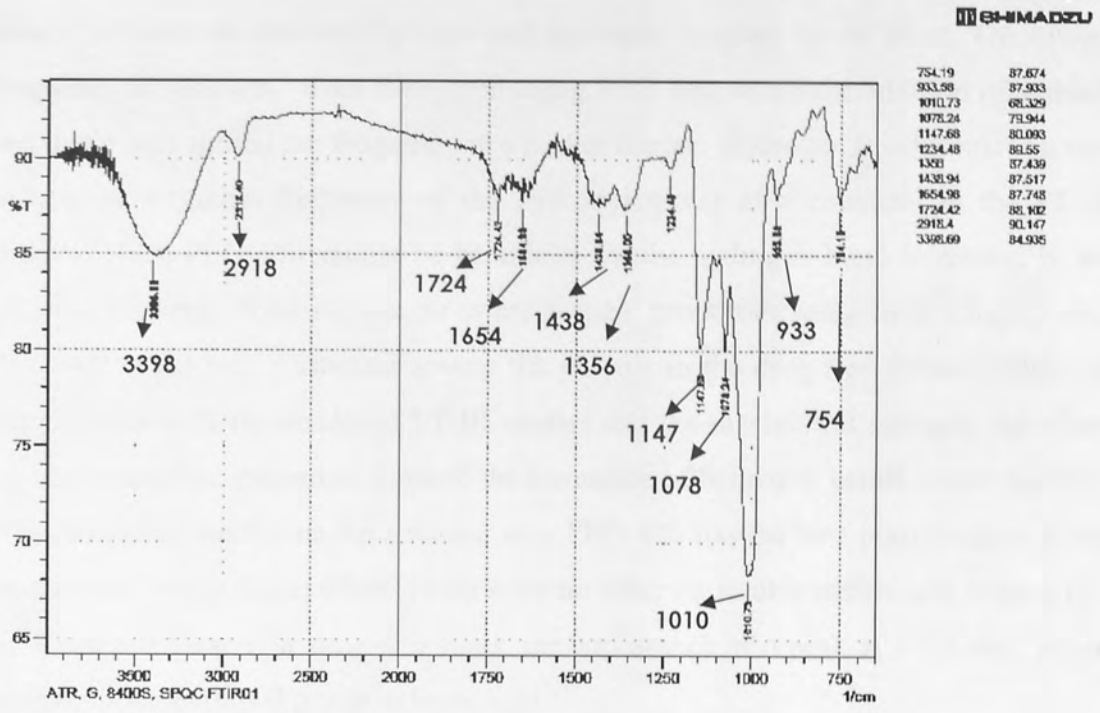
Major peaks: 3352, 2926, 1654, 1629, 1448, 1421, 1356, 1147, 1078, 1014, 933, 754 cm^{-1}.

Figure 5.4 The FT-IR spectrum of drug-free pullulan film containing 10% (w/w) sorbitol



Major peaks: 3396, 2924, 1654, 1438, 1356, 1147, 1078, 1003, 933, 754 cm^{-1}.

Figure 5.5 The FT-IR spectrum of drug-free pullulan film containing 10% (w/w) lactic acid



Major peaks: 3398, 2918, 1724, 1654, 1438, 1356, 1147, 1078, 1010, 933, 754 cm^{-1}.

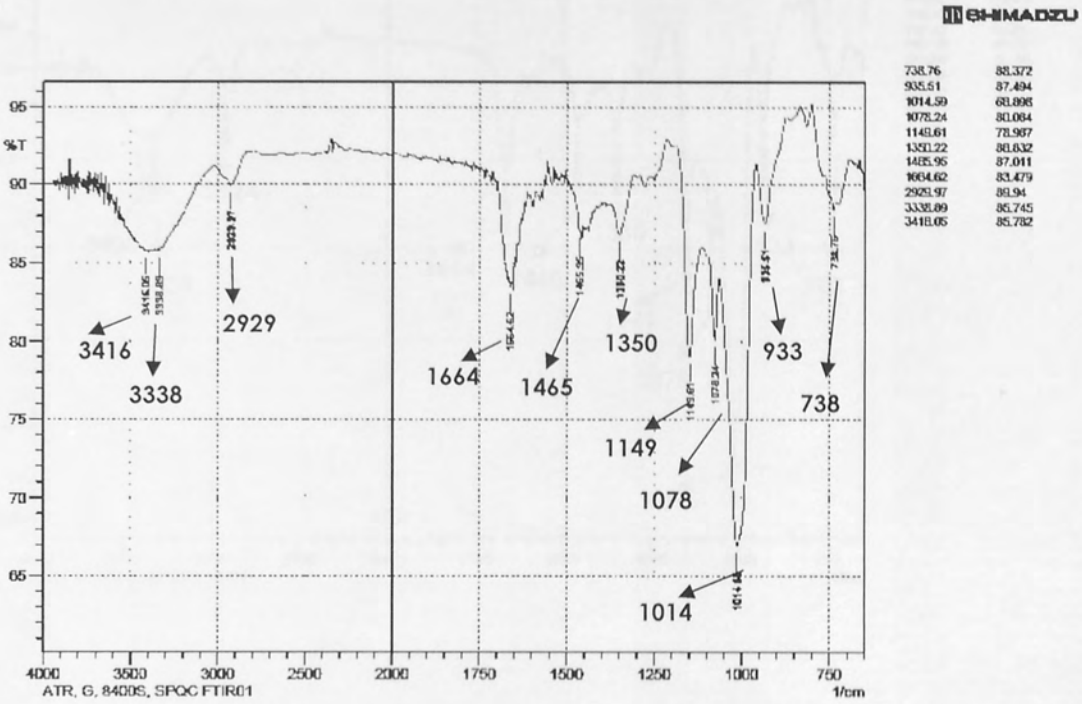
Table 5.1 The assignment of the major peaks in the FT-IR spectra of four drug-free formulations

	P100 (cm^{-1})	10% PEG400 (cm^{-1})	10% Sorbitol (cm^{-1})	5% LA (cm^{-1})
OH	3375	3352	3396	3398
CH2	2926	2926	2924	2918
COOH	N/A	N/A	N/A	1724
H-O-H	1654, 1637	1654, 1629	1654	1654
C-H	1448, 1419, 1363	1448, 1421, 1356	1438, 1356	1438, 1356
C-O, C-C, CCH, COH, HCO	1147, 1078, 1012, 933	1147, 1078, 1014, 933	1147, 1078, 1003, 933	1147, 1078, 1010, 933

Figures 5.3, 5.4, 5.5 show the FT-IR spectra of the drug-free pullulan films containing 5% (w/w) PEG 400, sorbitol, and lactic acid respectively. From Table 5.1, the most significant difference between these spectra is the broad peak qt 3300-3400 cm^{-1}, which indicates the hydroxyl groups and hydrogen bonding in the films. The lowest frequency is 3352 cm^{-1} from films containing PEG 400, while the addition of sorbitol and lactic acid shifted the frequency to a higher degree. Hydrogen bond formation will reduce the vibration frequency of the hydroxyl group of a chemical in the FT-IR spectra. Thus, PEG 400 should be beneficial for the hydrogen bond formation in the polymer network. From the results of mechanical properties research in Chapter two, PEG 400 is the best plasticizer among the polyols in the drug-free pullulan films. In combination with the results of FT-IR studies and the mechanical strength, the effect on improving film properties through the formation of hydrogen bonds within the PEG 400-containing matrix maybe a reason why PEG 400 has the best plasticization effect on the drug free pullulan films. There were no other noticeable differences among FT-IR spectra of these four drug-free films, the appearance of a peak at 1724 cm^{-1} which represents the carboxyl group in lactic acid.

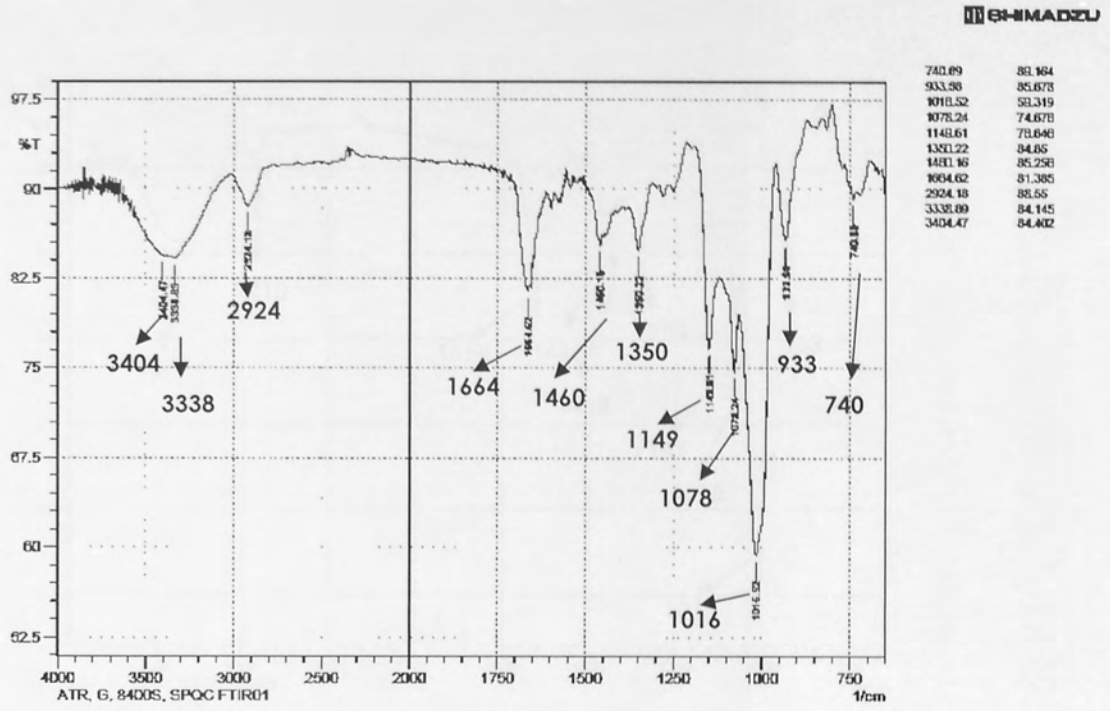
5.4.1.2. 20% (w/w) drug-loaded pullulan films

Figure 5.6 FT-IR spectrum of PD20



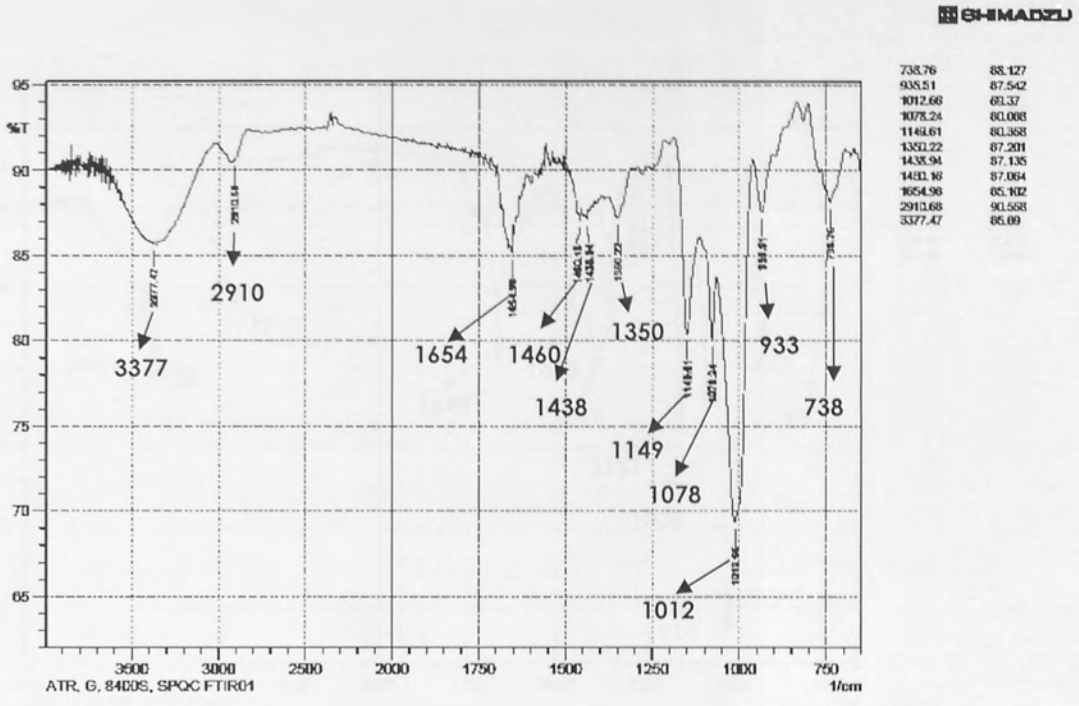
Major peaks: 3416, 3338, 2929, 1664, 1465, 1350, 1149, 1078, 1014, 933, 738 cm^{-1}.

Figure 5.7 The FT-IR spectrum of drug-loaded pullulan film containing 10% (w/w) PEG 400



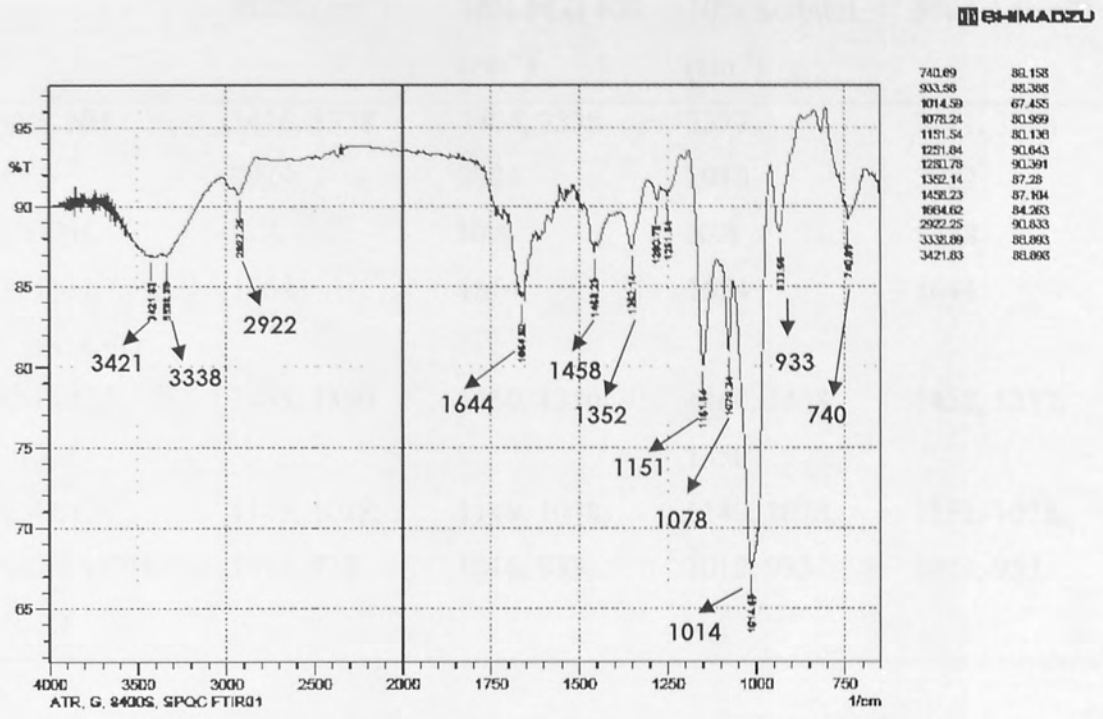
Major peaks: 3404, 3338, 2924, 1664, 1460, 1350, 1149, 1078, 1016, 933, 740 cm^{-1}.

Figure 5.8 The FT-IR spectrum of drug-loaded pullulan film containing 10% (w/w) sorbitol



Major peaks: 3377, 2910, 1654, 1460, 1438, 1350, 1149, 1078, 1012, 933, 738 cm^{-1}.

Figure 5.9 The FT-IR spectrum of drug-loaded pullulan film containing 5% (w/w) lactic acid



Major peaks: 3421, 3338, 2922, 1644, 1458, 1352, 1151, 1078, 1014, 933, 740 cm^{-1}.

Table 5.2 The assignment of the major peaks in the FT-IR spectra of four formulations containing 20% (w/w) drug

	PD20 (cm^{-1})	10% PEG 400 (cm^{-1})	10% Sorbitol (cm^{-1})	5% LA (cm^{-1})
OH, NH	3416, 3338	3404, 3338	3377	3421, 3338
CH_2	2929	2924	2910	2922
COOH	N/A	N/A	N/A	1718
H-O-H, C=C, C=N, C=O	1664	1664	1654	1644
C-H, C-N	1465, 1350	1460, 1350	1460, 1438, 1350	1458, 1352,
C-O, C-C, CCH, COH, HCO	1149, 1078, 1014, 933	1149, 1078, 1016, 933	1149, 1078, 1012, 933	1151, 1078, 1014, 933

Figures 5.7, 5.8, and 5.9 show the FT-IR spectra of drug-loaded (20% w/w) pullulan films containing 10% PEG400, 10% sorbitol and 5% lactic acid respectively. The significant difference is the absorption at 1664-1644 cm^{-1}; otherwise there are no frequency shifts caused by the presence of the drug. It is obvious that this strong absorption of 1664-1644 cm^{-1} resulted from the C=O, C=N groups of the drug yonkenafil (Figures 5.2, 5.7, 5.8 and 5.9). These results indicated that the concentration (20% w/w) of the drug in the films was still too low exhibits string absorptions due to the drug chemical structure, and overall spectra were dominated by pullulan. Therefore, FT-IR was not a useful technique to study interactions of the drug with PEG 400, sorbitol and lactic acid, nor with pullulan.

5.4.2. TGA and DSC

In this project, TGA was applied to measure the water content in the polymeric films.

Figure 5.10 The TGA graph of pullulan alone film

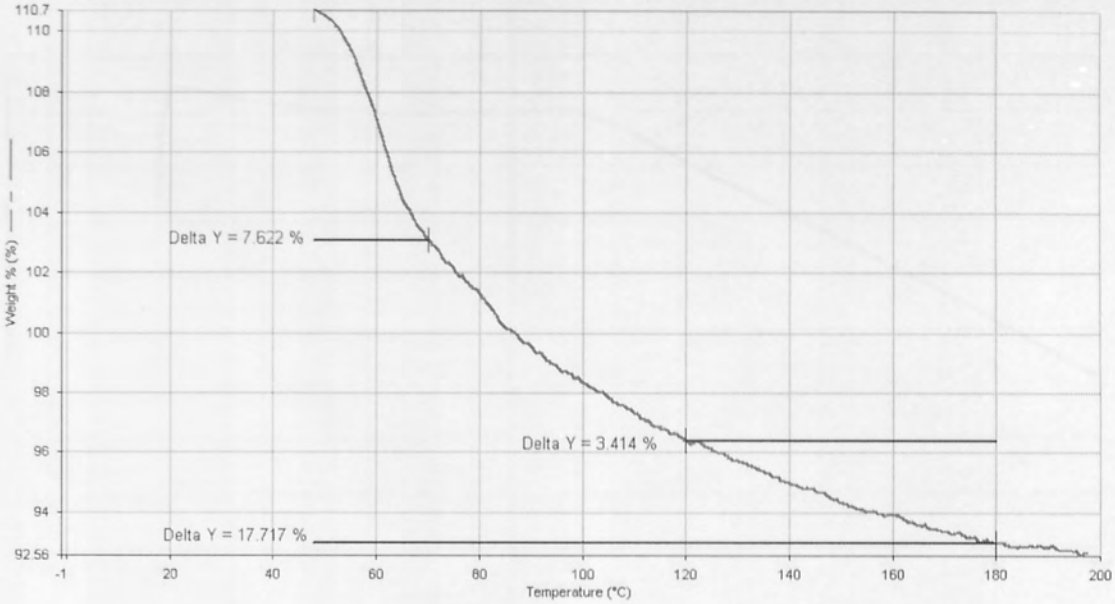


Figure 5.11 The TGA graph of HPMC alone film

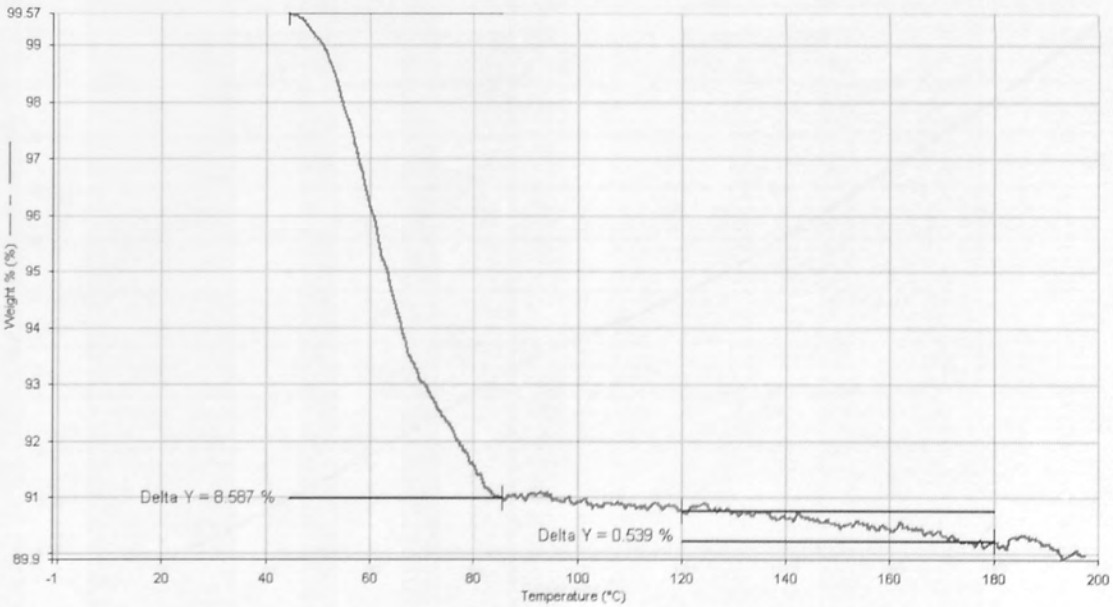


Figure 5.12 A DSC graph of pullulan alone film

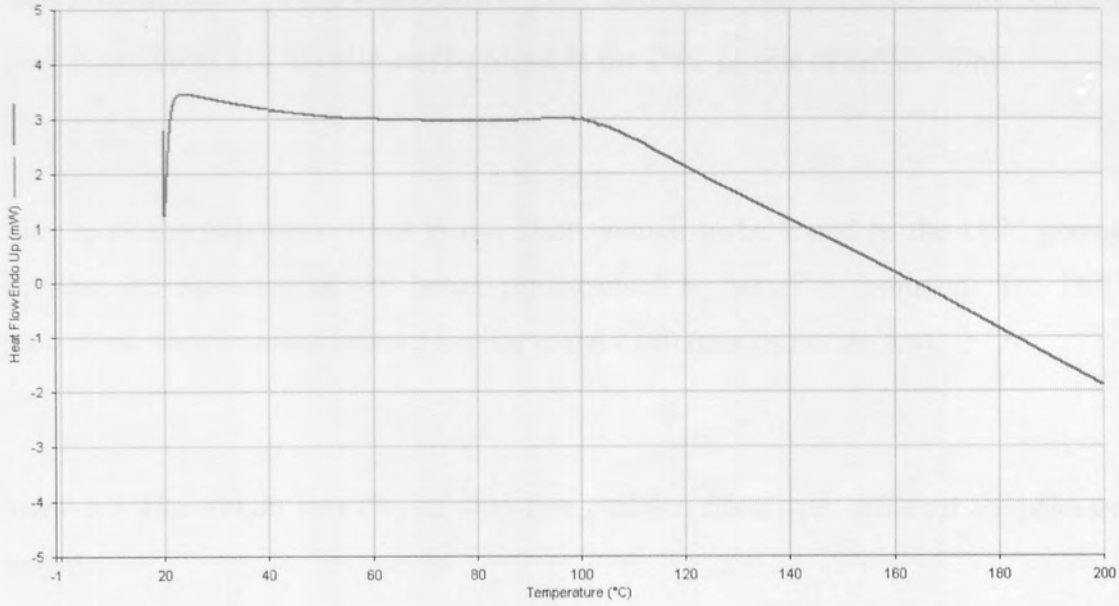


Figure 5.13 The DSC graph of alone HPMC film

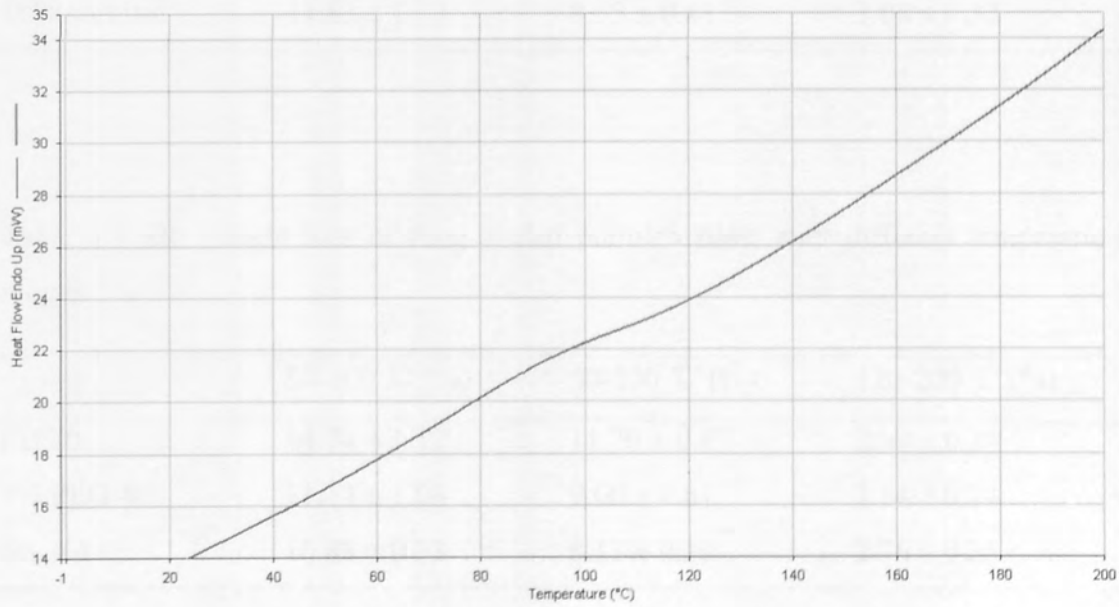


Figure 5.10, 5.11, 5.12, and 5.13 show the TGA and DSC profiles of the pullulan and HPMC alone films. The pullulan films exhibit weight loss from 50 – 200 ℃. The broad peak found in the DSC curve agrees with the TGA profile. However, the TGA for HPMC is quite different from that of pullulan. The water content in the HPMC

films was lost much faster than those in pullulan films. When the temperature was raised beyond 80 ℃, there was nearly not any the weight loss of HPMC films. A broad peak (from 60 to 110 ℃) also can be found in the DSC profile of HPMC films.

The T_g of the polymeric films is not clear enough to be found in the DSC graphs because the existence of the broad peak caused by water evaporation. The DSC profiles of other formulations are similar to the P100 (results not shown).

Table 5.3 The weight loss (%) of drug-free pullulan films over different temperature ranges

	50-200 ℃ (%)	50-120 ℃ (%)	120-200 ℃ (%)
P100	18.94 ± 1.32	15.72 ± 1.02	3.20 ± 0.30
10% PEG 400	14.82 ± 0.48	13.08 ± 0.37	6.36 ± 0.04
10% sorbitol	11.63 ± 1.13	8.55 ± 0.81	3.08 ± 0.32

Table 5.4 The weight loss of drug-loaded pullulan films over different temperature ranges

	50-200 ℃ (%)	50-120 ℃ (%)	120-200 ℃ (%)
PD 20	14.74 ± 1.12	11.76 ± 0.89	2.99 ± 0.24
5% PEG 400	11.14 ± 1.04	9.00 ± 0.81	2.14 ± 0.24
5% LA	10.88 ± 0.33	8.12 ± 0.19	2.76 ± 0.25

Table 5.5 The weight loss of drug-free HPMC films in different temperature range

	50-200 ℃ (%)	50-120 ℃ (%)	120-200 ℃ (%)
H100	8.32 ± 1.01	7.73 ± 0.88	0.59 ± 0.12
10% sorbitol	7.83 ± 0.96	7.46 ± 0.91	0.37 ± 0.05
10% PEG400	8.61 ± 0.57	7.43 ± 0.66	1.18 ± 0.10

Tables 5.3, 5.4, and 5.5 show the weight loss from the film samples on heating. For the pullulan films, adding plasticizer and drug reduced the water content in the films. It was divided to two processes of the water molecule evaporation from the films. The water evaporated from 50 – 120 ℃ was considered to be free water weakly bounded to the polymer in the films, and the water evaporated from 120 – 200 ℃ that water strongly bound to the polymer network (*Lin S.Y., Wang S.L., et. al. 2007*).

For the drug-free pullulan films, 10% PEG 400 showed a better plasticization effect (see section 2.4.2.2) than P100 and 10% sorbitol. However, the result from TGA seems that the water content did not have any relationship with the mechanical properties of the drug-free pullulan films. The plasticization effect of PEG 400 is probably contributed to the interaction with PEG 400 and pullulan. When the drug was added into the films, the most plastic formulation, that containing 5% w/v LA (see section 2.4.3.2) did not have a significant difference of water content with the other two formulations. Neither PEG 400 nor lactic acid has an effect on the hygroscopic absorption of the films (see Table 5.3, 5.4, 5.5). There is no evidence found in these experiments to indicate the PEG 400 and lactic acid affected the thermal properties of the pullulan films. Thus, the TGA results did not have could not be used to explain the mechanical properties of the pullulan films.

The water content in the HPMC films was much lower than in the pullulan films (Table 5.5). The low water content and fast evaporation from the HPMC films implied that the HPMC molecules did not have as strong an interaction with the water molecules, while pullulan is more hydrophilic. And there is no relationship between water content and tensile properties of the HPMC films either in my results.

5.4.3. Scanning electron microscopy (SEM) and optical microscopy

Figure 5.14 P100 film under SEM

A. Revolution = 10 μm B. Revolution = 20 μm

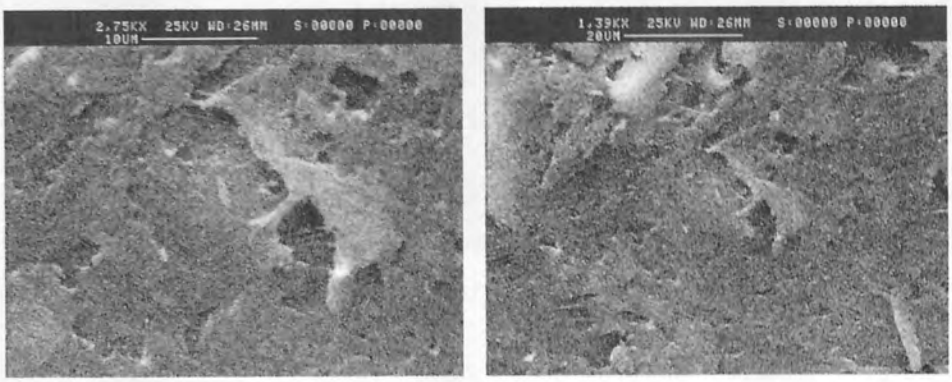


Figure 5.15 PD20 film under SEM

A. Revolution = 10 μm B. Revolution = 20 μm

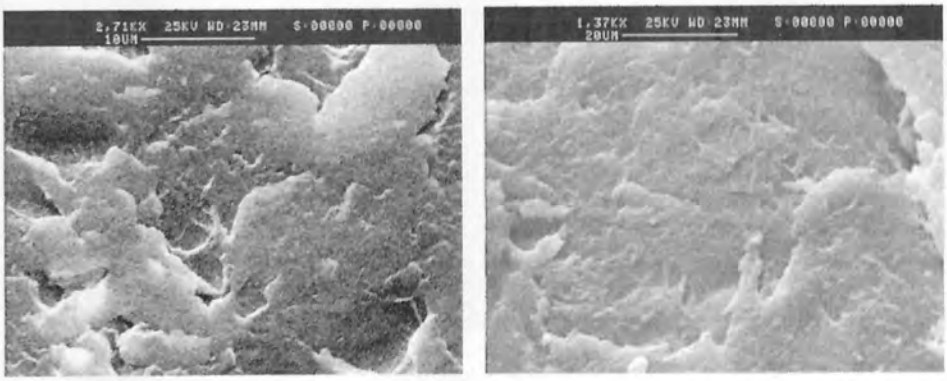


Figure 5.16 10% (w/w) PEG 400 drug free pullulan film under SEM

A. Revolution = 10 μm B. Revolution = 20 μm

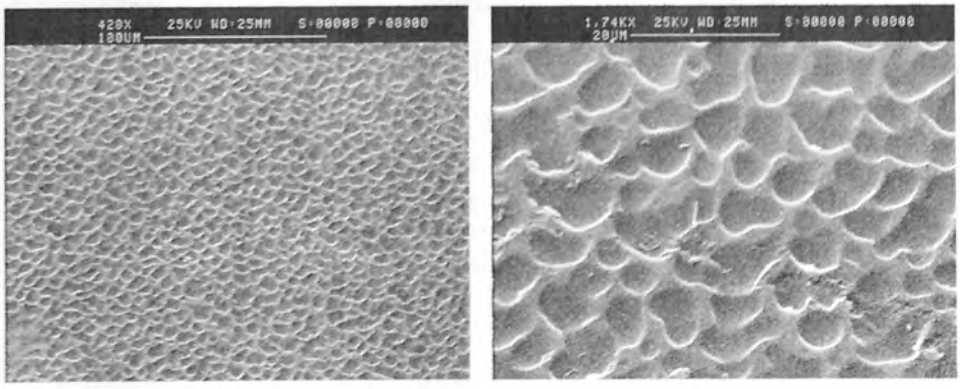


Figure 5.17 10% (w/w) PEG 400 20% (w/w) drug loaded pullulan films under optical microscopy

×200 ×500

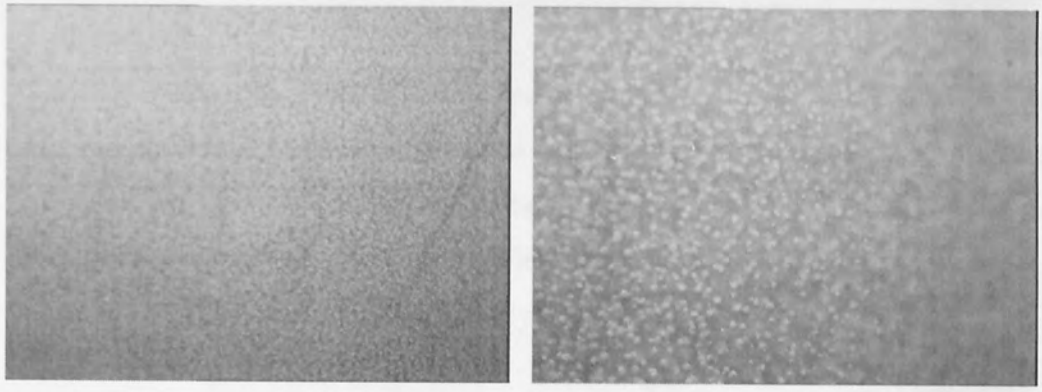
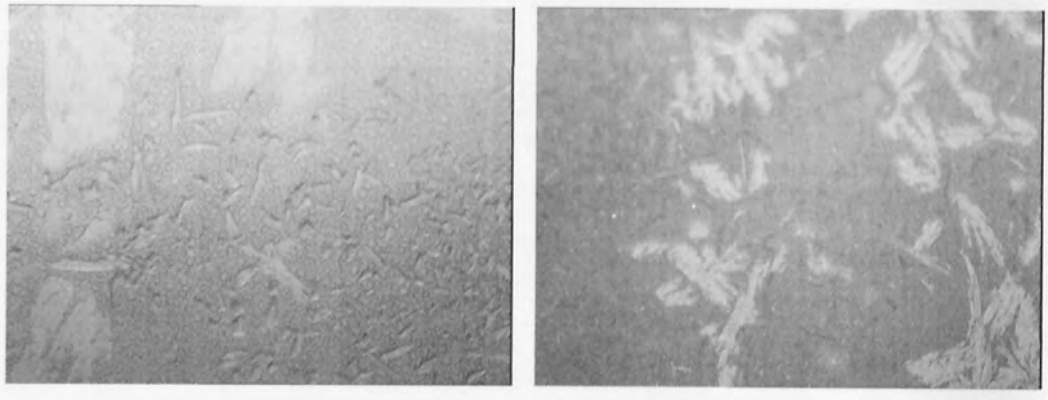


Figure 5.18 25% (w/w) drug loaded pullulan films under optical mocroscopy

×200 ×500



The drug-free 10% PEG 400 film exhibits a different surface structure compared with P100 and PD20 (figures 5.14, 5.15 and 5.16). Due to the films is not transparent, that structure may be formed by the pullulan deposited and rearranged the space structure during the drying process, under an unknown effect of PEG 400. When the drug loading is 25%, drug crystals could be observed under an optical microscopy (Figures 5.18).

5.5. Conclusion

DSC, TGA and FT-IR studies were carried out to study the thermodynamic properties of the pullulan films and the polymeric micro-structure of the films. Overall, addition of plasticizers, lactic acid, PEG400, sorbitol, into pullulan films interfered the polymeric matrix by interactions of polar groups from plasticizers with hydroxyl groups in pullulan, which was reflected in FT-IR spectra and changes in elongations and elastic modulus of the films. The thermal study using DSC and TGA did not find any reliable evidence to prove the relationship between the thermodynamic properties and the mechanical properties of the polymeric films.

Chapter Six

Conclusion and future work

6.1 Preparation of the polymeric films

In this project, the polymeric films were prepared with pullulan and HPMC by solvent evaporation method, using petri dishes as flat surface equipments at the room temperature. It has been recognized that a number of factors influenced the quality of the polymeric films during preparation processes, such as temperature, pressure, humidity, drying time and the concentration of a polymer in the solution. However, we found all these factors are related to drying methods applied into the preparation process. How to dry the polymeric solutions into films has long been considered to a key process for preparation high quality films. Kaeahara have proved that drying temperature influenced the mechanical properties of the pullulan films (*Kawahara et. al., 2003*). I tried to dry the solutions in an oven at 60℃ and found that the films were brittle and non-homogeneous. I also found when the solutions were dried under a vacuum, films will be deformed which was not a flat film anymore.

In addition, I found the physicochemical properties of a polymer and an additive, such as a drug or a plasticizer, are also deeply tangled with the drying process, particularly the phase transitions of the polymer and the additive from a solvent to a solid form during the drying process or interaction during of the polymer with the additive. Therefore, drying method or process could affect possible crystallizations of the additive and cross-linkage between the polymer and the additive. The status of the polymer and additive in the polymeric film is an important factor to the physicochemical properties, drug release, and storage of the films.

Future work:

(1) Find out an appropriate drying condition for drying the polymer solutions into films, using the shortest time and keeping the films flexible and uniform.
(2) Study the possibility of phase transition of the additives happening under different drying conditions.

6.2 Study of mechanical properties

In this project, I studied the mechanical properties of drug free pullulan and HMPC films, and drug-loaded pullulan films. Up to present, there is no a formal, official standard guiding for the evaluation of the mechanical properties of the polymeric edible films for the purpose of drug delivery. Most of the literatures use tensile strength, elongation and elastic modulus to describe the tensile properties of the films; however, it is difficult to compare the flexibility of the films made by different researchers, because the films were in different size, thickness, speed of pulling force, and storage conditions.

It is a complicated task to describe the tensile properties of a polymeric film, besides tensile strength, elongation and elastic modulus, since the type of the deformation of a film should be paid more attention to. The deformation of the films can be divided into elastic deformation and plastic deformation (Section 2.1.4.3). Because the plastic deformation happens after the elastic deformation, which implies that a film undergoing a plastic deformation should be more flexible with a low tensile strength, elastic modulus and high elongation. Thus, we cannot determine the tensile properties of the films by only one or two parameters, such as tensile strength, elongation and elastic modulus. The graph of Stress-Strain (Figure 2.2) of a polymeric film can show deformation process is more valuable to describe the tensile properties.

In my study, only polyols, acids and 20% (w/w) Yonkenafil were introduced into the films. From the results, only hydroxyl and carboxyl groups were noticed to influence

physicochemical properties of polymeric films and discussed in chapter two. When other excipients (penetration enhancers, flavors) or a different drug is applied, physicochemical properties of the resulted polymeric films should be different from the present one because the new excipients and drug contain different functional groups. Therefore, it is necessary to perform all studies all over again, that means the results from individual research could not serve as general guidance for film mechanical properties, including film flexibility, drug loading and drug release.

Future work:

(1) Build up a general evaluation standard to describe the tensile properties of the polymeric films for drug loading.
(2) Apply different drug into the films and increase drug loading.

6.3 Drug release and polymer dissolution

In this project, I studied the relationship of the drug release and polymer dissolution with different formulations by comparing the mathematics models and the curves of drug release and polymer dissolution. The release models are usually used in the controlled or sustained release system, to predict the mechanism of the drug release in these polymeric systems. My results showed that the application of the models at the relative fast release films (8 minutes) might be practicable. However, only one polymer matrix and drug were used in my experiments.

Future work:

(1) Loading yonkenafil into different films made of different polymer matrix to study how the polymer affects the drug release.
(2) Loading different drugs into the pullulan films to study the application the mathematic models to reveal the release mechanisms of the drug loaded pullulan films.

6.4 *in vitro* drug penetration through buccal mucosa

In the section of the penetration experiments, I could not find a suitable penetration enhancer which can be loaded into the polymeric films. Probably low solubility of menthol and lauric acid in water made them have no effect on enhancing drug penetration through the mucosa, while sodium dodecyl sulfate (SDS) might form micellar-bounded complex with the drug at high concentration. The penetration enhancers are generally lipophilic and slightly soluble in water. Therefore, to improve the solubility of the lipophilic penetration enhancers in saliva is critical for the application a penetration enhancer into a film for buccal drug delivery. In the case of SDS, it is important to maintain an appropriate concentration of SDS in the mucus to prevent the formation of micellar-bounded complex.

Future work:

(1) We used pig esophageal mucosa instead of the buccal mucosa for the *in vitro* penetration experiments. The histological differences between pig buccal mucosa and esophageal mucosa are minor and it has been reported that the pig buccal and esophageal mucosa had similar permeability to fentanyl (*Del Consuelo et. al., 2005*). The experiment to compare the permeability of pig buccal and esophageal mucosa to yonkenafil should be performed in the further study.

(2) Study the penetration enhancing effect of different concentration SDS solution.

(3) Compare the penetration enhancing effect of ethanol, menthol-ethanol solution and lauric acid-ethanol solution.

6.5 Thermal and FT-IR study

The glass transition temperature (T_g) of the polymeric films were not shown clearly in the DSC results because the interference of the broad peak of water evaporation. However, if drying the films further to water free, the physicochemical properties of the film will be away from the original film's; as a result, the glass transition temperature (T_g) of the polymeric films could reflect nature of the normal films. On the other hand, by changing the heat rate to get water evaporated first was proved to be not practical because high boiling point of water. The results of FT-IR did not provide much valuable information about the films chemical structure, because FT-IR sensitivity of the technology.

Reference

Amir H. S., Buccal mucosa as a route for systemic drug delivery: a review, J. Pharm. Pharmaceutic Sci., 1 (1): 15-30, 1998.

Amir H. S., Mansoor K., Gerald L., Reza K., Transbuccal permeation of a nucleoside analog, dideoxycytidine: effects of menthol as a permeation enhancer, Int. J. Pham. 192, 139-146, 1999.

Anonymous Press release. Hayashibara in worldwide license and supply agreement with Pfizer using pullulan for oral use. http://www.hayashibara.co.jp/eng/contents_hn.html, 2001.

Artursson P., Lindmark T., Davis S.S., and L. Illum. Effect of chitosan on the permeability of monolayers of intestinal epithelial cells (Caco-2). Pharm. Res. 11:1358-1361 1994.

Artusi M., Santi P., Colombo P., Junginger H.E., Buccal delivery of thiocolchicoside: in vitro and in vivo permeation studies. Int. J. Pharm. 250:203– 213, 2003

Aungst, B.J. and Rogers, N.J., Comparison of the effects of various transmucosal absorption promoters on buccal insulin delivery, Int. J. Pharm., 53:227-235, 1989.

Bajdik János; Regdon Géza; Marek Tamás; Eros István; Süvegh Károly; Pintye-Hódi Klára, The effect of the solvent on the film-forming parameters of hydroxypropyl-cellulose, Inter. J. Pharm. 301 192-198, 2005

Baker R.W., Lonsdale H.K., Controlled release: mechanisms and rates, in: A.C. Tanquary, R.E. Lacey (Eds.), Controlled Release of Biologically Active Agents, Plenum Press, New York, pp. 15–72, 1974.

Ballard SA, Gingell CJ, Tang K, et al: Effects of sildenafil on the relaxation of human corpus cavernosum tissue in vitro and on the activities of cyclic nucleotide phosphodiesterase isozymes. J Urol 159: 2164–2171, 1998.

Banya Y, Ushiki T, Takagane H, et al. Two circulatory routes within the human corpus cavernosum penis: a scanning electron microscopic study of corrosion casts. J Urol; 142: 879-83, 1989.

Barry B.W., Dermatological Formulations: Percutaneous Absorption, Marcel Dekker, New York, 1983.

Baszkin A., Proust J.E., Monsenego P., Boissonnade M.M., Wettability of polymers byc mucin aqueous solution. Biorheology 27:503-514, 1990.

Beavo JA, Conti M & Heaslip RJ. Multiple cyclic nucleotide phosphodiesterases. Molecular Pharmacology; 46: 399-405,1994

Beavo JA: Cyclic nucleotide phosphodiesterases: functional implications of multiple isoforms. Physiol Rev 75: 725–748, 1995.

Biliaderis C.G., Lazaridou A. and Arvanitoyannis I., Glass transition and physical properties of polyol-plasticized pullulan-starch blends at low moisture. Carbohydr Polym 40:29±47 1999.

Bologna, W.J.; Levine, H.L.; Cartier, P.; de Ziegler, D. Bioadhesive Progressive Hydration Tablets and Methods of Making and Using the Same. US Patent 6,248,358, June 19, 2001.

Boolell M, Allen MJ, Ballard SA, et al: Sildenafil: an orally active type 5 cyclic GMP-specific phosphodiesterase inhibitor for the treatment of penile erectile dysfunction. Int J Impot Res 8: 47–52, 1996.

Borra´s-Blasco J., Lo´ pez A., Morant M.J., Dı´ez-Sales O., Herra´ez-Domı´nguez M., Influence of sodium lauryl sulphate on the in vitro percutaneous absorption of compounds with different lipophilicity, Eur. J. Pharm. Sci. , 15–22, 1997.

Brook I.M., Tucker G.T., Boyes R.N., A lignocaine patch for dental analgesia safety and early pharmacology, J. Controlled Release 10, 183–188, 1989.

Cappello B., De Rosa G., Giannini L., La Rotonda M. I., Mensitieri G., Miro A., Quaglia F., Russo R., Cyclodextrin-containing poly(ethyleneoxide) tablets for the delivery of poorly soluble drugs: Potential as buccal delivery system, Int. J of Pharm. 319, 63-70, 2006.

Carslaw H.S., Jaeger J.C. (Eds.), Conduction of Heat in Solids, Clarendon Press, Oxford, 1959.

Carstensen JT, Van Scoik K., Amorphous-tocrystalline transformation of sucrose. Pharm Res 7:1278–1281, 1990.

Chen J.L., Cyr G.N., Compositions producing adhesion through hydration, in: R.S. Manly (Ed.), Adhesion in Biological Systems, Academic Press, New York, pp. 163–180, 1970.

Chen L.L.H., Chetty D.J., Chien Y.W., Amechanistic analysis to characterize oralmucosal permeation properties. Int J Pharm 184:63–72, 1999.

Childers RF, Oren PL, Seidler WMK, Film coating formulations. US Patent 5,015,480, 1991.

Christ GJ, Brink PR, Melman A, Spray DC. The role of gap junctions and ion channels in the modulation of electrical and chemical signals in human corpus cavernosum smooth muscle. Int J Impot Res; 5: 77-96, 1993.

Chu L.Q, Mao H.Q., Knoll W., In situ characterization of moisture sorption/desorption in thin polymer films using optical waveguide spectroscopy, Polymer 47, 7406-7413, 2006.

Conca K.R., Yang T.C.S., Edible food barrier coatings. In: Ching C, Kaplan D.L., Thomas E.L. (eds) Biodegradable polymers and packing. Technomic, Lancaster, pp 357-369, 1993.

Corbin JD, and Francis SH: Cyclic GMP phosphodiesterase-5: target of sildenafil. J Biol Chem 274: 13729–13732, 1999.

Crank J. (Ed.), The Mathematics of Diffusion, Clarendon Press, Oxford, 1975.

Cui Z., Mumper R.J., Bilayer films for mucosal (genetic) immunization via the buccal route in rabbits, Pharm. Res. 19, 947–953, 2002.

Cui Z., Mumper R.J., Buccal transmucosal delivery of calcitonin in rabbits using thin-film composites, Pharm. Res. 19, 1901–1906, 2002.

Danjo K., Higuchi F., Otsuka A., Release of lidocaine from polymer film dosage forms, Chem. Pharm. Bull. (Tokyo) 43, 1759–1763,1995.

Das N.G., Das, S.K., development of mucoadhesive dosage forms of bupreonorphine for sublingual drug delivery, Drug Del., 11:89-95, 2004.

Davis A.F., Hadgraft J., Effect of supersaturation on membrane transport. 1. Hydrocortisone acetone, Int. J. Pharm. 76, 1-8, 1991.

de Silva DJ, Olver JM., Hydroxypropyl methylcellulose (HPMC) lubricant facilitates insertion of porous spherical orbital implants. Ophthal Plast Reconstr Surg. ;21(4):301-2, 2005

del Consuelo I. D., Falson F., Guy R.H., Jacques Y., Transport of fentanyl through pig buccal and esophageal epithelia in vitro. Influence of concentration and vehicle pH. Pharm. Research Vol. 22 No. 9 2005. -b

del Consuelo I. D., Pizzolato G.-P., Falson F., Guy R. H., Jacques Y., Evaluation of pig esophageal mucosa as a permeability barrier model for buccal tissue, J Phar. Sci. Vol. 94 Iss. 12, 2777-2788 2005.

Der Marderosian, A.H., Pharmaceutical necessities. In: Gennaro, A.R., Medwick, T. (Eds.), Remington's Pharmaceutical Sciences, 18th ed. Mack Publishing, Easton, PA, pp. 1286–1329, 1990.

Derjahuin B.V., Smilga V.P., The electronic theory of adhesion. In: Adhesion: Fundamentals and Practice. University of Nottingham, England: Gordon & Breach, 1966.

Desphande M.S., Rale V.B. and Lynch J., Aureobasidium pullulans in applied microbiology: a status report. Enzyme Microb Technol 14:514-527, 1992.

di Gioia L., Guilbert, S. Amphipilic plasticizers. J. Agric. Food Chem., 47, 1254-1261, 1999.

Diab T. Biliaderis C.G., Gerasopoulos D., Sfakiotakis E., Physicochemical properties and application of pullulan edible films and coating in fruit preservation, J Sci Fodd Agric 81:988-1000, 2001.

Diaz del Consuelo, I., Falson, F., Guy, R. H., Jacques, Y., Ex vivo evaluation of bioadhesive films for buccal delivery of fentanyl. *Journal of Controlled Release*, 122 (2), pp. 135-140, 2007.

Dowty M.E., Knuth K.E., Irons B.K., Robinson J.R., Transport of thyrotropin releasing hormone in rabbit buccal mucosa in vitro. Pharm Res 9: 1113–1122,1992.

Duchene D., Touchard F., Peppas N.A.. Pharmaceutical and medical aspects of bioadhesive systems for drug administration. Drug Dev Ind Pharm. 14(2 & 3): 283-318, 1988.

Edgar W.M., Saliva and dental health. Clinical implications of saliva: report of a consensus meeting. Br Dent J.; 169; 96-8, 1990.

Eggerth R.M., Rashidbaigi Z.A., Mahjour M,Goodhart F.W., Fawzi M.B., Evaluation of hamster cheek pouch as a model for buccal absorption. Proceed Int Symp Control Rel Bioact Mater 14: 180–181, 1987.

Elias P.M., Brown B.E., Fritsch P., Goerke J., Gray G.M., White R.J., Localization and composition of lipids in neonatal mouse stratum granulosum and stratum corneum, J. Invest. Dermatol. 73, 339-348, 1979.

Elias P.M., Lipid and the epithelium permeability barrier, Arch. Dermatol. Res. 270, 95-117, 1981.

Enscore, D., Hopfenberg, H., Stannett, V., Effect of particle size on the mechanism controlling n-hexane sorption in glassy polystyrene microspheres. Polymer 18, 793–800, 1977.

Fekton L.A., Characterization of coating systems, AAPS PharmSciTech; 8(4) Article 112, 2007.

Firsov, S.P., Zhbankov, R.G., Petrov, P.T., Shingel, K.I., Tsarenkov, V.M., Carbohydrates. In: Greve, J., Puppels, G.J., Otto, C. (Eds.), Eighth European Conference on the Spectroscopy of Biological Molecules. Kluwer, Dodrecht, pp. 323–324, 1999.

Ford JL., Thermal analysis of hydroxypropylmethylcellulose and methylcellulose: Powders, gels and matrix tablets. Int J Pharm 179:209–228, 1999.

Fournier GR Jr, Juenemann KP, Lue TF, Tanagho EA. Mechanisms of venous occlusion during canine penile erection: an anatomic demonstration. J Urol; 137: 163-7, 1987.

Francoeur M.L., Golden G.M., Potts R.O., Oleic acid: its effects on stratum corneum in relation to (trans)dermal drug delivery, Pharm. Res. 7, 621–627, 1990.

Fujii N., Shinohara S., Polysaccharide produced by Aureobasidium pullulans FERM-P4257. II. Toxicity test and antitumor effect. Kenkyu Hokoku-Miyazaki Daigaku Nogakubu 33: 243-248, 1986.

Galey W.R., Lonsdale H.K., Nacht S., The in vitro permeability of skin and buccal mucosa toselected drugs and triated water. J Invest Dermatol 67:713–716, 1976.

Gallagher F.J., Taybos G.M., Terezhalmy G.T.J. Indiana Dent. Assoc., 64, 26-28, 1985

Gandhi R.B., Robinson J.R., Oral cavity as a site for bioadhesive drug delivery, Adv. Drug Deliv. Rev. 13, 43-74, 1994.

Ganem-Quintanar A., Kalia Y.N., Falson-Reig F, Buri P, Mechanisms of oral permeation enhancement, Int J Pharm 156:127-142, 1998.

Gao, S., Singh, J., In vitro percutaneous absorption enhancement of a lipophilic drug tamoxifen by terpenes. J. Control. Rel. 51, 193–199, 1998.

Ghanem A.H., Mahmoud H., Higuchi W.I., Liu P., Good W.R., The effects of ethanol on the transport of lipophilic and polar permeants across hairless mouse skin: methods/ validation of a novel approach, Int. J. Pharm. 78, 137– 156, 1992.

Giannola L. I., De Caro V., Giandalia G., Siragusa M. G., Campisi G., Wolff A., Current status in buccal drug delivery. Pharm Tech Europe May 01, 2008.

Gibbs P.A. and Seviour .RJ., Pullulan, in Polysaccharides in Medicinal Applications, Ed by Severian D, Marcel Dekker, New York, pp 59-86 1996.

Golden G.M., McKie J.E., Potts R.O., Role of stratum corneum lipid fluidity in transdermal drug flux, J. Pharm. Sci. 76, 25–28, 1987.

Gómez C., Costela Á., García-Moreno I., Llanes F., Teijón J. M., Blanco D., Laser treatments on skin enhancing and controlling transdermal delivery of 5-fluorouracil, Las. Sur. Med. 40:6-12, 2008.

Good R.J., Surface energy of solid and liquids: thermodynamics, molecular forces, and structure. J Colloid Interface Sci 59:398-419, 1977.

Gopal VK, Francis SH, and Corbin JD: Allosteric sites of phosphodiesterase-5 (PDE5). A potential role in negative feedback regulation of cGMP signaling in corpus cavernosum. Eur J Biochem 268: 3304–3312, 2001.

Grant D.A., Stern I.B., Listgarten M.A., editors. Saliva. In: Periodontics. 6th ed. St Louis: CV Mosby., p. 135-46, 1988.

Gu J.-M., Robinson J.R., Leung S.-H.S., Binding of acrylic polymers to mucin/epithelial surfaces: structure–property relationships, Crit. Rev. Ther. Drug Carr. Syst. 5, 21– 67, 1998.

Hao J.S., and Heng P. W. S., Buccal Delivery Systems, Drug Dev. and Ind. Pharm. Vol. 29, No. 8, pp. 821–832, 2003.

Harris D., Robinson J.R., Oral delivery via the mucous membranes of the oral cavity, J. Pharm. Sci. 81:1-10, 1992.

Harris, D. and Robinson, J.R., Drug delivery via the mucous membranes of the oral cavity, J. Pharm. Sci., 81:1-10,. Reproduced with permission of the American Pharmaceutical Association, 1992.

HEO. K.C., Lee J.J., Park S.Y., and Rhim J.W., Physical and mechanical properties of water resistant sodium alginate films, Department of Food Engineering, Mokpo National University, 61 Dorimri, Chungkyemyon, Muangun, Chonnam, 534-729, South Korea. 2003.

Hideo U., Kenji S., Yasunori M., Skin penetration-enhanceing effect of drugs by phonophoresis, J. Controlled Rel. 37, 291-297,1995.

Hietala S., Holmberg S., Näsman J., Ostrovskii D., Paronen M., Serimaa R., Sundholm F., Torell L., Torkkeli M., Angew. Makromol. Chem. 253, 151, 1997.

Higuchi T., Physical chemical analysis of percutaneous absorption process, J. Soc Cosmetics Chemists 11, 85-97, 1960.

Higuchi T., Rate of release of medicaments from ointment bases containing drugs in suspensions, J. Pharm. Sci. 50, 874–875, 1961.

Hijiya H, Shiosaka M., Adhesives and pastes. US Patent 3,873,333 1975. a

Hill M.W., Squier C.A., Linder J.E., A histological method for the visualization of the intercellular permeability barrier in mammalian stratified squamous epithelia. Histochem J 14:641–648, 1982.

Hopwood D, Logan K.R., Bouchier I.A.D., The electron microscopy of normal human oesophageal epithelium. Virchows Arch B Cell Pathol 26:345– 358, 1978.

Hopwood D., Milne G., Jankowski J., Howat K., Wormsley K.G., Uptake of horseradish peroxidase by human oesophageal explants over 24 h. Histochem J 23:409–411, 1991.

Howie N.M., Trigkas T.K., Cruchley A.T., Wertz P.W., Squier C.A., Williams D.M., Short-term exposure to alcohol increases the permeability of human oral mucosa, Oral Dis. 7, 349– 354, 2001.

Humphrey Sue P., RDH, MSEd, and Williamson R. T., DMD, A review of saliva: normal composition, flow, and function, J Prosthet Dent; 85: 162-9, 2001.

Iervolino M., Raghavan S.L., Hadgraft J., Membrane penetration enhancement of ibuprofen using supersaturation, Int. J. Pharm. 198, 229–238, 2000.

Ignarro LJ, Bush PA, Buga GM, Wood KS, Fukuto JM, Rajfer J. Nitric oxide and cyclic GMP formation upon electrical field stimulation cause relaxation of corpus cavernosum smooth muscle. Biochem Biophys Res Commun; 170: 843-50, 1990.

İkinci G., Şenel S., Akıncıbay H., Kaş, S., Erciş S., Wilson C.G., Hıncal A.A., Effect of chitosan on a periodontal pathogen Porphyromonas gingivalis, Int. J. Pharm. 235, 121– 127, 2002.

Ilango R., Kavimani S., Mullaicharam A.R., Jayakar B., Invitro studies on buccal strips of glibenclamide using chitosan, Indian J. Pharm. Sci. 59, 232– 235, 1997.

Ishida M., Nambu N., Nagai T., Highly viscous gel ointment containing carbopol for application to the oral mucosa., Chem. Pham. Bull., 31, 4561-4564, 1983.

Ishida M., Nambu N., Nagai T., Mucosal dosage form of lidocaine for toothache using hydroxypropyl cellulose and carbopol, Chem. Pharm. Bull. (Tokyo) 30, 980–984, 1982.

Jay, S.; Fountain, W.; Cui, Z.; Mumper, R.J. Transmucosal delivery of testosterone in rabbits using novel bi-layer mucoadhesive wax-film composite disks. J. Pharm. Sci., 91 (9), 2016–2025, 2002.

Jones D.S., and Medlicott N.J., Casting solvent controlling release of chlorhexidine from ethylcellulose films prepared by solvent evaporation, Inter. J. Pharm., 114, 257-261, 1995.

Kačuráková, M., Capek, P., Sasinková, V., Wellner, N., Ebringerová, A., FT-IR study of plant cell wall model compounds: pectic polysaccharides and hemicelloloses. Carbohydr. Polym. 43, 195–203, 2000.

Kačuráková, M., Mathlouthi, M., FTIR and laser-Raman spectra of oligosaccharides in water: characterization of the glycosidic bond. Carbohydr. Res. 284, 145–157, 1996.

Kaelble D.H., Moacanin J., A surface energy analysis of bioadhesion. Polymer 18:475-482, 1977.

Kamimori,G.H.;Karyekar,C.S.;Otterstetter,R.; Cox, D.S.; Balkin, T.J.; Belenky, G.L.; Eddington, N.D. The rate of absorption and relative bioavailability of caffeine administered in chewing gum versus. capsules to normal healthy volunteers. Int. J. Pharm., 234 (1–2), 159–167, 2002.

Kaplun-Frischoff, Y., Touitou, E., Testosterone skin permeation enhancement by menthol through formation of eutectic with drug and interaction with skin lipids. J. Pharm. Sci. 86, 1394–1399, 1997.

Kavita Khanvilkar, Maureen D. Donovan, Douglas R. Flanagan, Drug transfer through mucus, Advanced drug delivery reviews 48, 173-193, 2001.

Kawahara M., Mizutani K., Suzuki S., Kitamura S., Fukada H., Yui T., Ogaw K., Dependence of the mechanical properties of a pullulan film on the preparation temperature, Biosci. Biotechnol. Biochem., 67(4), 893-895, 2003.

Kimoto T., Shibuya T., Shiobara S., Safety studies of a novel starch, pullulan: chronic toxicity in rats and bacterial mutagenicity. Food Chem Toxicol 35: 323-329, 1997.

Kobayashi, D., Matsuzawa, T., Sugibayashi, K., Morimoto, Y., Kimura, M., Analysis of the combined effect of 1-menthol and ethanol as skin permeation enhancers based on a two-layer skin model. Pharm. Res. 11, 96–103, 1994.

Kohnle J., Wolff A., Intelligent drug delivery from the oral cavity, Med. Dev. Tech. 2007.

Kok Khiang Peh, Choy Fun Wong, Polymeric films vehicle for buccal delivery: swelling, mechanical, and bioadhesive properties, J. Pharm. Pharmaceuti. Sci., 2 (2): 53-61, 1999.

Kokate A., Li X.L., Singh P., Jasti B.R., Effect of thermodynamic activitiers of the unionized and ionized species on drug flux across buccal mucosa, Wiley InterSci. DOI 10.1002/jps.21301, 2007.

Kondo S., Sugimoto I., Enhancement of transdermal delivery by superfluous thermodynamic potential. I. Thermodynamic analysis of nifedipine transport across the lipoidal barrier, J. Pharmacobio-Dyn. 10, 587–594, 1987.

Kurosaki Y., Takatori T., Kitayama M., Nakayama T., and Kimura T., Application of propranolol to the keratinized oral mucosa: avoidance of first-pass elimination and the use of 1-dodecylazacycloheptan-2-one (Azone) as an absorption enhancer of bioadhesive film-dosage form, J. Phar.-Dyn. 11:824, 1988.

Lamb D.J., Douglas C.W.I.J. Den., Treatment of denture stomatitis by a sustained drug-delivery device: a preliminary study, 16, 219-221, 1988.

Langer R., Peppas N.A., Chemical and physical structure of polymers as carriers for controlled release of bioactive agents: a review, Rev. Macromol. Chem. Phys. C23, 61–126, 1983.

Lee J.W., Park J.H., Robinson J.R., Bioadhesive-based dosage forms: the next generation, J. Pharm. Sci. 89, 850-866, 2000.

Lehr C.-M., Bodde H.E., Bowstra J.A. and Junginger H.E., A surface energy analysis of mucoadhesion. II: Prediction of mucoadhesive performance by spreading coefficients. Eur. J. Pharm. Sci. 1, pp. 19–30, 1993.

Lehr C.-M., Bouwstra J.A., Schacht E.H., Junginger H.E., In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers, Int. J. Pharm. 78, 43– 48, 1992.

Lehr C.M., Bowstra J.A., Bodde H.E. and Junginger H.E., A surface energy analysis of muchoadhesion: contact angle measurements on polycarbophil and pig intestinal mucosa in physiologically relevant fluids. Pharm Res 9, pp. 70–75, 1992.

Leung S-HS, Leone RS, Kumar LD, Kulkarni N., Fast dissolving orally consumable films. PCT International Application WO 00/18365, 2000.

Leung S-S, Martin A, Leone RS., Bioadhesive antibacterial wound healing composition. US Patent 6,329,434, 2001.

Lin S.Y., Wang S.L., Wei Y.S., Li M.J., Temperature effect on water desorption from methylcellulose films studied by thermal FT-TR microspectroscopy, Sur. Sci. 601, 781-785, 2007.

Longer M.A., Robinson J.R., Fundamental aspects of bioadhesion, Pharm. Int. 7, 114–117, 1986.

Lourdin D, Ciognard L, Bizot P, Colonna P,. Influence of equilibrium relative humidity and plasticizer concentration on the water content and glassy transitions of starch materials. Polymer 38:5401–5406, 1997.

Lourdin, D., Bizot, H.; Colonna, P. "Antiplasticization" in starch-glycerol films J. Appl. Polym. Sci., 63, 1047-1053, 1997.

Lueßen H.L., Lehr C.-M., Rentel C.-O., Noach A.B.J., de Boer A.G., Verhoef J.C., Junginger H.E., Bioadhesive polymers for the peroral delivery of peptide drugs, J. Control. Release 29, 329– 338, 1994.

Lueßen H.L., Verhoef J.C., Borchard G., Lehr C.-M., de Boer A.G., Junginger H.E., Mucoadhesive polymers in peroral peptide drug delivery: II. Carbomer and polycarbophil are potent inhibitors of the intestinal proteolytic enzyme trypsin, Pharm. Res. 12, 1293– 1298, 1995.

Mak V.H., Potts R.O., Guy R.H., Percutaneous penetration enhancement in vivo measured by attenuated total reflectance infrared spectroscopy, Pharm. Res. 7, 835–841, 1990.

Mangavel, C.; Barbot, J.; Guéguen, J.; Popineau, Y. Molecular determinants of the influence of hydrophilic plasticizers on mechanical properties of cast wheat gluten films. J. Artic. Food Chem., 51, 1447-1452, 2003.

Mark R. P., Microneedles for trsnsdermal drug delivery, Adv. Drug Del. Reviews 56, 581-587, 2004.

Mashru R.C., Sutariya V.B., Sankalia M. G., Sankalia J.M., Effect of pH on in vitro permeation of ondansetron hydrochloride across porcine buccal mucosa, Pharm. Dev. Tech., 10:214-247, 2005. a

Mashru R.C., Sutariya V.B., Sankalia M.G., and Parikh P.P., Development and evaluation of fast-dissolving film of salbutamol sulphate, Drug Development and Industrial Pharmacy, 31:25-34, 2005. b

Megrab N.A., Williams A.C., Barry B.W., Oestradiol permeation through human skin and silastic membrane: effects of propylene glycol and supersaturation, J. Control. Release 36, 277–294, 1995.

Mikos A.G., Peppas N.A., Measurement of the surface tension of mucin solutions. Int J Pharm 53:1-5, 1989.

Minabe M., Takeuchi K., Tamura T., Hori T., Umemoto T., Subgingival administration of tetracycline on a collagen film, J. Periodontol. 60, 552– 556, 1989.

Miyamoto Y, Goto H, Sato H, Okano H, Iijima M., Process for sugar-coating solid preparation. US Patent 4,610,891, 1986.

Morgan T.M., O'Sullivan H.M., Reed B.L., Finnin B.C., Transdermal delivery of estradiol in postmenopausal women with a novel topical aerosol, J. Pharm. Sci. 87, 1226– 1228, 1998. b

Morgan T.M., Parr R.A., Reed B.L., Finnin B.C., Enhanced transdermal delivery of sex hormones in swine with a novel topical aerosol, J. Pharm. Sci. 87, 1219–1225, 1998. c

Morgan T.M., Reed B.L., Finnin B.C., Enhanced skin permeation of sex hormones with novel topical spray vehicles, J. Pharm. Sci. 87, 1213–1218, 1998. a

Moser K., Kriwet K., Froehlich C., Kalia Y. N., Guy R. H., Pharm. Res., 18, 1006—1011, 2001.

Nagai T.J., Adhesive topical drug delivery system Controlled release, 2, 121-134, 1985.

Nagai, T., Konishi R.J., Buccal/gingival drug delivery systems, Controlled Release, 6, 353-360, 1987.

Nagai, T.; Machida, Y. Buccal delivery systems using hydrogels. Adv. Drug Del. Rev., 11 (1–2), 179–191, 1993.

Nair M, Chien Y.W,. Buccal delivery of progestational steroids: I. Characterization of barrier, 1993.

Nakashio S, Tsuji K, Toyota N, Fujita F., Novel cosmetics containing pullulan. US Patent 3,972,997, 1976. b

Nakashio S., tSUJI k., Toyota N., Fujita F., Novel cosmetics containing pullulan. US Patant 3,972,997, 1976.

Narasimhan Balaji and Peppas Nikolaos A., Molecular analysis of drug delivery systems controlled by dissolution of the polymer carrier, Journal of Pharmaceutical Sciences Vol. 86, No. 3, 297-304, 1997.

Nazila S.-M., Chittchang M., Thomas P. J., The use of mucoadhesive polymers in buccal drug delivery, Advanced drug delivery reviews 57, 1666-1691, 2005.

Nicolazzo J. A. and Finnin B. C., In Vivo and In Vitro Models for Assessing Drug Absorption Across the Buccal Mucosa, Bio.: Phar. Asp. Vol. VII, 89-111, 2008.

Nicolazzo J. A., Reed B. L., Buccal penetration enhancers – How do they really work, Journal of Controlled Release 105,1-15, 2005. a

Nicolazzo J.A., Reed B.L., Finnin B.C., Enhancing the buccal mucosal uptake and retention of triamcinolone acetonide. J. Control. Release, 240-248, 2005. b

Nicolazzo J.A., Reed B.L., Finnin B.C., Modification of buccal drug delivery following pretreatment with skin penetration enhancers, J. Pharm. Sci. 93, 2054–2063, 2004. a

Nicolazzo, J. A., Reed, B. L., Finnin, B. C., Assessment of the effects of sodium dodecyl sulfate on the buccal permeability of caffeine and estradiol, J. Pharm. Sci.; 93(2):431-440, 2004. b

Nicoli Sara; Colombo Paolo; Santi Patrizia Release and permeation kinetics of caffeine from bioadhesive transdermal films. The AAPS journal; 7(1):E218-23, 2005.

Nielsen, H.M.; Rassing, M.R. Nicotine permeability across the buccal TR146 cell culture model and porcine buccal mucosa in vitro: effect of pH and concentration. Eur. J. Pharm. Sci., 16 (3), 151–157, 2002.

Nugraha E. Suyatma, Lan Tighzert, and Alain Copinet, Effects of hydrophilic plasticizers on mechanical, thermal, and surface properties of chitosan films, J. Agric. Food Chem., 53, 3950-3957, 2005.

Oguchi M., Shikama N., Sasaki S., Gomi K., Katsuyama Y., Ohta S., Hori M., Takei K., Arakawa K., Sone S., Mucosaadhesive water-soluble polymer film for treatment of acute radiation-induced oral mucositis, Int. J. Radiat. Oncol. Biol. Phys. 40, 1033–1037, 1998.

Okabe, H., Takayame, K., Ogura, A., Nagai, T., Effect of limonene and related compounds on the percutaneous absorption of indomethacin. Drug Des. Deliv. 4, 313–321, 1989.

Okamoto H., Nakamori T., Arakawa Y., Iida K., Danjo K., Development of polymer film dosage forms of lidocaine for buccal administration: II. Comparison of preparation methods, J. Pharm. Sci. 91, 2424– 2432, 2002.

Okamoto H., Taguchi H., Iida K., Danjo K., Development of polymer film dosage forms of lidocaine for buccal administration: I. Penetration rate and release rate, J. Control. Release 77, 253–260, 2001.

Omar, L.; El-Barghouthi, M.; Masoud, N.; Abdoh, A.; Al Omari, M.; Zughul, M.; Badwan, A., Inclusion Complexation of Loratadine with Natural and Modified Cyclodextrins: Phase Solubility and Thermodynamic Studies, Journal of Solution Chemistry, Volume 36, Number 5, pp. 605-616(12), 2007.

Park H., Amiji M., Park K., Mucoadhesive hydrogels effective at neutral pH, Proc. Int. Symp. Control. Release Bioact. Mater. 16, 217–218, 1989.

Park H., Robinson J.R., Mechanisms of mucoadhesion of poly(acrylic acid) hydrogels, Pharm. Res. 4, 457– 464, 1987.

Park, C.R.; Munday, D.L. Development and evaluation of a biphasic buccal adhesive tablet for nicotine replacement therapy. Int. J. Pharm., 237 (1–2), 215–226, 2002.

Peppas N.A., Buri P.A., Surface, interfacial and molecular aspects of polymer bioadhesion on soft tissues, J. Control. Release 2, 257–275, 1985.

Peppas N.A., Sahlin J.J., A simple equation for the description of solute release. III. Coupling of diffusion and relaxation, Int. J. Pharm. 57, 169–172, 1989.

Perioli Luana; Ambrogi Valeria; Angelici Fausta; Ricci Maurizio; Giovagnoli Stefano; Capuccella Marinella; Rossi Carlo, Development of mucoadhesive patches for buccal administration of ibuprofen. Journal of controlled release : official journal of the Controlled Release Society; 99(1):73-82, 2004.

Ping Z.H., Nguyen Q.T., Chen S.M., Zhou J.Q., Ding Y.D., States of water in different hydrophilic polymers – DSC and FTIR studies, Polymer 42, 8461-8467 (7), 2001.

Ponchel D, Duchêne D., Development of a bioadhesive tablet. In: M Szycher, ed. High performance Biomaterials. Lancaster, PA: Technomic Publishing Company, pp 231-242, 1991.

Rana Abu-Huwaij; Shereen Assaf; Mutaz Salem; Alsayed Sallam, Mucoadhesive Dosage form of Lidocaine Hydrochloride: I. Mucoadhesive and Physicochemical Characterization, Drug Development and Industrial Pharmacy, 1520-5762, Volume 33, Issue 8, 437-448, 2007.

Rathbone M.J., Hadgraft J., Absorption of drugs from the human oral cavity, Int. J. Pharm. 74, 9-24, 1991.

Rekha M.R. and Chandra P. Sharma, Pullulan as a Promising Biomaterial for Biomedical Applications: A Perspective, Trends Biomater. Artif. Organs, Vol 20 (2), pp 000-000, 2007.

Remuñán-López, C.; Portero, A.; Vila-Jato, J.L.; Alonso, M.J. Design and evaluation of chitosan/ethylcellulose mucoadhesive bilayered devices for buccal drug delivery. J. Contr. Rel., 55 (2–3), 143–152, 1998.

Repka, M.A.; Repka, S.L.; McGinity, J.M. Bioadhesive Hot-Melt Extruded Film for Topical and Mucosal Adhesion Applications and Drug Delivery and Process for Preparation Thereof. US Patent 6,375,963, April 23, 2002.

Resio AC, Aguerre RJ, Suarez C., Analysis of the sorptional characteristics of amarant starch. Food Eng 42:51–57, 1999.

Rhee G.J., Lee D.K., Sin K.H., Park C.B., Formulation and pharmaceutical properties of mucoadhesive films containing dipotassium glycyrrhizate, J. Korean Pharm. Sci. 29, 127– 136, 1999.

Ritger P.L., Peppas N.A., A simple equation for description of solute release. I. Fickian and non-Fickian release from non-swellable devices in the form of slabs, spheres, cylinders or discs, J. Controlled Release 5, 23–36, 1987. a

Ritger P.L., Peppas N.A., A simple equation for description solute release II. Fickian and anomalous release from swellable devices, J. Controlled Release 5, 37–42, 1987. b

Ritschel W.A., Ritschel G.B., Forusz H., Kraeling M., Buccal absorption of insulin in the dog, Res. Commun. Chem. Pathol. Pharmacol. 63, 53– 67, 1989.

Roller N.W., Ship I.I., Lidocaine topical film strip for oral mucosal biopsies, J. Oral Med. 30, 55–58, 1975.

Rossi S., Sandri G, Caramella C.M., buccal drug delivery: a challenge already win?, Drug Dis. Today: Tech. Vol. 2 No. 1, 59-65, 2005.

Rossi S., Sandri G., Ferrari F., Bonferoni M.C., Caramella C., Buccal delivery of acyclovir from films based on chitosan and polyacrylic acid, Pharm. Dev. Technol. 8, 199– 208, 2003.

Roth G, Calmes R, editors. Salivary glands and saliva. In: Oral biology. St Louis: CV Mosby; p. 196-236, 1981.

Saenz de Tejada I, Goldstein I, Azadzoi K, Krane RJ, Cohen RA. Impaired neurogenic and endothelium-mediated relaxation of penile smooth muscle from diabetic men with impotence. N Engl J Med; 320: 1025-30, 1989.

Sakata Y. and Otsuka, Evaluation of relationship between molecular behaviour and mechanical strength of pullulan films, Inter. J. Pharm. 374, 33-38, 2009.

Sandri G., Rossi S., Ferrari F., Bonferoni M.C., Muzzarelli C., Caramella C., Assessment of chitosan derivatives as buccal and vaginal penetration enhancers. Eur J Pharm Sci 21:351–359, 2004.

Sano T, Uemura Y, Furuta A., Photosensitive resin composition containing pullulan or esters thereof. US Patent 3,960,685, 1976.

Sasago M, Endo M, Takeyama K, Nomura N., Water-soluble photopolymer and method of forming pattern by use of the same. US Patent 4,745,042, 1988.

Save T., Shah M.U., Ghamande A.R., Venkitachalam P., Comparative study of buccoadhesive formulations and sublingual capsules of nifedipine, J. Pharm. Pharmacol. 46, 192– 195, 1994.

Sehgal, A,, Srivastava, J., Arora, V., K., Nasally administrable bioavailable pharmaceutical composition of loratadine, US patent, WO 2004/082589, 2004.

Semalty M., Semalty A., Kumar G., Formulation and Characterization of Mucoadhesive Buccal Films of Glipizide, Indian journal of pharmaceutical sciences, vol. 70, no1, pp. 43-48, 2008.

Şenel, S.; İkinci, G.; Kaş, S.; Yousefi-Rad, A.; Sargon, M.F.; H|ncal, A.A. Chitosan films and hydrogels of chlorhexidine gluconate for oral mucosal delivery. Int. J. Pharm., 193 (2), 197–203, 2000.

Senzaki H, Smith CJ, Juang GJ, et al: Cardiac phosphodiesterase 5 (cGMP-specific) modulates beta-adrenergic signaling in vivo and is down-regulated in heart failure. FASEB J 15: 1718–1726, 2001.

Shimizu T, Moriwaki M, Shimoma W., Condenser. Japanese Patent 58,098,909, 1983.

Shingel, K.I., Determination of structural peculiarities of dextran, pullulan and γ-irradiated pullulan by Fourier-transform IR spectroscopy. Carbohydr. Res. 337, 1445–1451, 2002.

Siepmann J., Peppas N.A., Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC), Adv. Drug Del. Rev. 48, 139-157, 2001.

Spychal R.T., Marrero J.M., Saverymuttu S.H., Northfield T.C., Measurement of the surface hydrophobicity of human gastrointestinal mucosa. Gastroenterology 97, pp. 104–111, 1989.

Squier C.A., Kremer M.J., Biology of oral mucosa and esophagus. J Natl Cancer Inst Monogr 29:7–15, 2001.

Stablein M.J., Meyer J., The vascular system and blood supply, in: J. Meyer, C.A. Squier, S.J. Gerson (Eds.), The structure and function of oral mucosa, Pergamon Press, Oxford, pp. 237-256, 1984.

Stief CG, Uckert S, Becker AJ, et al: Effects of sildenafil on cAMP and cGMP levels in isolated human cavernous and cardiac tissue. Urology 55: 146–150, 2000.

Sugiyama A, Satoh Y, Shiina H, et al: Cardiac electrophysiologic and hemodynamic effects of sildenafil, a PDE5 inhibitor, in anesthetized dogs. J Cardiovasc Pharmacol 38: 940–946, 2001.

Tabor D., surface forces and surface interactions. J Colloid Interface Sci 58: 2-13. 1977.

Tanaka, Y., Edible film (Pullulan film). Housou Gijyutsu (JPI Journal) (in Japanese), 29, 852-856, 1991.

Tsujisaka Y., Mitsuhashi M., Pullulan. In: Whistler RL, Bemiller JN (eds) Industrial gums. Polysaccharide and their derivatives. 3rd edn. Academic Press, San Diego, pp 447-460, 1993.

Tsukada N, Hagihara K, Tsuji K, Fujimoto M, Nagase T., Protective coating material for lithographic printing plate. US Patent 4,095,525, 1978.

Tsutsumi K, Obata Y, Takayama K, Isowa K, Nagai T., Permeation of several drugs through keratinized epithelial-free membrane of hamster cheek pouch. Int J Pharm 177:7–14, 1999.

Tuovinen Laura, Peltonen Soili, Jarvinen Kristiina, Drug release from starch-acetate films, Journal of Controlled Release 91, 345-354, 2003.

Turunen T.M., Urtti A., Paronen P., Audus K.L., Rytting J.H., Effect of some penetration enhancers on epithelial membrane lipid domains: evidence from fluorescence spectroscopy studies, Pharm. Res. 11, 288–294, 1994.

Uekama K., Design and evaluation of cyclodextrin-based drug formulation, Chem. Pharm. Bull. 52(8) 900-915, 2004.

Uekama K., Hirayama F., Irie T., Cyclodextrin drug carriersystem. Chemical Reviews, 98, 2045-2076, 1998.

Vermeersch JT, Coppens PJ, Hauquier GI, Schacht EH., Lithographic base with a modified dextran or pullulan hydrophobic layer. US Patent 5,402,725, 1995.

Voorspoels, J.; Remon, J.P.; Eechaute, W.; DeSy, W. Buccal absorption of testosterone and its esters using a bioadhesive tablet in dogs. Pharm. Res., 13 (8), 1228–1232, 1996.

Voyutskii S.S., A utohesion and adhesion of high polymers. 1st ed. HF Mark, EH Immergut eds. Polymer Reviews. Vol 4. New York: John Wiley & Sons, p 272., 1963.

Wallenfells, K., Bender, H., Keilich, G., and Bechtler, G., über pullulan, das schleimhülle von Pullularia pullulans. Angew, Chem., 73, 245-246, 1961.

Wallis RM, Corbin JD, Francis SH, et al: Tissue distribution of phosphodiesterase families and the effects of sildenafil on tissue cyclic nucleotides, platelet function, and the contractile responses of trabeculae carneae and aortic rings in vitro. Am J Cardiol 83(suppl 5A): 3C–12C, 1999.

Wang Guocheng, Ph.D. thesis: Design, synthesis and development of PDE5 inhibitors, , Aston Univerity, Birmingham, UK, 2009.

Williams RO, Sykora MA, Mahaguna V, Method to Recover a Lipophilic Drug From Hydroxypropyl Methylcellulose Matrix Tablets. AAPS PharmSciTech.; 2(2): article 8, 2001.

Williams, A.C., Barry, B.W., Terpenes and the lipid– protein-partitioning theory of skin penetration enhancement. Pharm. Res. 8, 17–24, 1991.

Yamaguchi, T., Caldwell, J., Farmer, P.B., Metabolic fate of [3H]-1-menthol in the rat. Drug Metab. Dispos. 22, 616–624, 1994.

Yamamura K., Ohta S., Yano K., Yotsuyanagi T., Okamura T., Nabeshima T., Oral mucosal adhesive film containing local anesthetics: in vitro and clinical evaluation, J. Biomed. Mater. Res. 43, 313–317, 1998.

Yuen S, Pullulan and its applications. Process Biochem 22:7-9, 1974.

Yuen S. Pullulan and its applications. Process Biochem 9:7-9, 1974.

Zografi G., States of water associated with solids. Drug Dev Ind Pharm 14:1905–1926, 1988.