An Investigation Into The Use Of Zein Proteins As Pharmaceutical Excipients For Modified Drug Release Applications

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For Mum and Dad

Abstract

Zein, the major storage protein of corn, has several interesting properties as a pharmaceutical excipient. This study aims to acquire an understanding of the potential uses of zein, with particular emphasis on its use in modified-release formulations and specifically in colon-targetted drug delivery systems. The drug release from zein-based pellets followed first order kinetics but the release did not comply with the Biopharmaceutics Classification System (BCS) guidelines for immediate release products. At the same time, they showed no real evidence of a sustained-release profile. Zein alone coatings showed an effectively zero-order release all the way through the upper gastrointestinal tract (GIT). These coatings were resistant to degradation in simple buffers of acidic (pH 1) and more basic (pH 6.8) pH over the test duration. The release pattern was found to remain constant regardless of the pH of the medium. Zein was susceptible to the digestive action of pepsin and rupture of the coat was observed. However, the drug release from tablets coated at 20% total weight gain was very low (circa 2%) over the residence time of a solid dosage form (2 hours) in the stomach. Digestion was not observed to the same extent in the presence of pancreatin. Thus, zein-based coated substrates might be expected to enter the colon intact, potentially making them good candidates for colon-specific drug delivery. The incorporation of polysaccharides into pseudolatex-based zein coatings altered the performance of the coated substrates in conditions simulating the upper GIT and their susceptibility to the colonic microbiota. The incorporation of pectin led to a premature drug release along with film cracking in the upper GIT. However, the incorporation of heat-treated acetylated high amylose maize starch (HAS) allowed sufficient control of the drug release in the upper GIT and resulted in a significant increase ($p < 0.05$) in release rate and extent under colonic conditions, as compared to the zein alone coatings.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

Acetonitrile	MeCN
Acetylated High Amylose Maize Starch	HAS
Area under the curve	AUC
Aspect Ratio	AR
Atomic Force Microscopy	AFM
Attenuated Total Reflectance-Fourier Transform Infra-Red	ATR-FTIR
Biopharmaceutics Classification System	BCS
British Pharmacopoeia	BP
Caffeine	CAFF
Colony Forming Unit	CFU
Corn Gluten Meal	CGM
Degree of Substitution	DS
Dibasic Calcium Phosphate	DCP
Ethyl Cellulose	EC
Fasting State Simulated Intestinal Fluid	FaSSIF
Fourier Transform Infra-Red	FTIR
Gastrointestinal tract	GIT
Generally Recognized As Safe	GRAS
Glass transition temperature	Tg
Glycerol	GLY
High Performance Liquid Chromatography	HPLC
Hydroxypropyl Methylcellulose	HPMC
Isopropyl Alcohol	IPA
Kilo Daltons	kDa
Laser Diffraction	LD
Localized Thermomechanical Analysis	LTA

Loss On Drying	LOD
Mean Dissolution Time	MDT
Mega Pascale	MPa
Methyl Parabens	MP
Microcrystalline Cellulose	MCC
Minimum Film Formation Temperature	MFT
Modulated Temperature Differential Scanning Calorimetry	MTDSC
Nano-Thermal Analysis	n-TA
Newton	N
Paracetamol	PCT
Particle Size Distribution	PSD
Phosphate-Buffered Saline	PBS
Poly Vinyl Pyrrolidone	PVP
Polyethylene Glycol 400	PEG 400
Polypropylene Glycol 400	PPG 400
Polyvinyl Alcohol	PVA
Polyvinyl Pyrrolidone	PVP
Propyl Parabens	PP
Propylene Glycol	PG
Relative Humidity	RH
Scanning Electron Microscope	SEM
Simulated Colonic Fluid	SCoF
Simulated Gastric Fluid	SGF
Simulated Intestinal Fluid	SIF
Small-Angle X-Ray Scattering	SAXS
Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis	SDS-PAGE
Sodium Lauryl Sulphate	SLS
Standard Deviation	SD

Tapping mode in AFM imaging	TM-AFM
Thermogravimetric analysis	TGA
Total weight gain	TWG
Triethylglycerol	TEG
Ultraviolet-Visible Spectrophotometry	UV-VIS
United States Pharmacopoeia	USP
X-Ray Powder Diffraction	XRPD
α-Lactose Monohydrate	Lact

1 Chapter 1 Introduction

Biodegradable plastics and films based on plant-derived proteins, being both renewable and biodegradable, are of great interest and offer environmental benefits over petroleum-based materials (Parris et al., 1997; Gillgren et al., 2009). Zein, the major storage protein of corn, was first isolated by Gorham in 1821 (Lawton, 2002). Zein films have been used in food packaging and coating of fruit and vegetables (Gennadios et al., 1990; Weller et al., 1998; Bai et al., 2003). Moreover, zein has been investigated for its potential use as a pharmaceutical excipient with particular initial interests in encapsulation (Katayama et al., 1992; Wang et al., 2005) and coating (Winters et al., 1958; Beck et al., 1996; O'Donnell et al., 1997). These studies revealed some promising drug delivery properties of zein with controlled-release characteristics being attained. Modifying the drug release rate from a solid dosage form can be achieved by various technologies including the use of barrier coatings to control the body fluid access to the drug, thereby controlling both drug dissolution and drug diffusion rates from the dosage form. Alternatively, one may embed the drug in a hydrophobic or hydrophilic matrix system (Ansel et al., 1999). The following discussion will give an overview of zein protein, its characteristics and potential uses, and of modified-drug delivery, site-specific targetting, the importance of targetted delivery and how it may be achieved. Two techniques including extrusion/spheronization and conventional coating will be discussed as means of producing zein-based multiparticulates (pellets) and zein-coated dosage forms, respectively. The former is an example of a matrix-controlled drug delivery device, whereas the latter represents a reservoir drug delivery system. Finally, the objectives of the project will be outlined.

1.1 Zein

1.1.1 Introduction

Zein proteins are the major storage proteins of corn (Shukla et al., 2001), and are organized in protein bodies located in the endosperm (Cabra et al., 2007). Zein proteins serve as a nitrogen source for embryos during germination and they compromise circa 45-50% of the total protein in corn (Shukla et al., 2001) and constitute 44-79% of the endosperm proteins (Lawton, 2002). Zein is typically isolated from corn gluten meal (CGM), a protein-rich by-product of starch production by wet milling, by solvent extraction usually with

isopropanol. Zein has several interesting properties, potentially making it a good candidate for being used as a pharmaceutical excipient. Firstly, zein can form tough, glossy and hydrophobic coatings which are resistant to microbial attack. Secondly, it is soluble in aqueous alcohol solutions but not in water (Shukla et al., 2001), signifying its use as a potential vehicle for controlled drug release (Georget et al., 2008). In addition, it is biodegradable and biocompatible (Dong et al., 2004) and has strong adhesive properties (Kim, 2008). Indeed, zein has been widely used in the food industry, including packaging and coating, but little is known about using this biopolymer in pharmaceutical products (Georget et al., 2008).

1.1.2 Characteristics of Zein

1.1.2.1 Composition

Zein proteins are prolamins, a term which means a protein rich in proline and glutamine and/or aspargine (Esen, 1986). Zein is particularly rich in hydrophobic amino acids (more than 50%), especially aliphatic amino acids, with the highest levels corresponding to leucine (20%), proline (10%) and alanine (10%) (Gianazza et al., 1977; Shukla et al., 2001; Cabra et al., 2006). This structure reflects high aliphatic indexes and high surface hydrophobicity (Cabra et al., 2005). Zein is deficient in acidic and basic amino acids but rich in glutamic acid (21-26%) (Shukla et al., 2001); however, almost all the β- and γ-carboxyl residues of glutamic and aspartic acids are amidated (glutamines and aspargines, respectively) (Righetti et al., 1977; Cabra et al., 2006). The zein amino acid constitution shows low amounts of polar and a high proportion of nonpolar amino acids which explains their high insolubility in water and their tendency to aggregate. It also denotes the low amount of aromatic residues, and the absence of tryptophan and lysine residues. The latter feature explains the negative nitrogen balance of zein. Zein's insolubility in water and its poor nutritional quality limit its use in human food products (Shukla et al., 2001).

Zein is known to be a hydrophobic protein (Cabra et al., 2006). A number of scales has been developed to determine the hydrophobicity of proteins. Goldman-Engelman-Steitz (GES) hydrophobicity scale classifies amino acids on the basis of the water-oil transfer free energies of the hydrophobic and hydrophilic components in the amino acid side chains (Engelman et al., 1986). According to the GES scale, 76.25% of the amino acid residues of

zein are hydrophobic, with the highest level corresponding to alanine, leucine, proline and serine. The highly hydrophobic character of zein can be also described by the aliphatic index which refers to the relative volume occupied by the aliphatic side chains. The grand average of hydropathicity index (GRAVY) classifies proteins according to their solubility in water; hydrophobic proteins have positive values while hydrophilic proteins show negative GRAVY indices. The amino acid sequence of zein yields a high aliphatic index (119.3) and a positive grand average of hydropathicity (0.27). The relative hydrophobicity of zein can be also determined by calculating its free energy of hydration based on its amino acid sequence. In comparison with albumins and globulins, the hydration free energy calculated for zein is less negative indicating higher hydrophobicity (Cabra et al., 2006). Despite the abundance of hydrophobic and uncharged amino acids, however, the structure of zein comprises both hydrophobic and hydrophilic domains allowing it to behave as a polymeric amphiphile (Wang et al., 2008). Zein is therefore able to self-assemble into several mesophases as controlled by the hydrophilic-hydrophobic balance (HLB) of the system. The swelling behaviour and water sorption of zein (Beck et al., 1996; Oh et al., 2003) suggest some protein-water interaction which is generally driven by the polar side chains of amino acid residues of proteins that provide much of the attraction for the adsorbed water molecules (Pauling, 1945).

Biologically, zein is a mixture of different peptides of various molecular size, solubility and charge (Shukla et al., 2001; Lawton, 2002). There is a large number of protein fractions identified by various methods by several authors which has created confusion in the nomenclature of zein polypeptides (Figure 1.1). Gianazza et al. (1977) fractionated zein into two broad fractions, one soluble in alcohol (named Z1) and the other soluble in alcohol containing a reducing agent (Z2) which were further resolved into four polypeptide chains having molecular weights (kilo Daltons, kDa) of 23 (Z23), 21 (Z21), 13.5 (Z13.5) and 9.6 (Z9.6). The last two components are only present in the Z2 fraction and are rich in sulphur-containing amino acids and are therefore thought to be responsible for zein granule formation via disulphide bridges. Esen (1986) fractionated whole zein into three separate fractions by differential solubility in solutions containing between 0 and 95 % isopropyl alcohol (IPA), with or without reducing agents and/or buffers. These three fractions are α-zein, β-zein and γ-zein. α-Zein was the fraction soluble in 50 to 95% IPA, but insoluble in 30% IPA/30 mM Na-acetate. β-Zein was the fraction soluble in solutions of 30 to 95% IPA

containing a reducing agent, but insoluble in both 90% IPA (without the reducing agent) and 30% IPA/30 mM Na-acetate. γ-Zein is soluble in 0 to 80% IPA in the presence of a reducing agent as well as soluble in 30% IPA/30 mM Na-acetate. Wilson (1991) separated zein into six classes using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). These classes are called A and B zeins (equivalent to α or 22 and 19 kD zeins, respectively), C zein (β or 14-15 kD zein), D zein (δ or 10 kD zein), E (γ or 27 kD zein) and F (γ or 16 kD zein).

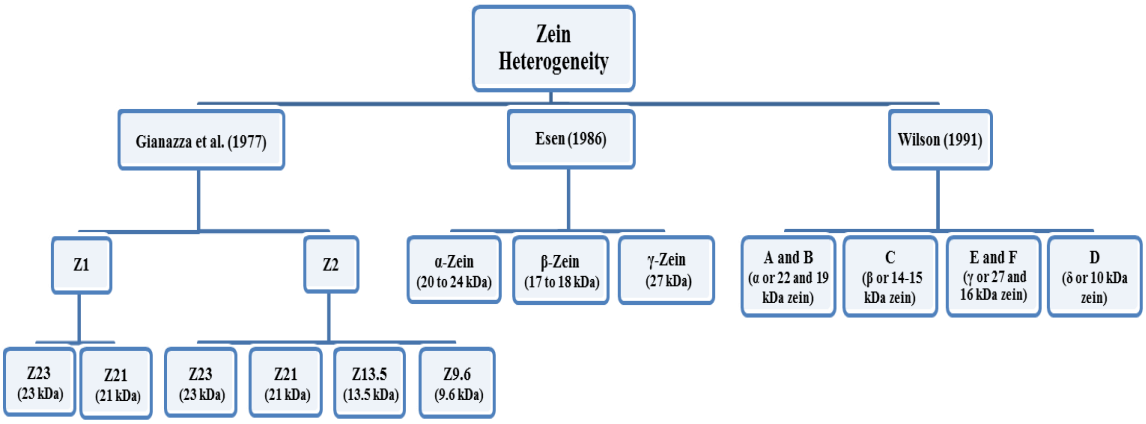


Figure 1.1 A diagram showing the fractions of zein identified by different authors.

In short, zeins can be classified on the basis of their solubility and molecular weight into α, β, γ and δ zeins (Esen, 1986; Wilson, 1991). α-Zein accounts for 75-85% of the total zein and is divided into two types: Z19 and Z22 of approximate weights of 19 and 22 kDa, respectively. β-Zein contains two 17-18 kDa methionine-rich polypeptides and accounts for 10-15% of the total zein. γ-Zein are the next most abundant zein (approximately 20% of the total zein) (Lawton, 2002) and include two proline-rich polypeptides of 27 and 16 kDa. δ-Zein is a minor fraction of the total zein and has an approximate weight of 10 kDa. Commercial zein is, however, made up of α-zein as the other types of zein (β, γ and δ) are thought to contribute to gelling (Lawton, 2002).

1.1.2.2 Zein Structure

The primary structure, i.e. the amino acid sequence, of α-zein has been deduced from chromatography on zein itself and the determination of the genes that code the protein (Lai et al., 1999). Z19 and Z22 consist of 210 and 245 amino acids, respectively. Both of these

zein fractions have sequence homology where non-repetitive N- (of about 10 residues) and C-terminal sequences (36-37 residues) are separated by 9 or 10 domains of repetitive sequences of about an average of 20 residues in Z19 and Z22 zeins, respectively (Tatham et al., 1993). α-Zeins were among the first proteins to be studied by physical and chemical methods (Tatham et al., 1993; Forato et al., 2004). The conformation of zein in alcoholic solutions has been investigated by ultracentrifugation, viscometry, birefringence, and dielectric constant studies. All early studies, in agreement with the recently conducted SAXS (small-angle X-ray scattering) experiments (Tatham et al., 1993; Matsushima et al., 1997), suggested that α-zeins behave as asymmetrical particles in solution. Furthermore, those earlier studies indicated elongated molecular structures with high axial ratios that vary according to the author and the method used. The variation can be assigned to different factors including the use of total or crude zein fractions, which may contain oligomers, monomers and proteins other than the α-zein, the use of different methods that measure different protein properties (Tatham et al., 1993), and finally the use of the α-zein mixture instead of its individual components, i.e. Z19 and Z22 (Forato et al., 2004). Subsequently, the secondary structure of α-zein in solution was verified by optical rotation (OR), optical rotatory dispersion (ORD) and circular dichroism (CD), all implying high α-helices content with an average content of 50-60% (Argos et al., 1982). Using FTIR and solid-state C-NMR spectroscopy, Forato et al. (2003) demonstrated that the secondary structure of α-zeins in the solid state, i.e. the high helical content of the protein of about 43%, is consistent with the α-helices content (40-59%) calculated by CD and ORD in an alcohol solution. However, a higher β-sheet content compared to that calculated in solution was quantified, and they suggested that the difference is due to the different properties measured by the two methods. They also concluded that alcohol solubilisation does not affect zein's secondary structure.

Factors that influence the secondary structure of zein have been investigated. The effect of temperature was investigated by several authors (Duodu et al., 2001; Cabra et al., 2006); an increase in the β-sheet component possibly at the expense of some α-helical structure was detected as temperature was increased. This change was regarded as a characteristic of heat or solvent denatured and aggregated protein (Tomer et al., 2002; Gao et al., 2005). It was suggested that heating stimulates the formation of higher molecular weight, disulphide-bonded protein oligomers and polymers, leading to changes in the protein secondary

structure and to irreversible protein denaturation (Duodu et al., 2001; Cabra et al., 2006). They explained that the energy applied during cooking (heating) would disrupt the hydrogen bonds that originally stabilized the α-helices. Subsequently, the polypeptides may become loosened and arranged next to each other forming the intermolecular β-sheet structure and allowing for more disulphide crosslinking and aggregation to take place (Cabra et al., 2006). The data showed a predominance of the α-helical structure when the pH was more alkaline than the isoelectric point. When the pH is greater than the isoelectric point of zein (pI = 6.8), Z19 will have a net negative charge. This fact suggests that the protein favours the α-helical structure, when it has less positive electrostatic charges (i.e. a net negative charge). The obtained results implied that the denaturation speed is greater at higher pH values (more alkaline), thus signifying a relationship between the speed and pH. This observation can be explained by the protein net negative charge due to the transformation (deamidation) of glutamine into glutamate at high pH values, resulting in electrostatic interactions and structural instability. It is worth noting that the pI value of zein might influence the drug release from zein-based dosage forms at different pH values, possibly by promoting some electrostatic interactions with charged drugs (Xiao et al., 2011).

The high resolution techniques such as NMR and X-ray diffraction have not established a three-dimensional structure of α-zeins yet. However, several models have been suggested. One of the previously proposed structures is the Argos model (Argos et al., 1982). These authors suggested a structural model that consists of nine topologically antiparallel and adjacent helices grouped within a distorted cylinder of oval cross-section with the helical and cylindrical axes aligned. The structure was stabilized by intramolecular hydrogen bonding maintained by the two polar segments of the mainly hydrophobic helical wheel. The formation of a molecular plane of zein is established via the intermolecular bonding of the third polar segment of the helical wheel and the interaction of the glutamine residues in the turn regions that exist at the caps or ends of the cylindrical clusters. Figure 1.2 (a) suggests a possible nine-helical zein protein structural model. A possible model for the arrangement of zein proteins within a plane as well as for the stacking of molecular planes is shown in Figure 1.2 (b). This model suggests a relatively globular form with an axial ratio of 2:1 which is incompatible with the elongated molecular structure suggested by the physico-chemical studies (Matsushima et al., 1997).

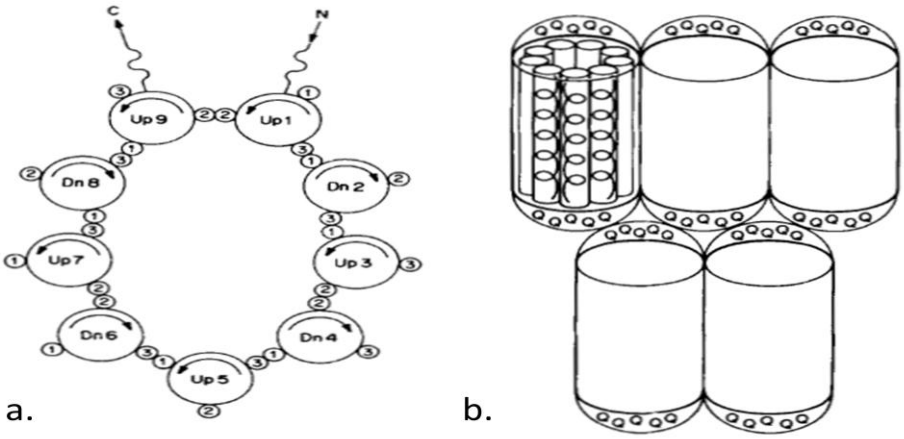


Figure 1.2 A possible nine-helical zein protein structural model (a) and a possible model for the arrangement of zein proteins within a plane as well as for the stacking of molecular planes (b) (Argos et al., 1982).

More recently, small-angle X-ray scattering (SAXS) has been used to study the dimensions and shape of zein in alcoholic solutions. Tatham et al. (1993) studied α-zeins in solutions of 70% (v/v) of aqueous methanol. Both SAXS and viscometric results suggest that α-zein proteins exist as elongated asymmetric structures, approximating to prolate ellipsoids or rods, in solution. A prolate ellipsoid of length 19.6 nm and cross-section diameter 0.7 nm, or a rod of length 15.3 nm and diameter 1.38 nm were interpreted based on SAXS data. Viscometric measurements resulted in values of 23.8 and 1.8 nm for the length and diameter, regardless of the shape. Two possible structures were predicted. The first one has the α-helix folded back on itself, either partially or in half, developing a two-helix bundle. The second one has a linear α-helix that is distorted in a way to shorten the protein apparent length. The rigid structure predicted by SAXS, suggested that the α-helical sections are associated by rigid structures that could be reverse turns or other undefined structures.

Furthermore, Matsushima et al. (1997), using SAXS, suggested that the antiparallel helices of the tandem repeat units (each formed by a single α-helix and joined by glutamine rich turns or loops) linearly stack in the direction perpendicular to the helical axis (Figure 1.3). The suggested structural model of α-zein adopts an elongated rectangular prism shape with the length of 13 nm and an approximate axial ratio of 6:1. SAXS results also suggested that the reduced α-zein exist as an aggregate of four zein molecules rather than monomers in aqueous ethanol solution.

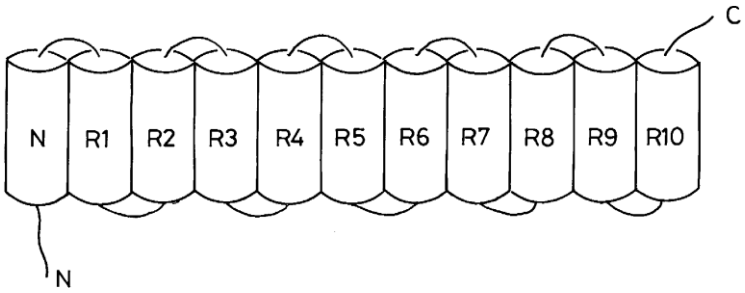


Figure 1.3 A possible structural model of α-zein (Z22). Each cylinder represents a tandem repeat unit formed by a single α-helix and the glutamine rich turns or loops joining them are represented by the curves (Matsushima et al., 1997).

Based on NMR and SAXS studies in alcoholic solutions and FTIR spectroscopy in the solid state, Forato et al. (2004) suggested a hairpin model that is composed of short helical structures arranged in an extended way and linked by loops, turns, or sheets. The helices would either fold back upon themselves or be extended according to the environment.

More recently, Momany et al. (2006) stated that the previously published structures of α-zein do not agree with the experimental data and they created a totally new model using molecular mechanics and dynamics simulations. They suggested a three superhelical (triple helix) structures; each consisting of three helical segments aligned in an antiparallel direction to each other and connected by a glutamine rich bend or turn (Figure 1.4). Momany et al. (2006) found that only the coiled-coil conformations can be used to build up this superhelical segment. All the nonpolar faces are on the interior of the triple-helix segment, while the glutamine side chains pointed away from the helical region. Lutein (the yellow pigment of corn) is suggested to be bound to the totally nonpolar core of the superhelix. The resulting structure has an axial ratio of ≈ 6 or 7:1 which is consistent with the experimental data.

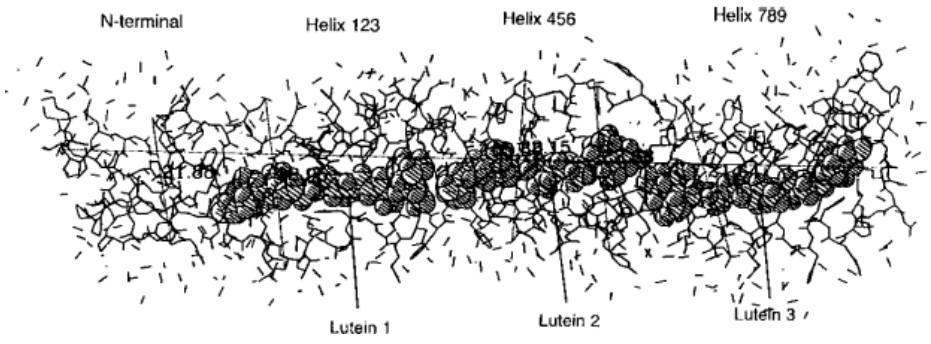


Figure 1.4 Complete structure of Z19 after coupling all triple-superhelix segments and adding the N-terminal segment (Momany et al., 2006).

1.1.2.3 Solvents

1.1.2.3.1 Non-aqueous Solvents

Based on the predominance of nonpolar amino acids, solvents for zein should possess mixed characteristics, containing both ionic and non-ionic polar groups as well as nonpolar groups, either by their structure (in case of pure solvents) or by their composition (for mixed solvents) (Shukla et al., 2001). Zein is insoluble in water alone, but is soluble in the presence of alcohol, high concentrations of urea, high concentrations of alkali (pH 11 or above) or anionic detergents. The phenolic hydroxyl group of tyrosine and the amide rich amino acids account for its alkaline solubility. Zein is insoluble in anhydrous alcohol, except in methanol or at high temperatures.

Zein is known for its solubility in binary solutions consisting of lower aliphatic alcohols and water, such as aqueous ethanol and aqueous isopropanol (Lawton, 2002). The incorporation of secondary liquids such as water and aromatic hydrocarbons, would improve the solvating power of anhydrous alcohols. Different combinations are reported in which aliphatic alcohols, ketones or glycols can be used as primary solvents along with secondary components such as water, aromatic or chlorinated hydrocarbons, nitroparafins, aldehydes, or cyclic ethers. The power of binary solvents depends on the ratio of the two components and the temperature. For example, zein is only soluble in 50 to 90% aqueous ethanol; however, zein can be dispersible in higher concentrations (90 to 100%) and in lower concentrations of alcohol (40%) at high temperatures. Moreover, Chan et al. (1993) stated that the extent of solubilisation, for an insoluble protein, is determined by the solvent composition, exposure to oxygen, and solubilisation time where the solubility reached a maximum upon the inclusion of a reducing agent and when oxygen was excluded. Shukla et al. (2001) reported that no limit to zein's solubility in an alcohol water mixture, once a suitable one was found, could be determined. Indeed, the only limitation on the amount of zein that can be dissolved is the viscosity that can be handled by the mixing equipment. They suggested that acetone, dioxane and dioxolane can form good solvent mixtures with water for zein and the resulting viscosity would be far less than those with alcohol. As they could not find a saturated solution for zein, they were uncertain if it was a true solution and the mixture was therefore believed to be more related to colloidal systems. Therefore, they used the term 'cloud point' to describe the temperature at which the zein solution turns turbid. The cloud point temperature is dependent on the binary solvent and the ratio of the

two components. Aqueous n-propanol was reported to be the most effective solvent for zein due to its low cloud point and wide range of mixture ratios.

Primary solvents of zein are also available such as glycols, glycol-ethers, amino-alcohols, nitro-alcohols acids, amides, and amines. The solvating power of primary solvents depends on the balance of polar and nonpolar groups within its structure. For instance, propylene glycol can dissolve zein at room temperature whereas glycerol needs to be heated to 150 °C.

1.1.2.3.2 Aqueous Solvents

The solubility of zein in water can be increased by either acid or alkali treatments with HCl (pH < 1) or NaOH (pH > 12), respectively; these are commonly used to convert the glutamine and aspargine amino acids to the acid or salt form. On one hand, these treatments consume large quantities of chemicals and may cause significant degradation of the protein. On the other hand, hydrolysing the amide groups to carboxylic groups enhances zein solubility at lower pH and suggests the possibility of further modification. For example, zein or deamidated zein can be esterified or reacted with fatty alcohol to form fatty acid esters or fatty acylated zein (Shukla et al., 2001).

Alternatively, enzymatic modification with alcalase (a protease) was used to prepare water soluble zein (Mannheim et al., 1993). Zein was partially hydrolysed by the alcalase in an organic solvent, followed by an aqueous phase hydrolysis with the same protease, and the ultrafiltration of the reaction mixture. Zein hydrolysates showed higher moisture sorption capacity than the unmodified zein due to the exposure of the ionisable polar amino acids such as glutamate, which is abundant in zein, upon the enzymatic modification of the ternary and quaternary structure of the protein.

1.1.2.4 Aggregation, Oligomerization and Gelation

Zein proteins are known for their native tendency to aggregate. The four types of zein α, β, γ, and δ aggregate and are stably deposited in membrane vesicles named protein bodies (Matsushima et al., 1997). Nunes et al. (2005) proposed that zein proteins are organised in oligomers which are resistant to high temperatures and treatment with reducing agents and

tend to form high molecular weight aggregates. Moreover, heating of the protein e.g. via cooking promotes the formation of these stable oligomers. Similarly, Cabra et al. (2006) detected the presence of highly stable (resistant to highly denaturing conditions) 45 and 66 kDa oligomers in the Z19 α-zein sample. They stated that the Z19 protein exists as monomers as well as dimers, trimers and oligomers. They found that the disulphide-crosslinking and the hydrophobic nature of zein are the causes of the oligomerization process. In addition to the hydrophobicity, the high glutamine content could lead to the formation of intermolecular hydrogen bonds that would contribute to the oligomerization process and the formation of more stable structures. The sulfhydryl groups and disulfide content affect the protein flexibility, thermal stability and tendency to oligomerization (Cabra et al., 2006; Cabra et al., 2008). Most of the sulphhydryl (-SH) groups form disulphide bonds, while only 2.47% of the total -SH groups are free and all of them are surface-located. Cabra et al. (2008) suggested that these disulphide interactions are the driving force for the Z19 α-zein aggregation; these bonds favour the interactions that precede the aggregation. The non-covalent interactions, particularly the hydrophobic interactions, contribute much less to the aggregation process. In agreement, Cabra et al. (2006) found that direct alkylation of the free -SH groups, without reducing the disulphide bonds, avoided the formation of higher order high molecular weight aggregates, whereas the reduction of disulphide bonds and the later alkylation of -SH groups inhibited almost 95% of the protein aggregation.

The Z19 α-zein tendency towards oligomerization and aggregation is strongly dependent on temperature (Cabra et al., 2006; Cabra et al., 2008). The disulphide-sulphhydryl exchange reaction is a chemical aggregation process that results in covalent disulphide linked aggregates. It has been suggested that the -SH group acts as a reducing agent for the intramolecular disulphide bonds. Subsequently, the free SH group will form a new disulphide bond with a recently liberated –SH group (Cabra et al., 2008). It is well known that the speed of a chemical reaction increases as the temperature increases. Similarly, heating induces the formation of more disulphide crosslinked oligomers and polymers (Cabra et al., 2006). Wet cooking has been proposed to induce formation of disulphide-bonded protein polymers in maize (Batterman-Azcona et al., 1998; Duodu et al., 2001). It was also suggested that this polymerization reaction results in changes in the protein secondary structure, i.e. a greater intermolecular β-sheet component, possibly at the

expense of some α-helical component, and explains the irreversible thermal denaturation of the Z19 protein.

The degree of aggregation also depends on the composition of the solvent mixture. Conventionally, 70-90% aqueous ethanol is used to dissolve zein; however, a size variation of zein molecules in that range of aqueous ethanol was detected as a result of the aggregation process (Kim et al., 2008). The viscosity of the zein solution decreased as the ethanol concentration increased from 50% to 90%, indicating that the apparent molecular weight of zein decreases at a higher ethanol content.

Zein solutions and dispersions gel easily with time and heat (Shukla et al., 2001; Lawton, 2002). Gelation could be a result of denaturation of zein and/or due to the presence of insoluble 'bodies' that act autocatalytically to precipitate zein (Swallen, 1941). The time required to gel depends on the solvent and the concentration of the solvent; nonaqueous mixtures were less prone to gel compared to aqueous mixtures and the latter ones gelled at slower rate at lower water proportions. Gelation is faster at higher temperatures and is accelerated by agitation, even in dilute solutions of zein. Gelling of zein also depends on pH. Primary solvents provide better protection against gelling than those containing water. However, zein solutions can be stabilized against gelling by the addition of a third organic component such as acid resins (e.g. rosin or shellac) or an aldehyde (Shukla et al., 2001; Lawton, 2002), or by addition of stabilizers such as propylene glycol (Shukla et al., 2001). Guo et al. (2009) found out that pure zein-based dry coated tablets formed a gelatinous layer on the surface that prevented their disintegration during the dissolution test. They suggested that this gelation can be due to disulphide-mediated polymerization of zein. Similar results were reported by Gao et al. (2007).

1.1.2.5 Plasticizers

Plasticizers of zein, similar to its solvents, need a proper balance between polar and nonpolar groups. Triethylglycerol (TEG) was found to be an effective plasticizer for zein (Lawton, 1992; Lawton, 2002; Wang et al., 2003b; Selling et al., 2004). Although water was not added for its plasticization effect, Lawton (1992) and Madeka et al. (1996) revealed that water is an excellent plasticizer for zein. Lawton (1992) studied the effect of water on zein's glass transition temperature (Tg); the Tg of zein decreases rapidly with

increasing water content, but the rate of change evens out at approximately 15% water (Figure 1.5). They noticed that the Tg of hydrated zein does not go much below room temperature at any moisture (plasticizer) contents used. As a result, sorbed or lost moisture (depending on the relative humidity) must be considered when evaluating the plasticization effect on zein.

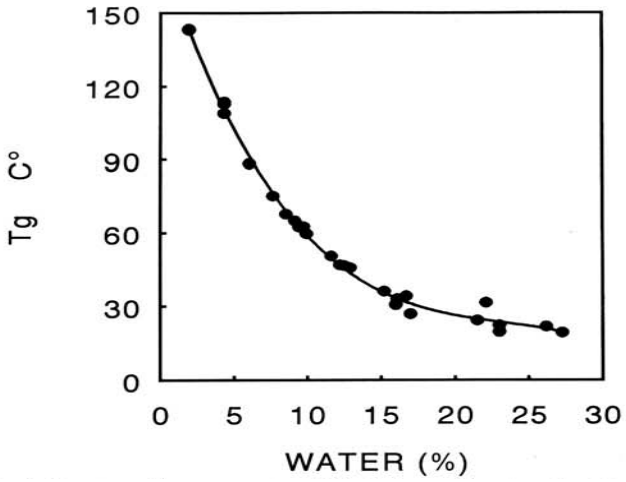


Figure 1.5 Glass transition temperature (Tg) of zein as a function of moisture content (Lawton, 1992).

Other plasticizers of zein include polyols such as glycerol (Gao et al., 2006; Ghanbarzadeh et al., 2006; Gillgren et al., 2009; Li et al., 2010), fatty acids such as oleic acid (Swallen, 1941; Kanig et al., 1962; Wang et al., 2003b), polyethylene glycol 400 (PEG 400) (Parris et al., 1997; Guo et al., 2008; Li et al., 2010), polypropylene glycol 400 (PPG 400) (Parris et al., 1997), propylene glycol (PG) (Oshlack et al., 1994; Parris et al., 1997) or combinations of any such as PEG 400/glycerol or PPG 400/glycerol (Parris et al., 1997).

1.1.3 Use of Zein So Far

In the mid-20th Century, zein was used to coat pharmaceutical tablets based on its film-forming properties and microbial resistance (Winters et al., 1958). Zein coated tablets were superior to sugar-coated tablets in terms of resistance to heat, abrasion, and humidity. The coatings also masked the taste and odours of the original tablets. Kanig et al. (1962) studied some film-forming materials used in pharmaceutical applications including zein, methylcellulose, ethylcellulose and a combination of ethylcellulose and polyvinyl pyrrolidone. Zein films exhibited the lowest water vapour transmission, possibly due to the

large number of nonpolar side chains, and are therefore expected to offer good protection against moisture when applied to dosage forms. The moisture absorption was 1.5% or less when stored in the relative humidity range of 10 to 52%, but the film was considerably softened after 1 week at a relative humidity of 93%. Gennadios et al. (1994) reported that zein films plasticized with 20% w/w glycerol (based on zein amount) can absorb > 20% of its dry weight when stored a water activity less than 0.8. The amount of water sorbed by zein films is comparable to that of ethylcellulose with a degree of substitution in the range 1.2 to 1.5 (Beck et al., 1996). Water has an effective plasticizing (Lawton, 1992) and swelling (Oh et al., 2003) effect on zein and would therefore influence the physical and barrier properties of zein films. Consequently, an understanding of water sorption properties of zein films at different relative humidities is necessary for its application as a coating agent in the pharmaceutical and food industries.

Zein films have low permeability to oxygen and carbon dioxide, a property particularly important for coating and packaging applications in the food and pharmaceutical industries. The oxygen permeability value of zein films is higher than those of other plant proteins-based films (wheat gluten- and wheat gluten/soy protein isolate-based films (Gennadios et al., 1993)). However, these films had lower permeability values compared to those of common plastic films, such as polyethylene and polyvinyl chloride. Moreover, zein films had an oxygen permeability coefficient that is two-folds less than that of highly substituted (degree of substitution circa 2.5) ethylcellulose-based films (Beck et al., 1996). Accordingly, zein films provide excellent resistance to oxygen permeability and can be used to protect ingredients from oxidative degradation. However, the oxygen barrier characteristics of edible protein films were expected to be influenced by the relative humidity and temperature values as both factors are expected to enhance the molecular motion of the polymer and hence increase its permeability values. Zein has been used as a food coating in order to increase the gloss and to prevent oxidation and development of off-odours (Bai et al., 2003). Zein has also been used as an active packaging i.e. it would be able to release an active ingredient such as antimicrobials into food (Mastromatteo et al., 2009).

Directly compressed tablets of physical mixture or wet granules (Georget et al., 2008), or of spray-dried zein and drug mixtures (Katayama et al., 1992) were developed for

controlled release purposes. The use of an aqueous pseudolatex of zein for controlled release coatings of solid dosage forms (Oshlack et al., 1994; O'Donnell et al., 1997; Guo et al., 2008; Li et al., 2010) and zein-based dry coating of tablets (Guo et al., 2009) have been investigated. Zein was also used as a coating material to achieve better encapsulation efficiency and controlled-release profiles of drug-loaded nanoparticles (Luo et al., 2010).

Zein possesses the additional benefits of being renewable and biodegradable. Therefore, zein has been used to prepare films and tablets composed of microspheres (Dong et al., 2004; Liu et al., 2005; Wang et al., 2005; Hurtado-Lopez et al., 2006; Chen et al., 2010). Films of zein and drug, used to deliver the drug to vascular devices, proved to be biocompatible (Wang et al., 2005). Furthermore, Dong et al. (2004) demonstrated that zein is a promising biomaterial with good biocompatibility for the development of tissue engineering. Injectable in situ gels of zein for extended release were also developed (Gao et al., 2007).

On the other hand, zein has been used in numerous nonedible applications based on its film forming ability. It possesses an outstanding resistance to grease making it suitable for use in food wrappings and containers. It can also be used as an adhesive, and for coating papers (Swallen, 1941). Zein has also been investigated for use as a fibre (Miyoshi et al., 2005). Zein adsorption from aqueous 2-propanol solutions onto hydrophilic surfaces was greater than that onto hydrophobic ones (Wang et al., 2004). Based on zein's strong adhesive properties and its good adsorption to hydrophilic surfaces, it can be mixed with other biopolymers to form biodegradable composites to replace petroleum-based polymers/plastics (Kim, 2008). Fatty acid-zein composites are produced by plasticizing zein with oleic acid and precipitating in cold water (Lai et al., 1999) or by extrusion (Wang et al., 2003a) to evaluate zein's potential use as industrial polymeric material. The incorporation of fatty acids such as oleic acid in zein films is expected to retard water absorption and lower its water vapour permeability. Cross-linking of zein is also thought to increase its toughness, mechanical strength, and water resistance (Swallen, 1941; Sessa et al., 2007).

1.2 Modified Drug Release Systems

1.2.1 Introduction

Modified release dosage forms are designed to achieve drug release characteristics over a specific time course and/or at a specific location that can offer certain therapeutic or convenience objectives over conventional dosage forms. Modified drug release devices aim either to decrease or increase the release rate compared to conventional dosage forms (Siepmann et al., 2008). Rate-controlled release in drug delivery is an advanced technology that aims to deliver the drug at some predetermined rate (Hillery, 2001), whereas targetted-drug delivery aims to deliver the drug to specific sites. Controlled-release dosage forms are associated with greater clinical efficacy by reducing blood level fluctuations and avoiding subtherapeutic plasma concentrations, a lower rate of side effects by avoiding peak concentrations of drug in plasma, and lower dosing frequency and thereby improved patient compliance. This technology would therefore decrease total treatment costs and provide economic value compared to immediate-release dosage forms (Saks et al., 1997; Thombre, 2005). Controlled-release dosage forms are not limited to zero-order delivery but also include pulsed and delayed delivery offering the possibility to simulate night time dosing or circadian rhythms (chronotherapy). Despite the wide applicability of controlling drug release rate, there are only a small number of mechanisms by which this is achieved which include (Hillery, 2001):

- Diffusion-controlled release mechanisms

In these systems, the drug must diffuse through either a polymeric membrane, and they are known as diffusion-controlled reservoir devices, or through a polymeric matrix where they are known as diffusion-controlled matrix (or monolithic) devices.

- Dissolution-controlled release mechanisms

The drug release is controlled by the dissolution rate of an employed polymer. These systems can be further divided into reservoir devices in which the drug is surrounded by a polymeric membrane and matrix devices where the drug is distributed throughout a polymeric matrix.

- Osmosis-controlled release mechanisms

These systems are based on osmosis phenomena and the increase in pressure in the solution that accompanies the movement of water. The excess pressure is known as the osmotic pressure and can be used to pump out a drug at a constant rate from the dosage form. Such devices work independently of the environment. Osmotically controlled systems are a subset of diffusion-controlled systems, but are often categorized separately (Liu et al., 2006b).

- Mechanical-controlled release mechanisms

This mechanism is controlled by external programmable pumps, and it is commonly used for the intravenous administration of drugs in either a zero-order or intermittent drug release.

- Bio-responsive-controlled release mechanisms

The drug release from these devices is stimulated by changes in the external environment such as pH values, ionic strength or blood levels of certain metabolites.

In practice, a combination of mechanisms might simultaneously control the drug release from a specific delivery device. However, diffusion occurs in almost all modified drug delivery systems with different species being able to diffuse including water, drug, soluble polymer chains and additional excipients. Section 1.2.2 therefore focuses on the diffusion-controlled mechanism that controls the release from reservoir and matrix devices made of either biodegradable or non-biodegradable polymers.

1.2.2 Diffusion-controlled release mechanism

Diffusion is defined as a process by which molecules move spontaneously from one region to another to equalize chemical potential or thermodynamic activity. The migrating molecules are named diffusants (also permeants or penetrants). The diffusional barrier designates the membrane or the matrix in which the diffusant migrates and which separates the drug molecules from the external phase (the medium). The concentration gradient of the diffusant within the diffusional barrier is the driving force for diffusion. Diffusion can also be driven by osmotic pressure or matrix swelling (Leong et al., 1988).

1- Reservoir-type non-degradable polymeric systems

In such systems, a drug reservoir is separated form an external phase by a polymeric membrane which functions as a rate-controlling barrier and has either a compact or porous structure. The drug core can be in the solid or liquid state. For compact membranes, the diffusion of drug molecules is initiated by the penetration of a solvent into the drug reservoir, followed by the dissolution (if the drug is solid) and diffusion of the drug within the reservoir, dissolution or partitioning of the drug between the reservoir and the barrier, diffusion through the barrier and lastly partitioning between the barrier and the external medium (Pothakamury et al., 1995). In a reservoir maintaining a saturated state of the drug, the driving force remains unchanged and hence the transport of the drug across the membrane will be kept constant obtaining zero order kinetics, whereas first order release kinetics are obtained when the drug concentration in the inner compartment decreases with time. The rate of drug diffusion follows Fick's law Eq. (1.1):

$$\frac{dm}{dt} = \left(\frac{Dk}{h} \right) \cdot A \cdot \Delta C \qquad \text{Eq. (1.1)}$$

where *dm/dt* is the rate of drug diffusion, *D* is the diffusion coefficient of the drug in the membrane, *k* is the partition coefficient of the drug into the membrane, *h* is the membrane thickness, *A* is the available surface area and *ΔC* is the concentration gradient, i.e. C_d - $C_{r,}$ where C_d and C_r signify the drug concentrations in the donor and receiver compartments, respectively.

As sink conditions ($\Delta C \approx C_d$) apply and with further substituting:

$$\frac{dm}{dt} = K_1 \cdot C_d \qquad \text{Eq. (1.2)}$$

where K_1 is a pseudo-rate constant and is dependent on the factors *D*, *A*, *k* and *h*. Eq. (1.2) presents the familiar form of a first-order rate equation and suggests that the diffusion rate is proportional to drug concentration. However, the drug concentration in the donor compartment C_d is assumed to remain constant, so that Eq. (1.2) simplifies to:

$$\frac{dm}{dt} = K_2 \qquad \text{Eq. (1.3)}$$

where K_2 is a constant and is dependent on C_d. Eq. (1.3) represents a zero-order rate equation and implies that the release rate of a drug from this system does not vary with time.

In some cases, the rate controlling polymeric barrier is not compact but porous. In such systems, the pathway of drug transport becomes tortuous. Accordingly, Eq. (1.2) is modified as follows:

$$\frac{dm}{dt} = D_s \cdot A.C_s \cdot \varepsilon / \tau h \qquad \text{Eq. (1.4)}$$

where C_s, the drug solubility in a solvent, is the product of K and C_r, D_s is the drug diffusion coefficient in the solvent and τ and ε are the tortuosity and porosity of the membrane, respectively. However, the surface area of the membrane and the drug concentration in the donor compartment remain constant, thus a zero-order release rate is attained.

2- Matrix-type non-degradable polymeric systems

In a matrix-type system, the drug is either dissolved or dispersed throughout a polymeric matrix. The total loading of the drug determines its physical state in a polymer; the drug is soluble at a concentration equal to or less than saturation, whereas loading the drug above the saturation gives rise to dispersion (Sah et al., 2001). The release is, however, governed by the geometry of the device, the type of the carrier material and the drug loading.

Based on rate controlling materials, matrix-systems can be further divided into two categories, hydrophobic and hydrophilic systems. In a hydrophobic matrix, the rate controlling components are water insoluble in nature and exhibit negligible swelling and dissolution, thereby helping to maintain the physical dimensions of the matrix during the drug release process. Regardless of a drug's physical state, the release rate from the polymeric matrix is expected to decrease over time. This can be explained by the increase in distance the drug molecules must travel before they reach the medium. The release rate

of up to 50-60% of the total drug content is proportional to the square root of time according to Eq. (1.5):

$$\frac{M_t}{M_\infty} = kt^{1/2} \qquad \text{Eq. (1.5)}$$

where M_t/M_∞ is the fraction of drug released, k is the kinetic constant (with units of time$^{-0.5)}$ and t is the release time.

Eq. (1.5) should not be confused with the square root of time relationship introduced by Higuchi (1963) for thin films with very high initial drug concentration compared to drug solubility, Eq. (1.6):

$$Q_t = \sqrt{Dt}(2A - C_s)C_s \qquad \text{Eq. (1.6)}$$

where Q_t is the amount of drug released after time t per unit exposed area, C_s is the drug solubility in the matrix, A is the drug loading per unit volume, D is the diffusivity or diffusion coefficient and t is the time. Very often, pores form within the hydrophobic matrix upon the release of the active ingredient. Diffusion of drug molecules can therefore occur through these pores in addition to permeation through the polymeric phase and thus Eq. (1.6) can be modified as:

$$Q_t = \sqrt{\frac{D\varepsilon}{\tau}}(2A - \varepsilon C_s)C_s t \qquad \text{Eq. (1.7)}$$

where τ and ε are the tortuosity and porosity of the matrix, respectively. The tortuosity is introduced to account for an increase in diffusion path length due to branching and bending of the pores. Porous monolithic systems maintain the square-root-of time release profile described by Eq. (1.6); however, the release from such a system is proportional to drug loading. The latter, together with the particle size of the drug determines the extent and size of the pores generated within the matrix and would therefore influence the release kinetics. In a general way it is possible to simplify the Higuchi model as:

$$Q = k_H t^{1/2} \qquad \text{Eq. (1.8)}$$

where Q is the amount of drug released, t is the time and k_H is a constant reflecting the design variables of the system.

On the other hand, hydrophilic, initially glassy polymers swell and form a gel layer on contact with aqueous solution. Hydrophilic matrices dominate today's oral controlled release market, and hydroxypropyl methylcellulose (HPMC) is most commonly used. Swelling of the hydrophilic polymer, which involves a transition from a glassy to a gel state upon interaction with the medium, is essential for drug diffusion to occur as the polymeric chains are more mobile allowing the drug to diffuse more rapidly. Swelling of the polymer particles induces structural changes including change of the mobility of the macromolecular chains, macromolecular relaxations and changes of the porous structure including the shape and size distribution of the pores (Korsmeyer et al., 1983). These controlled-release devices where the release mechanism is dependent on solvent penetration can also be referred to as swelling-controlled release systems. The drug release can therefore occur via pure Fickian diffusion characterized by an initial $t^{1/2}$ time dependence of the drug released (described by Eq. (1.5)), can be controlled by polymer relaxation (referred to as case II transport), or via a coupling of diffusion and relaxation phenomena. A simple exponential equation has been widely used to differentiate the contribution of both mechanisms (Korsmeyer et al., 1983; Ritger et al., 1987a; Ritger et al., 1987b).

$$\frac{M_t}{M_\infty} = k_{kp} t^n \qquad \text{Eq. (1.9)}$$

where M_t/M_∞ is the fraction of drug released, k_{kp} is the kinetic constant (with units of time^{-n}), t is the release time and n is the diffusional exponent which is indicative of the transport mechanism. For a thin polymeric film, when the value of n is 0.5, the drug is released by the usual molecular diffusion due to a chemical potential gradient (Fickian diffusion). When the value of n approaches unity, diffusion is described as Case II diffusion where the drug release is independent of time and follows zero-order kinetics. Case II diffusion is associated with state transition (glass-to-gel transition) and relaxation of hydrophilic

polymers which swell in water or biological fluids. Under an analogous condition where diffusion is a combination of Fickian and non-Fickian diffusion, the value of *n* falls between 0.5 and 1. Super case II is associated with *n* values > 1. It is worth mentioning that this equation can be used to fit the first 60% of a release curve, regardless of geometric shape, where the assumption of one-dimensional diffusion under sink conditions is valid. However, the relationship between the diffusional exponent *n* and the corresponding release mechanism is dependent on the geometry as shown in Table 1.1.

Table 1.1 Diffusion exponent and solute release mechanism (Peppas et al., 1989).

Diffusion exponent (*n*)			**Mechanism**
Film	**Cylinder**	**Sphere**	
0.50	0.45	0.43	Fickian diffusion
$0.50 < n < 1$	$0.45 < n < 0.89$	$0.43 < n < 0.85$	Analomous (non-Fickian) transport
1	0.89	0.85	Case II transport

3- Biodegradable polymeric systems

As for non-degradable polymeric systems, biodegradable ones are further divided into reservoir and matrix devices. Two forms of degradation can be distinguished: biodegradation and bioerosion. Biodegradation refers to chemical or enzymatic degradation of the polymer structure that results in chain scission, by which the polymer chains are cleaved into oligomers and finally monomers. In contrast, bioerosion involves material loss from the polymer bulk including monomers, oligomers, parts of the polymer backbone or the polymer bulk (Siepmann et al., 2008). Moreover, erosion can be heterogenous or homogenous. Heterogenous erosion occurs when degradation is confined to the surface of the delivery system, whereas homogenous erosion occurs when degradation occurs at a uniform rate throughout the polymeric system. The type of erosion depends on the hydrophobicity of the polymer. Heterogeneous erosion is more common with hydrophobic polymers and it is more favoured as it can result in constant release rate, whereas homogenous erosion is common with hydrophilic polymers (Pothakamury et al., 1995).

In such systems, the drug release is controlled by the degradation of the polymer membrane or matrix, diffusion or a combination of both. If the rate of degradation is slow

compared to the rate of drug diffusion, drug release mechanisms and kinetics are analogous to those obtained with non-degradable systems. However, in other cases drug release occurs in parallel with polymer degradation and thus the drug release mechanism is more complicated and occurs by drug diffusion, polymer degradation and/or polymer dissolution. The permeability of the drug through the polymer increases with time as the polymeric matrix or membrane is being gradually degraded.

1.2.3 Drug targeting: Basic Concepts and Strategies

Unlike rate-controlled systems, which deliver the drug at a predetermined rate but are generally unable to control the fate of the drug once it is in the body, drug targeting has been developed to attain site-specific drug delivery. These advanced technologies aim to minimize side-effects caused by drug action at non-target sites, and to enhance drug efficacy and patient compliance (Hillery, 2001).

In the simplest form, drug targeting can be achieved by local administration of the drug such as applying a cream or ointment for skin diseases or direct injection of an anti-inflammatory agent into a joint. However, more sophisticated technologies have been developed, particularly for oral and parenteral routes. For oral delivery, systems are available to target specific regions within the gastrointestinal tract such as the small intestine and the colon.

1.2.4 Colon-specific Drug Delivery Systems

1.2.4.1 Introduction

The colon, despite its simple function of water and electrolyte absorption and the formation, storage and expulsion of faecal material, can be liable to many pathological conditions, such as constipation, Crohn's disease, ulcerative colitis, irritable bowel syndrome, carcinomas and infections. Colon-specific drug delivery is therefore necessary to transport drugs in adequate concentrations to the disease site, maximize their pharmacological action and reduce the administered doses and associated systemic side effects. In addition to local treatment, the colon can be a site for systemic drug delivery. Despite the small area of absorption, the viscous environment and the strong barrier

properties of the colonic epithelium, the colon has some unique characteristics which make it attractive for site-specific drug delivery. These include the lower peptidase activity and the longer transit time (can last for up to 78 hours) compared to the upper gastro-intestinal (GIT) which allows the delivery of unstable peptides and drugs of low permeability via the colon. The colonic microbiota is another feature unique to this part of the GIT (Leopold, 2001).

1.2.4.2 Physiological characteristics of the colon

The large intestine is composed of the caecum, the ascending colon, the transverse colon, the descending colon, the sigmoid colon and the rectum, making up approximately 1.5 m of the 6 m GIT. The colon is folded but, unlike the small intestine, is devoid of villi and microvilli and thus it provides a smaller surface area (circa 1/30 th) than the small intestine (Ashford, 2007). The human colon hosts over 1,000 species of bacteria (Hoyles et al., 2012), with a population of 10^{11} - 10^{12} colony forming unit/m (CFU/mL) compared to 10^{2} and 10^{4} CFU/mL in the stomach and small intestine (McConnell et al., 2008b), respectively. *Bacteroides*, *Bifidobacterium*, *Eubacterium* and *Lactobacillus* constituting count for over 60% of the total flora (Yang, 2008). The dramatic increase in bacterial population upon reaching the large intestine has been of particular interest for colonic targetting. These bacteria are mainly anaerobic or facultative anaerobic microorganisms (Leopold, 2001), and produce a wide spectrum of reductive and hydrolytic enzymes involved in carbohydrate and protein fermentation, bile acid and steroid transformation and metabolism of xenobiotic substances. The composition of colonic bacteria and enzymes can be affected by many factors including age, diet, diseases, medication such as antibiotics, and geographic regions (Yang, 2008). The redox potential of the colon, in the range of - 250 to - 480 mV, demonstrates its ability to support the growth of anaerobic bacteria. The pH of the colon ranges from a slightly acidic environment in the proximal colon (pH 6.0 to 6.4), owing to the degradation of polysaccharides to short chain fatty acids, to a neutral or slightly alkaline environment in the distal colon (pH 7.0 to 7.4) (Leopold, 2001).

Water is efficiently absorbed in the colon and therefore the colonic environment is generally viscous. The low-fluid environment of the colon would thus influence the drug release from the administered dosage forms; a modified-release dosage form could be

exposed to anything between 1 and approximately 100 mL of free fluid in the colon (McConnell et al., 2008a). McConnell et al. (2008a) stated that the total fluid volume in the proximal colon is approximately 187 mL and the average free fluid volume in fasted state is 13 mL. Water available for dissolution is maximal in the ascending colon, whereas the transverse colon is characterized by low water and high gas levels (the gas is produced by fermentation in the ascending colon) which limit the access of contents to water. By the time the luminal content reaches the descending colon, the mass is too solid to allow drug dispersion or absorption in this region. Therefore, specific drug delivery to the large bowel should be targetted toward the proximal colon (Wilson, 2008). The understanding of colonic motility is limited due to its inaccessibility and regional differences in structure and function. Nevertheless, the most common pattern of contractile activity is non-propulsive segmental or haustral contractions brought about by the contraction of the circular muscle and associated with mixing intraluminal content and shifting it over short distances (Ashford, 2007; Yang, 2008). On the other hand, the contraction of the longitudinal muscle and the associated propulsive movements occur only 3 - 4 times per day in normal individuals. The colonic transit of pharmaceuticals is long and variable depending on the type of dosage form, diet and disease state. Drug delivery within the colon is highly dependent on the rate of transit through this region. The colonic transit of solutions and particles is slower than that of large single dosage forms such as capsules and tablets, and is approximately 30 to 40 hours and 20 to 30 hours, respectively (Lee et al., 2001). This can be due to the entrapment of the finer particulates in the folds of the colon which results in an increase in their retention time (Wilson, 2008).

1.2.4.3 Approaches to Colon-specific Drug Delivery

Colon-specific drug delivery via the oral route is simple in concept; however, it is challenging in practice. The formulation must reach the colon intact preventing premature drug release in the stomach and intestine but initiating drug release in the colon. However, the colon is the most distal part of the GIT, and therefore the formulation would be exposed to a range of conditions on its way down the gut, including pH, enzymes, electrolytes, transit time and pressure (Basit, 2005). These conditions considerably vary among individuals and are affected by factors such as age, sex and diseases. Characteristics unique to the colon have been identified and exploited for colon-specific drug delivery and these include pH, transit time, pressure and microbiota. Both single unit and multi-unit

systems were investigated for use as modified drug release formulations with the latter being more advantageous for colonic delivery. Multiparticulates exhibit more uniform gastrointestinal transit, and provide larger surface area for drug release; this particularly facilitates enzymatic attack by the colonic microbiota and enhances the release of drugs, especially poorly water soluble ones, in this low-fluid environment.

1.2.4.3.1 pH-dependent Delivery

This strategy uses pH-sensitive polymers having a threshold pH for dissolution that is higher than those for conventional enteric coating polymers are used (Basit, 2005). This type of pH-responsive delivery can be more accurately referred to as "ileo-colonic" drug delivery rather than colonic delivery, as the pH is highest at the ileocaecal junction (McConnell et al., 2008a). Such systems are however subjected to inter and intrasubject variability and are complex; they are influenced by physiological parameters, such as the fluid composition and volume, transit time and retention time at the appropriate pH. Moreover, the luminal colonic pH can be altered by some pathological conditions such as inflammatory bowel diseases that result in a drop to pH values between 2.5 and 4.7 (Leopold, 2001).

1.2.4.3.2 Time-dependent Delivery

Time-controlled drug release relies on the relatively constant small intestinal transit time of approximately 3 to 4 hours. These systems are expected to release the drug after a predetermined lag phase of 5 to 6 hours (Basit, 2005), the time needed for the dosage form to reach the colon, before which no measurable drug release should occur. Such a delayed release can be induced by swelling, pressure or erosion (Leopold, 2001). Although the small intestinal transit time is reported to be relatively constant, the gastric emptying is much more variable. Moreover, the rate of transit of solid oral dosage forms through the GIT is subjected to inter- and intra-subject variability and diurnal rhythms, and is influenced by diseases (Basit, 2005). The drug might therefore be released at the pre-determined time point irrespective of the site of the GIT, thus limiting the utility of such systems for colonic targetting purposes.

1.2.4.3.3 Pressure-dependent Delivery

Pressure-controlled drug release was developed based on the high intraluminal pressure generated in this lower part of the large intestine via the action of haustral contractions coupled with the viscous environment. These pressure-sensitive formulations release the drug as soon as a certain pressure value is achieved (Leopold, 2001). However, not enough data are available on the luminal pressure of the gastrointestinal tract and hence it is not a well-established means of colonic-specific drug delivery (Basit, 2005).

1.2.4.3.4 Bacteria-dependent Delivery

The abrupt increase of the bacterial population and associated enzymatic activities in the ascending colon represents a non-continuous event independent of GIT transit time and pH and provides an alternative means for colonic targetting. These enzymes are reductive and hydrolytic in nature and include the azoreductase, various glycosidases, esterases and peptidases. As a result, pro-drugs and coating polymers linked by an azo-bond have been developed to selectively deliver drugs to the colon after enzymatic cleavage of degradable formulation components or drug carrier bonds: sulfasalazine is an example of such a pro-drug. However, such systems are highly drug-specific. Therefore, the need to find a universal system useful to deliver any drug to the colon and to overcome the toxicity issues associated with synthetic polymers, led to the establishment of bacteria-sensitive natural materials (Leopold, 2001).

Polysaccharides are naturally available polymers and some are selectively degraded in the colon, thus offering an alternative substrate for colonic microbiota. Such natural materials include amylose, chitosan, guar gum and pectin. These polymers are hydrophilic, which makes them either soluble or swellable in aqueous environments, and are therefore mixed with water-insoluble polymers to prevent premature drug release in the upper GIT. The use of glassy amylose in combination with the water-insoluble polymer ethylcellulose has been investigated as an organic-based (Siew et al., 2000; Siew et al., 2004; Wilson et al., 2005) and aqueous-based (Milojevic et al., 1996a; Milojevic et al., 1996b; McConnell et al., 2007) film coating material that can be applied to single and multi-unit dosage forms. Other forms of resistant starch have been developed through physical (Freire et al., 2009b; Freire et al., 2010a; Freire et al., 2010b) and chemical alterations (Chen et al., 2007) and proved successful for colonic targetting. Similarly, pectin has been exploited as a carrier

for drug targetting to the colon, being applied as a compression coat (Ashford et al., 1993) or a conventional coat in combination with ethylcellulose (Wakerly et al., 1997; Wei et al., 2008).

1.3 Extrusion/ Spheronization for Matrix-Controlled Drug Delivery Systems

1.3.1 Introduction

Extrusion/spheronization is a multi-step process that is used to produce uniformly sized spherical multiparticulates commonly named pellets or spheres (Erkoboni, 2003; Summers et al., 2007). Several methods for producing drug-loaded pellets are available such as solution or suspension spraying onto an inert core, spray congealing (spraying a melt of fats and waxes from the top into a cold tower), spray-drying a drug solution or suspension forming pellets due to the fluid phase evaporation, spraying a binder solution into the whirling powder using a fluidized bed, and extrusion/spheronization (Vervaet et al., 1995). Extrusion/spheronization was reported by Lustig-Gustafsson (1999) and Summers et al. (2007) to be the method of choice for incorporating high levels of active ingredients without producing an excessively large particle, indicating that minimal excipients are required. Additionally, Erkoboni (2003) stated that the process is easier and more efficient than other techniques for producing pellets.

Extrusion/spheronization, as shown in Figure 1.6, is a process that involves at least five operations including dry mixing, wet granulation, extrusion, spheronization, drying and/or screening (an optional step) (Erkoboni, 2003). First, dry mixing results in a homogenous powder dispersion that will be wet granulated to produce a sufficiently plastic wet mass (a). The wet mass is then extruded (b) to form rod-shaped particles (c) of uniform diameter that will be spheronized (d) and rounded off into spheres (e). The spheres are then dried to achieve the desired moisture content and optionally screened to attain a selected size distribution. Moreover, a coating step may also be required where controlled-release is to be achieved.

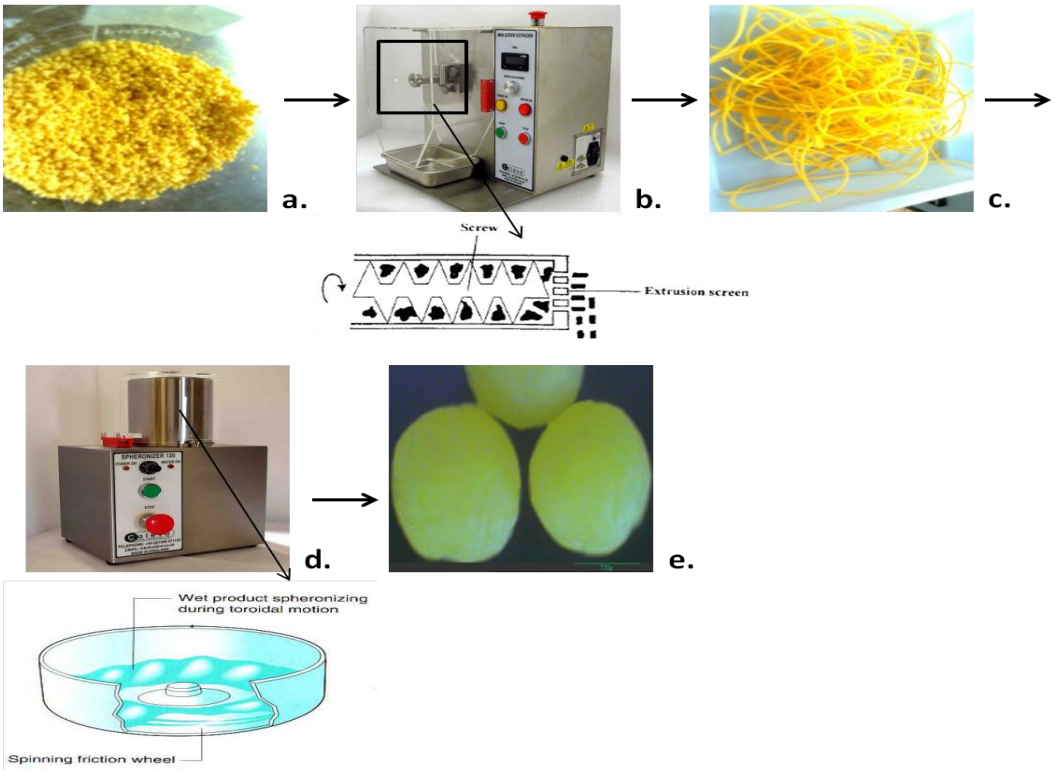


Figure 1.6 The extrusion/spheronization cycle starts with the wet mass (a), which is extruded by a single-screw extruder (b) to form rod-shaped extrudates (c) that will be spheronized on a friction plate (d) and rounded off into spheres (e).

The use of multiparticulate spherical pellets for drug delivery offers a wide number of options. These pellets can be used for both immediate- or controlled-release applications, and can be filled into hard gelatin capsules or compressed into tablets. Two or more actives can easily be combined in any ratio in the same dosage unit, in cases where pellets contain incompatible actives or actives of different target release profile, or in cases where blending polymers is not possible. Small pellets can be used to reduce drug migration for low-dose actives. The physical properties such as flowing properties of the active ingredients and excipients can be modified by pelletization. Moreover, dense multiparticulates disperse evenly within the gastrointestinal tract, therefore they can be used to minimize variation caused by gastric emptying, and to prolong gastrointestinal transit time. In addition, pellets, could improve the bioavailability, decrease the local irritation of the GIT and reduce the risk of dose dumping.

Each application will have very specific requirements, so one should have a thorough understanding of the effects of formulation and process variables in order to tailor the process to achieve the desired effects (Erkoboni, 2003).

1.3.2 Process Design and Parameters

1.3.2.1 Dry Mixing

Dry mixing is required to achieve a uniform mix prior to wet granulation (Erkoboni, 2003). The dry mixing step is usually taken for granted as it is followed by wet massing; however, the uniformity of the dry blend markedly affects the quality of the granulation and, subsequently, the spherical particles produced. Uneven distribution of materials of different properties such as size and solubility can lead to localized overwetting during granulation, as more soluble and finely divided components will dissolve and become part of the granulating fluid. This fluid, rich in soluble compounds, can either remain as overwet regions, which will result in pellets of a broad size-distribution, or be redistributed with continued wet massing. Erkoboni (2003) concluded that pellet uniformity, in terms of shape and size, is dependent on the uniform distribution and composition of the granulating fluid, which includes both the solvent and any dissolved ingredients. The dry mixing process parameters include both the equipment type and the mixing time.

1.3.2.2 Wet massing/Granulation

A wet mass having the requisite plasticity or deformation characteristic is produced by the wet granulation step (Erkoboni, 2003). Wet granulation involves the massing of a mix of dry primary powder particles using a granulating fluid, resulting in an initial particle aggregation (Summers et al., 2007). The granulation fluid consists of a solvent alone or, more usually, in combination with a binding agent to ensure particle adhesion upon drying. The solvent must be volatile, in order to be removed by drying, and non-toxic such as water, ethanol, and isopropanol either alone or in combination. Water is commonly used for economic and ecological reasons since it is non-flammable and hence no expensive safety precautions are required. However, water may negatively affect drug stability and it needs longer drying time than organic solvents, again affecting the stability. Therefore, organic solvents are used as an alternative to dry granulation in the case of water-sensitive drugs or when rapid drying is needed. Although there are a few differences, this step is similar to conventional granulation for compression (Erkoboni, 2003). The major differences are the amount of granulating fluid needed and the importance of achieving a uniform fluid dispersion (Erkoboni, 2003; Summers et al., 2007). The amount of granulation fluid to produce spheres of uniform size and sphericity is greater than that

needed for a similar tablet granulation. Similarly, the mixing time required to obtain adequate granulating fluid uniformity and, in turn, high-quality pellets, is greater than that needed for a tableting granulation. Furthermore, overgranulation and the resulting dense particles produced are not an issue in extrusion/spheronization, as it is in typical granulations for compression.

Batch type mixers/granulators are generally used, and include planetary mixers, vertical or horizontal high shear mixers, and sigma blade mixers (Erkoboni, 2003). The type and capacity of granulating mixers have a marked effect on the work input and time needed to produce a cohesive mass, adequate liquid distribution and intragranular porosity of the granular mass (Summers et al., 2007). Regardless of the mixer type used, the downstream steps of extrusion/spheronization are dependent on the granulating fluid level and the quality of its dispersion in the granulation. Vervaet et al. (1995) stated that the homogenous distribution of the liquid phase throughout the granulated mass is a distinctive element of the granulation step. Pellets of poor quality will be produced in the event of poor liquid distribution (Summers et al., 2007). Some authors reported that the water would equilibrate throughout the mix when the wet mass was left for 12 hours prior to extrusion (Bains et al., 1991; Fielden et al., 1992; Pinto et al., 1992).

The granulation process parameters include the equipment type, the granulating fluid level and type, and the wet mass mixing time (Erkoboni, 2003). The granulating fluid level and the wet mass mixing time significantly influence the pellet size. For example, low granulating fluid levels along with longer mixing times will lead to a slight increase in the pellet size, as longer mixing time results in more effective water distribution and thus more cohesive granule surfaces. On the other hand, longer mixing time at higher granulating fluid levels will distribute the water efficiently within the pore structure of the particles, reducing or even eliminating overwet surfaces while approaching a sufficiently plastic mass. As a result of water distribution and surface water reduction, a smaller mean size and a narrower distribution are achieved.

1.3.2.3 Extrusion

Extrusion is the third step of the process where the wet mass is shaped into rod-shaped particles (Erkoboni, 2003). It is used in the pharmaceutical industry as well as the food,

ceramics and polymer industries (Vervaet et al., 1995). During extrusion, the wet mass is forced through the die and shaped into small cylindrical (rod-shaped) particles of uniform diameter known as extrudates. The extrudates break at similar length under their weight, and thus they should possess enough plasticity to deform but not so much to avoid the adherence to each other when collected or rolled in the spheronizer (Summers et al., 2007).

Based on the feed mechanism, Summers et al. (2007) divided the extruders into three classes: screw-feed (axial or endplate, dome and radial) (Figure 1.7), gravity-feed (cylinder, gear and radial), and piston-feed (ram) extruders. Screw extruders have either one (single) or two (twin) screws that transfer the wet mass from the feed area to the extrusion zone. The single-screw extruder is the simplest and most widely used extrusion system in the world (Steiner, 2003).

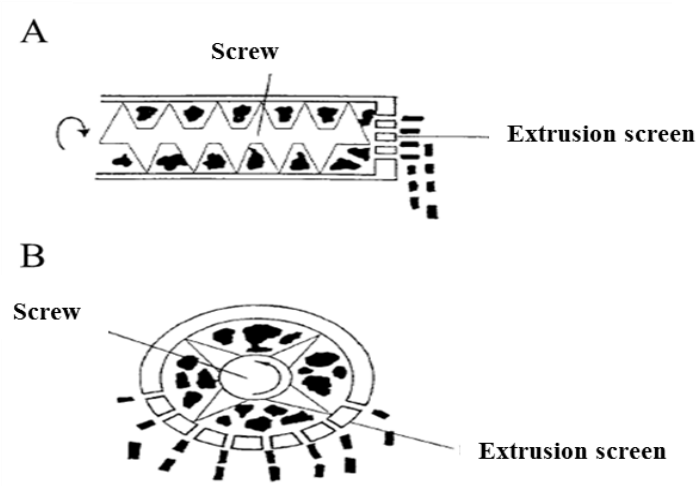


Figure 1.7 A schematic view of a screw extruder: (A) Axial type and (B) radial type (Vervaet et al., 1995).

The primary extrusion process parameters are the extruder type, the die diameter, and the extrusion speed. In addition, the granulating fluid content of the granulation is very important as the plasticity, cohesiveness and lubricity of the wet mass determine the quality of the extrudate and the resulting pellets (Erkoboni, 2003).

1.3.2.3.1 Extrusion Aid

As mentioned earlier, the extrusion/spheronization process is the method of choice for producing pellets with high drug loading (Lustig-Gustafsson et al., 1999). However,

successful processing is dependent on producing extrudates that fulfil certain criteria. Fielden et al. (1992) suggested that the extrudates must have sufficient mechanical strength to maintain its structure when wet, but yet brittle enough to break down into shorter cylinders of uniform length during spheronization. It must be plastic enough to enable the rods to be rounded into spheres by the friction disc. Additionally, it must not be adhesive so as to get discrete spherical granules and to prevent the formation of a static bed upon spheronization. Accordingly, the extrusion process involves the addition of an excipient to support the rheology of the wet mass, i.e. its plasticity and cohesiveness.

Microcrystalline cellulose (MCC) is the excipient used in almost all the formulations. Fielden et al. (1992) stated that MCC is able to hold within its structure a large quantity of moisture by either adsorption and/or hydraulic isolation (physical entrapment). They suggested that MCC exhibits a dual function. Firstly, it facilitates the extrusion process and the shaping during spheronization by modifying the mixture rheological properties so as to achieve the proper plasticity. Secondly, it controls water movement through the plastic mass and inhibits phase separation when the mass is subjected to forces during either extrusion or spheronization stages. Podczeck et al. (2008) found that assessing the water retention capacity of a formulation is essential both at the extrusion stage where water migration can lead to variable water levels in the extrudate and the spheronization stage where water migration to the pellets surface can result in agglomeration during the rotation on the plate.

MCC is mostly used at a level of 50% (Lustig-Gustafsson et al., 1999); however, Bains et al. (1991) revealed that achieving extrudates and spheres of the desired quality would be of a greater significance when using high drug content. Therefore, Podczeck et al. (2008) developed a modified microcrystalline cellulose in order to prepare pellets with higher drug amounts using the extrusion-spheronization technique. They modified the performance of MCC (Avicel PH grades or Avicel RC grades) by the addition of various levels of sodium carboxymethylcellulose (SCMC) to the wet cake prior to drying. Unlike the standard Avicel PH 101, SCMC containing formulations produced pellets with 80% of the drug systems under study including those of low water solubility such as ibuprofen. The new MCC types are able to hold greater amounts of water and thus restrict water migration during the extrusion and spheronization processes. These features of the new

MCC types made it possible to produce pellets from low solubility drugs (ibuprofen), as water migration was restricted by the modified cellulose (water migration is the main cause for not producing pellets from a low water soluble drug when using a standard MCC grade), however, some water migration still occurs at lower extrusion speeds.

1.3.2.4 Spheronization

The function of the spheronization process, the fourth step in the process, is to round off the rod-shaped extrudates produced by extrusion into spheres (Summers et al., 2007). The extrudates are rounded due to frictional forces generated by particle-particle and particle-equipment collisions. The apparatus is quite simple and consists of a bowl with fixed side walls and a rapidly rotating bottom plate or disc; called the friction plate (Figure 1.8, a). The friction plate has a grooved surface to increase the frictional forces. Two geometric patterns are available: the cross-hatched geometry where the grooves form right angles and the radial geometry where the grooves run radially from the centre of the disc (Figure 1.8, b).

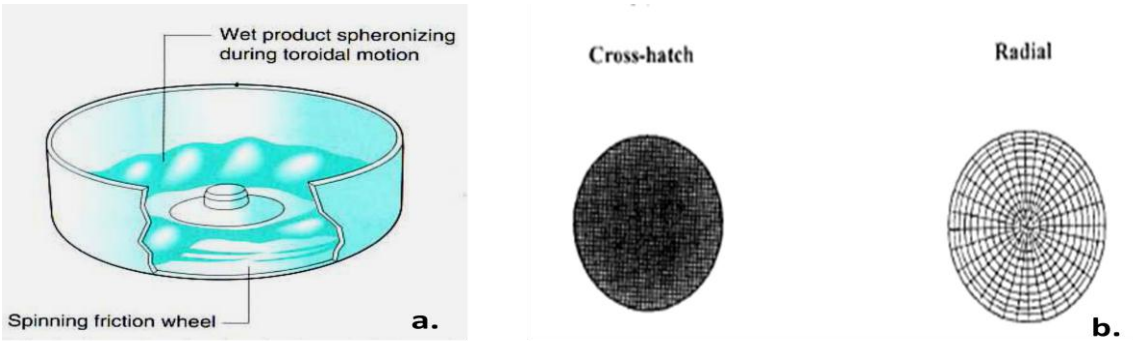


Figure 1.8 A spheronizer showing: a) the characteristic toroidal (rope-like) movement of the forming pellets in the spheronizer bowl during operation (Summers et al., 2007) and b) cross-hatch and radial geometry of the spheronisation plate (Vervaet et al., 1995).

During the spheronization step, the transition from rod-shaped particles into spherical particles occurs in various stages and it is highly dependent on the extrudates properties (Erkoboni, 2003). As the extrudate is charged to the spheronizer it is drawn to the walls of the extruder due to the centrifugal forces. Considering ideal conditions, the extrudate breaks into smaller more uniform pieces, and approximately equal diameter and length are achieved within short period of time. The angular movement of the disc along with the

differential in particle velocity, as the pieces move outward to the walls, climb the walls, and fall back onto the rotating bed, produces a ropelike (toroidal) movement (Figure 1.8, a). This movement can be used as an indicator of the quality of the granulation or extrudate. For instance, the formation of a static bed as the disk rotates indicates an overwet condition. This condition could be a result of an initially overwet granulation or as a result of fluid migration during extrusion or spheronization. The different stages of the spheronisation process will be discussed in Chapter 3. Erkoboni (2003) reported that substances that are soluble in the granulation fluid might migrate to the pellet surface during the spheronization or the drying process, leading to inhomogeneous distribution throughout the pellet. The migration process can be reduced by the addition of a surfactant such as sodium lauryl sulphate (SLS). The primary spheronization process variables include spheronizer size, charge (load), disc speed and residence time.

1.3.2.5 Drying

The drying step is the final step of the process, excluding optional steps such as screening and coating, required to obtain the desired moisture content of the pellets. The pellets can be dried by any dryer used for conventional wet granulation, including fluidized-bed dryers and tray dryers (Summers et al., 2007). The pellets can be dried at room temperature (Vervaet et al., 1995) or at elevated temperature in a fluidized-bed (Bains et al., 1991; Pinto et al., 1992; Baert et al., 1993a; Baert et al., 1993b) or in an oven (Millili et al., 1990). Erkoboni (2003) stated that the drying process must be chosen according to the desired particle properties since different drying techniques exhibit different rates of fluid removal; the drying process may thus affect the final pellet quality. For instance, tray drying is the slowest whereas fluidized bed driers result in much more rapid drying rates. In addition to their effect on the drying rate, the different types of drying techniques influence the migration process. The author revealed that the drying methods with rapid drying rates such as fluidized-bed driers will restrict the migration process. On the other hand, tray drying is a static and slow process which facilitates the migration of the drug to the surface where it recrystallizes as the solvent evaporates. Consequently, migration and recrystallization will influence a number of the particle properties. Firstly, the initial rate of dissolution will be increased as a result of the increased active concentration at the surface. Secondly, the recrystallization process will damage the smooth surfaces obtained by spheronization which might decrease the adhesion of film coats. Finally, slow drying

techniques result in stronger pellets, as the slow recrystallization in the static bed develops crystal bridges as the fluid is removed and the solute recrystallizes.

1.3.2.6 Factors affecting the final pellet quality

Different process and formulation variables, at the various process stages, may influence the pellet structure and properties, resulting in pellets of either good or poor quality. Therefore, these variables must be balanced throughout the whole process in order to obtain good pellets in terms of sphericity, size and physical properties (Schmidt et al., 1998). However, determination of the process and formulation parameters is not simple as modifying one factor can simultaneously result in positive and negative influence on the pellets quality.

Vervaet et al. (1995) stated that the moisture content is an extremely important factor that determines the powder mass plasticity needed to obtain good extrudates and consequently pellets of high quality. Pellets of an acceptable quality can be formed over a certain range of moisture content. If the moisture content is less than the lower limit or more than the upper limit, it will result in large yield of fines or agglomerates, respectively, during spheronization. Increasing the water content resulted in more spherical granules of smoother surfaces, larger mean particle size, greater yield, lower porosity and friability, higher mechanical strength and better flowability (Otsuka et al., 1994). Slower drug release was obtained from pellets upon increasing the water content in the granulating fluid of ethanol/water mixtures owing to the increase in pellet hardness and density (Millili et al., 1990).

Smaller spheres were produced at higher extrusion speeds. This can be explained by the even water distribution throughout the mass achieved at high extrusion speeds (Pinto et al., 1992). Similarly, increasing the spheronization time resulted in a decrease in the average diameter of the granules. Although the average diameter decreased rapidly at the initial spheronization process, constant values were reached after being spheronized for certain time period indicating an optimal spheronization time (Otsuka et al., 1994). Sinha et al. (2007) concluded that increasing the retention time in the spheronizer even at a lower speed subjects the pellets to a greater attrition force and causes more rounding than does the spheronization for less time, even at higher speed.

1.4 Film coating for Reservoir-Controlled Drug Delivery Systems

1.4.1 Introduction

Coatings are applied to a wide range of oral solid dosage forms, including tablets, capsules, and multiparticulates; however, tablets represent the most commonly coated dosage form (Porter, 2007). Coating of pharmaceutical oral solid dosage forms confer specific benefits that include protecting the active ingredient from environmental conditions, particularly light and moisture, and thus improving product stability, masking unpleasant taste of drug substances, improving the ease of swallowing, improving product appearance and facilitating product identification and modifying drug release from the dosage form.

Film coating is the most popular technique; however, other coating processes are also available such as sugar coating, and compression coating. Coating is a complex process that starts with droplet formation, contact, spreading, coalescence and evaporation (Dewettinck et al., 1999). Particles have to pass through this sequence many times before a complete single layer of coating can be formed on the surface. Turton (2008) described film coating as the repeated exposure of a substrate to a spray containing solute and solvent. The cycle starts with the passage of the particles through the spray zone formed by the atomization of a coating liquid (solution or suspension) using pressurized air, where deposition and spreading of the coating material onto the surface occur. The particle then leaves the spray zone into a region where a sufficient energy in the form of heated drying air is applied to evaporate the solvent, leaving a thin deposit of coating material around each particle. The coating equipment should promote the regular movement of particles through the active coating area or the spray zone as particles may bypass the spray reducing the frequency of circulation through the spray zone.

1.4.2 Components of Coating Solution

Film-coating formulations typically consist of a polymer, a solvent or vehicle, and additives such as plasticizers and colourants. The solvent comprises a major component of a coating liquid; however, a significant proportion is removed by the drying process that is concurrent with the spraying process (Porter, 2007).

1.4.2.1 Polymers

The characteristics of film-coating polymers including polymer solubility, viscosity, permeability and mechanical properties should be tailored to the intended use and the functional properties a film coating is expected to possess. Polymers used as immediate-release coatings should be soluble in aqueous fluids and thus ensure rapid dissolution of the active ingredient following ingestion. Cellulose, vinyl derivatives and aminoalkyl methacrylate copolymers are examples of such coatings. Hydroxypropyl methylcellulose (HPMC), polyvinyl pyrrolidone (PVP) and polyvinyl alcohol (PVA), and aminoalkyl methacrylate are the most commonly used derivatives of these polymers, respectively. Cellulose derivatives of higher level of substitution and methylmethacrylate copolymers are used for extended-release purposes. Extended-release coatings are water insoluble and are used to deliver the drug in a consistent manner over a relatively long period of time and thus reduce the frequency of doses taken by patients. Ethyl cellulose (EC) is an example of such polymers and can be applied as a solution in organic solvents or as an aqueous polymeric dispersion. Polymers intended for delayed (or enteric) release coatings exhibit a pH-dependent solubility, i.e. they are insoluble at low pH (stomach conditions) but gradually become soluble as the pH rises to neutrality (upper part of small intestine), and are employed to either protect the drug from the stomach conditions or prevent release of drugs such as gastro-irritant drugs in the stomach. These polymers include methacrylic acid copolymers and phthalate esters such as hydroxypropylmethylcellulose phthalate (HPMCP), cellulose acetate phthalate (CAP) and polyvinyl acetate phthalate (PVAP). Polymer blends can be also applied in order to achieve broad ranges of drug release patterns other than immediate, delayed or extended release (Lecomte et al., 2004a). A well-established example is mixing water soluble and insoluble polymers.

1.4.2.2 Solvents

1.4.2.2.1 Organic Systems

Film-coating processes were initially carried out using organic solvents in order to achieve rapid drying. However, the use of organic solvents is accompanied by environmental and safety issues due to their high flammability and associated explosive hazards. These will in turn lead to financial issues related to the need to build explosion-proof processing areas and suitable storage areas. Moreover, the solvent residue which is the amount of organic

solvent retained in the film structure can be a concern, and thus increases the pressure to quantify and limit the residue levels (Porter, 2007).

1.4.2.2.2 Aqueous Colloidal Dispersions

Film-coating polymers used in modified-release coatings are generally water-insoluble, a key attribute to achieve their intended functionality. Thus, solutions of these polymers in organic solvents have been used. However, the disadvantages of organic systems mentioned in section 1.4.2.2.1 induced the demand for a method to incorporate these polymers into aqueous media forming aqueous polymer dispersions (Porter, 2007). Aqueous colloidal dispersions are classified into true latexes and pseudolatexes based on the technique of manufacture (Wheatley et al., 1997). A true latex is made by emulsion polymerization of a monomer or monomer blend. This process is limited to precursor monomers that are polymerizable in an aqueous medium with the aid of anionic or non-ionic surfactants and in the presence of free radical initiators. Pseudolatexes are prepared by emulsification of an existing thermoplastic water-insoluble polymer, i.e. by preparing a polymer solution in an organic solvent, which is then emulsified in an aqueous phase containing surfactants and stabilisers. Homogenisation followed by solvent removal forms the aqueous colloidal dispersion. Pseudolatexes of ethylcellulose, cellulose acetate phthalate, and other cellulose derivatives are preferred owing to their utility in controlled release dosage forms. In spite of this difference, both latexes and pseudolatexes are colloidal dispersions containing spherical solid or semisolid particles that are less than 1 µm in diameter, and typically less than 0.1 µm. Both are fluid at a polymer concentration as high as 30% and both form films by the same mechanism. The particle size is a key element to pseudolatex stability, or resistance to settling and sedimentation, and subsequent film forming mechanisms. The tendency for colloidal particles to sediment is counterbalanced by their Brownian motion and the convection currents arising from small temperature gradients in the sample. The intensity of Brownian motion is inversely proportional to the particle size. Raising the viscosity of the water phase is a way of reducing the sedimentation rate of the particles

Pseudolatexes offer several technical advantages over polymer organic solutions owing to both the rheological properties of a dilute polymer dispersion and the unique method of film formation. The concentration-viscosity relationship is an advantage of pseudolatexes;

the viscosity of the pseudolatex is independent of the molecular weight and concentration of the dispersed polymer. Therefore, much higher polymer concentrations can be used in the aqueous coating formulation, exhibiting relatively low viscosities compared to the respective organic-based coating solutions (Lecomte et al., 2004b). As a result of using more concentrated polymer solutions, a shorter processing time is required as less water needs to be evaporated. This feature minimizes the core penetration of the solvent (water) and is important for moisture- or heat-sensitive drugs. Another advantage, related to rate of film deposition, is the rate of solvent loss. Pseudolatexes give up water more rapidly and independently of the solid concentration due to the rheological properties of dilute polymer dispersions. However, the rate of solvent loss from a polymer solution is inversely influenced by the solid concentration and the solution viscosity. As a result, higher solution concentrations are associated with a decrease in the rate of solvent loss. Moreover, the permeability properties of the formed pseudolatex films provide other advantages of such systems. Wheatley et al. (1997) stated that the water vapour transmission rates of plasticized ethylcellulose pseudolatex films were about one-half the value of organic-based ethylcellulose films.

A disadvantage of aqueous dispersions is their sensitivity to different factors, such as temperature, pH and addition of electrolytes, potentially resulting in the coagulation of the dispersions (Bodmeier et al., 1994).

1.4.2.2.3 Mechanism of Film Formation

The film forming process differs between organic solvents and aqueous based polymer systems. In organic solvent-based systems, the organic solvent begins to evaporate from the solution and when the polymer concentration reaches a certain concentration, the polymer solution undergoes a solution to gel transition. The solvent continues to evaporate eventually causing the polymer particles to become immobile (which is known as the solidification point), at which the polymer film is formed.

On the other hand, aqueous dispersions consist of discrete submicron-size spheres, each containing hundreds of polymer chains, and are suspended and separated by electrostatic repulsion. Thus, a strong driving force is required to overcome the repulsive forces, deform the spheres and force the particles to fuse. The evaporation of water rises up the interfacial

tension between water and polymer. Capillarity caused by the high interfacial tension of water generates the driving force to push the particles into contact and allow complete fusion and coalescence into a continuous film. A plasticizer is often added to reduce minimum film formation temperature (MFT, the minimum temperature needed for films to coalesce), softening the polymer spheres and facilitating their coalescence (Wheatley et al., 1997; Lecomte et al., 2004b).

It is worth noting that the differences in film formation can have an effect on the drug release behaviour. Lecomte et al. (2004b) compared polymer blends of ethylcellulose and Eudragit® L cast from aqueous dispersions and organic solutions. The swelling of Eudragit® L and subsequently the drug release were more effectively hindered in films prepared from organic solutions compared to those prepared from aqueous dispersions. This result can be ascribed to the high mobility of the two types of polymers in organic solution and the associated high degree of polymer-polymer interpenetration in comparison with the restricted mobility of the polymers within the colloidal particles and the incomplete interdiffusion of the polymeric chains. Similarly, Siew et al. (2000) reported differences in the drug release from amylose/ethylcellulose film coatings cast from aqueous dispersions and organic solutions.

1.4.2.2.4 Curing

Aqueous film coats often need a curing step after the film coating process. Curing involves thermal treatment at a temperature above the MFT of the polymer in either an oven or by leaving the substrates in the fluidised bed. Curing aims to accelerate the film formation process, to achieve a better coalescence of the colloidal polymer particles and hence to avoid stability and aging problems upon storage, i.e. further gradual coalescence of the colloidal polymer particles and the subsequent changes in drug release profiles (Wesseling et al., 1999). Curing of the coated particles has a significant influence on the drug release with retardation in the drug release being expected due to the completion of coalescence and film formation process. However, an increase in drug release was also reported due to migration of the drug (Bodmeier et al., 1994) or the plasticizer (Hutchings et al., 1994), with the extent being dependant on the drug type and curing conditions (curing temperature and time). The high plasticization levels might eliminate the need for a curing step. Moreover, the drug release from beads coated with organic-based ethylcellulose

solution was not affected by the curing step, whereas a reduction in drug release from those coated with Aquacoat® (a commercially available aqueous dispersion of ethylcellulose) was detected, indicating the importance of post coating heat treatment in achieving a complete coalescence of the polymer particles (Wesseling et al., 1999).

1.4.2.3 Additives

1.4.2.3.1 Plasticizers

Film-coating polymers are known to be brittle in nature, and therefore plasticizers are generally added to the formulation to modify the physical properties of the polymer (Porter, 2007). A basic requirement of any plasticizer is its compatibility and miscibility with the polymer. A plasticizer is known to be a substantially non-volatile, high-boiling, non-separating substance that functions by aligning its molecules between the polymer molecules, thus increasing free volume, decreasing the cumulative intermolecular forces along the polymer chains, and facilitating polymer chain motion within the coating structure (Marcilla et al., 2004). The reduction in cohesion forces between polymer chains decreases the tensile strength, lowers the softening temperature, and decreases the glass transition temperature (T_g). As a result, the film flexibility increases and the residual stresses within the coating upon drying and the film brittleness reduce. With aqueous colloidal dispersions, plasticizers are added to reduce the MFT of the polymer which is achieved by reducing the glass transition temperature (Wheatley et al., 1997). Plasticizers also have a pronounced effect on the drug release kinetics as they alter the permeability of the polymeric film for the drug (Lecomte et al., 2004a).

Plasticizers are divided into water-soluble and insoluble plasticizers. Plasticizers are directly incorporated into polymeric solutions, but the incorporation process into aqueous dispersion is more complex. Bodmeier et al. (1997) investigated the process of plasticizer uptake by the aqueous colloidal dispersions. They explained that water-soluble plasticizers are dissolved, while the insoluble plasticizers have to be emulsified in the aqueous phase of the polymer dispersions. The plasticizer should then partition into the dispersed particles to help softening of the particle, to promote particle deformation and coalescence into a film upon drying. Therefore, sufficient time should be allowed for the plasticizer uptake by the colloidal dispersion especially with water-insoluble ones. The uptake of water-soluble

plasticizers is not affected by the plasticization time. On the other hand, a water-insoluble plasticizer primarily exists in either the polymer phase or in emulsified form in the aqueous phase, with only a minor portion dissolved in the aqueous phase. The dissolution of the plasticizer from the emulsified droplets into the aqueous phase is the rate limiting step of the plasticizer uptake. Thioune et al. (2000) stated that the plasticizer should be mixed with a highly concentrated latex dispersion in order to incorporate the maximum quantity of plasticizer within the particles, i.e. the plasticizer uptake increases with increasing the solid content of the pseudolatex.

1.4.2.3.2 Other Additives

Several other additives are sometimes needed in the formulation. Colourants are often added for several reasons including improving the appearance of final dosage form, to facilitate product identification or to confer protection for photo-sensitive drugs. Pharmaceutically acceptable colourants include water soluble dyes and water-insoluble pigments. Iron oxide pigments, titanium dioxide, and aluminium lakes are examples of available colourants. Glidants, such as talc and magnesium stearate, are often added, particularly in aqueous coating dispersions, to avoid tackiness. Preservatives and surfactants, such as Tween 80 and sodium lauryl sulphate, can also be incorporated. Pore formers might be also incorporated to manipulate the drug release kinetics.

1.4.3 Coating techniques

The choice of proper equipment and processing conditions is as important to achieve a good film coating as the optimization of the appropriate coating formulation. Equipment used in film coating is classified into three general categories: conventional pans, perforated pans and fluidized bed coaters. The main difference between these systems is the process of solid mixing (Kleinbach et al., 1995). The mixing of solids can be achieved by the movement of the apparatus itself, as is the case with conventional and perforated pans, or mixing can take place in a fluidized bed formed by a fluidising gas as in fluidized bed coaters. These coating systems offer a drying step concurrently to the coating process, the fluidized bed being well known for its drying efficiency (Mehta, 1997).

1.4.3.1 Fluidized-bed Coating

Fluidized-bed coating, also referred to as air suspension coating, is categorized by three different configurations, including top spray, bottom spray (Wurster) or tangential spray (rotating fluidized-bed) (Mehta, 1997). In a top spray fluidized-bed coater, the substrate is fluidized up to the nozzle, which sprays the coating material countercurrently to the substrate. Figure 1.9 below shows an annotated picture of the Caleva mini coater-drier 2, which is a bench-top fluid bed coater/drier used to coat a number of substrates on a laboratory scale. The substrates are placed in the vibrating coating chamber and (preheated) air is forced into the bottom of the chamber to keep the particles fluidized and concurrently provide sufficient energy for the drying of the coated products. The coating solution can be placed on a hot plate stirrer to avoid sedimentation and to pre-heat the coating solution or dispersion. A peristaltic pump is used to pump the coating material to the spray head where the solution is mixed with atomising air and is then forced through a needle valve via compressed air, which produces a fine spray directed at the substrates in the fluidised bed. There are several parameters that can be controlled through the operating parameter controls and these include the fan speed, bed temperature and pump speed.

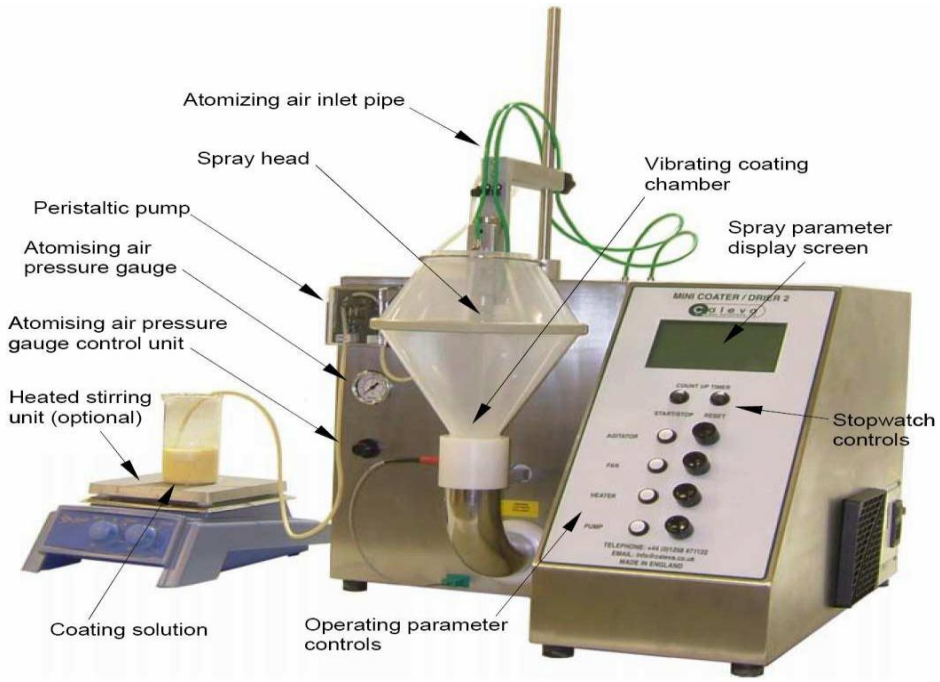


Figure 1.9 Caleva Mini Coater Drier 2 (reproduced from Caleva Mini Coater/Drier 2, Installation and Operation Manual).

Numerous variables are involved in film coating and a thorough understanding of their action is essential to optimise the final quality of the coating and to achieve its intended functionality. According to Link et al. (1997), the process parameters needed to obtain an optimum coating quality can be determined by experimental means only. Increasing the gas velocity resulted in more homogenous and fully intact coating, whereas at lower gas velocity the coating was inhomogenous and some material of the particles to be coated was incorporated in the coating (Kleinbach et al., 1995). This can be explained by the partial dissolution of the particle material during coating, possibly due to the high moisture content in the spray zone. The spreading of the coating material can be improved by using more diluted solutions or dispersions, although a too diluted coating liquid can lead to partial dissolution of the particles to be coated. Moreover, finer droplets of the coating material should enhance the coating quality; however, the spreading of these droplets might be reduced by the premature drying of the droplets before they hit the particles, and hence deterioration in the coating quality. The bed temperature affects the drying rate of the coating material deposited on the particles and can also lead to sticking of the coating material. There is therefore a maximum allowable liquid loading, i.e. spraying rate, at a constant bed temperature. The spraying rate depends on the evaporation capacity of the air, the tackiness of the coating material and the particles velocity (Dewettinck et al., 1999). The evaporation rate of the solvent of the coating material depends on the fluidization air velocity, bed temperature and humidity.

The atomisation pressure has a complex effect on coating efficiency. It affects the size and speed of the droplets thus affecting the probability of contact between the droplet and the particle to be coated, and the bed temperature (Dewettinck et al., 1998). Increasing the atomisation pressure of the nozzle creates smaller droplets, reduces the bed temperature and increases the speed of the droplets. The quality of coating can be improved by reducing the droplet size; however, the reduction in droplet size could promote premature droplet evaporation and therefore deteriorate the coating efficiency. In addition to the atomising air pressure, the droplet size is also influenced by the coating solution characteristics such as viscosity. The air used for atomisation contributes to the evaporation of the coating solvent which would increase the viscosity of the droplet and thus negatively affect the spreading and coalescence of the coating material upon contact with the core material.

The droplet viscosity is also influenced by the distance the droplet travels before it contacts the fluidized particles. However, spraying the coating liquid countercurrently onto the fluidized particles makes it difficult to control the distance the droplets travel before contacting the substrate and might result in coating imperfections due to premature droplet evaporation (Dewettinck et al., 1999). This is particularly a problem in case of organic solvent based coating materials as they have much lower heat of evaporation than aqueous based systems. The droplet size should be selected relative to the size of the particles to be coated. Adhesion of the droplet to the surface of particles would not occur if the droplets had dried before colliding with the particles; ideally the droplet must wet the particle surface and spread across the surface to the greatest extent possible (Werner et al., 2007). Coherent coatings are expected to form at low drying rates and if droplets were moist upon contact with particles owing to improved spreading and coalescence. Droplet drying can be controlled by manipulating temperature and velocity of the drying air, the atomiser air velocity, the proximity of the nozzle to the particles and the initial droplet viscosity.

1.4.4 Coating Defects

Coating defects can be as simple as defects in the visual appearance of the coated substrate or, more importantly, these defects can disturb the film continuity and thus influence its function. It is quite understandable that the more functionality the coat provides the more important is the mass and morphology uniformity of the coating, as imperfections in the film coating can lead to significant variability in the desired performance of the coat (Turton, 2008). Some defects can be solved by changing some of the coating process parameters such as fan speed or bed temperature and these include blistering, chipping, cratering, picking, and pitting. Other defects can be fixed only by changing both process parameters and film-coating formulation; these defects include blooming, blushing, colour variation, infilling, mottling and orange peel. More critical defects can occur and demand a more fundamental remedy including changing the tablet core as well as the coating process parameters and formulation conditions. High internal stresses within the film coating give rise to such defects and they include bridging, cracking, flaking, peeling and splitting (Rowe, 1997).

1.5 Objectives of project

Biopolymers, such as zein proteins, are currently investigated for their potential role as industrial polymeric materials. Formation of biodegradable zein films and plastics for packaging and coating is one of their most promising applications in industry (Shukla et al., 2001). Zein has been widely used in the food industry including packaging and coating, but little is known about using these biopolymers in pharmaceutical products. Its use in the pharmaceutical fields, encapsulating and coating in particular, has been progressively increasing. The main interest in zein proteins resides in its hydrophobic nature and its corresponding potential uses ranging from gas and moisture protection in food packaging to modified-release applications in drug delivery devices. However, a proper understanding of the properties of zein is required if zein-based formulations are to be achieved. The literature available on zein is confusing. The relationship between the drug release from zein-based systems and the pH value of the release medium is not well-defined in literature. The susceptibility of zein proteins to digestion by the enzymes of the upper GIT also needs to be verified. In addition, no work has been done on the susceptibility of zein to the colonic microbiota although a study has been performed on beads of zein/pectin intended for colonic targetting where zein was mainly used to maintain the structural integrity of the dosage form (Liu et al., 2006a).

The main objective of this project is therefore to investigate the behaviour of zein in conditions simulating the upper and lower GIT in an attempt to have a complete understanding of the influence of the pH range and enzymes content of GIT on zein functionality. This work thus aims to elucidate the potential use of zein proteins as a pharmaceutical excipient in developing modified drug release formulations, with particular reference to colon-specific drug delivery. Some studies were carried out on zein-based pellets produced via the extrusion/spheronization technique. However, the use of zein as a coating material, both in organic solutions and aqueous dispersions was mainly investigated.

In Chapter 2, the materials and methods used in this work are presented. Chapter 3 investigates the potential use of zein as a carrier in a matrix drug delivery device prepared using extrusion/spheronization. The use of zein as a film coating material: a) applied as an organic solution and b) as an aqueous dispersion (pseudolatex) is explored in Chapter 4

and Chapter 5, respectively. Chapter 5 also assesses the influence of incorporating polysaccharides on the functionality of aqueous-based zein coatings. The potential use of zein coatings for colon-specific drug delivery and the influence of film-coat incorporated polysaccharides are investigated using a batch culture fermentation model in Chapter 6.

2 Chapter 2 Materials and Methods

2.1 Introduction

Pellets and tablets were the main substrates used in this work and were produced using two techniques: extrusion/spheronization and tableting, respectively. Pellets and tablets were then coated with zein proteins; however, some studies were carried out on uncoated zein pellets. Organic-based zein solutions, aqueous dispersions of pure zein and those of zein/polysaccharides were used for coating the substrates. These formulations were tested for their release characteristics using *in vitro* dissolution studies. Model drugs including caffeine and paracetamol were quantified using either ultraviolet-visible spectrophotometry (UV-VIS) or high performance liquid chromatography (HPLC). Free films of pure zein and zein/polysaccharides were prepared by casting method of relevant aqueous dispersions.

Several imaging and thermal techniques were used for characterization studies. Scanning electron microscope (SEM) and nano-thermal analysis (n-TA), an application of atomic force microscopy (AFM), provided imaging information related to surface topography and to the thermal properties and miscibility of free films and coated tablets. Modulated temperature differential scanning calorimetry (MTDSC) and thermogravimetric analysis (TGA) were used to characterize the thermal properties of the raw materials and the produced films. Other techniques such as Attenuated Total Reflectance-Fourier Transform Infra-Red (ATR-FTIR), X-ray powder diffraction (XRPD) and laser diffraction (LD) were also used.

This Chapter will give a summary of the used materials, preparation and characterization techniques, their principles and experimental parameters.

2.2 Materials

This section describes the materials used in this project and they are subdivided according to their use into substrate core (Section 2.2.1), film coating (Section 2.2.2) and dissolution media (Section 2.2.3) materials. Table 2.1 summarizes the materials used in Sections 2.2.1

and 2.2.2, their chemical structure and solubility in water. Materials used to prepare the dissolution media, their chemical formula and their supplier are listed in Table 2.2.

2.2.1 Substrate Core Materials

2.2.1.1 Microcrystalline Cellulose (MCC)

Microcrystalline cellulose (MCC) is an odourless, white or almost white, fine or granular powder. It is practically insoluble in water, in dilute acids and in anhydrous ethanol. It is purified, partly depolymerised cellulose prepared by treating alpha-cellulose, obtained as pulp from fibrous plant material, with mineral acids (British Pharmacopoeia, 2011). Celluloses such as MCC are biocompatible, chemically inert but hygroscopic, and show good tablet-forming and disintegrating properties and are therefore widely used as fillers in solid pharmaceutical formulations (Alderborn, 2007). MCC can be used either in a direct compression tablet formulation or in a wet granulation formulation. It can be added extragranularly to enhance the compactibility of the granules or intragranularly as a granulation aid based on its hydrophilic and water holding capacity (Cantor et al., 2008). Similarly, it can be also used as an extrusion aid for pellet formation (Kleinebudde, 1997). The type of MCC used in this study is Avicel PH 301 and it was obtained from FMC BioPolymer (Brussels-Belgium, and distributed by IMCD UK limited) and used as received.

2.2.1.2 Alpha-Lactose Monohydrate (α-Lactose Monohydrate)

Alpha-lactose monohydrate (α-lactose monohydrate) is a white or almost white, crystalline powder that is freely but slowly soluble in water, and practically insoluble in ethanol (96%) (British Pharmacopoeia, 2011). Lactose is the most commonly used filler in tablets due to its good compactability, solubility in water, pleasant taste, and non-hygroscopic and fairly non-reactive character (Alderborn, 2007). It was purchased from Sigma-Aldrich (USA) in a reagent grade and used as received.

2.2.1.3 Dibasic Calcium Phosphate (DCP)

Dibasic calcium phosphate (DCP) is white or almost white powder with a molecular weight of 136.06 g/mol. It is practically insoluble in water (0.1 $g.L^{-1}$ at 25 ºC) and ethanol

(96%), but dissolves in dilute hydrochloric acid (British Pharmacopoeia, 2011). It is used in tablet products, in both direct compression and wet granulated formulations (Lin et al., 1995). Anhydrous calcium phosphate dibasic of 97% purity was purchased from Acros Organics (New Jersey, USA) and used as received.

2.2.1.4 Model Drugs

2.2.1.4.1 Caffeine (CAFF)

Caffeine (1, 3, 7-trimethyl- 3, 7-dihydro-1*H*-purine-2, 6-dione) is a purine derivative that works as a central nervous system stimulant. Caffeine appears as a white or almost white, crystalline powder or silky, white or almost white, crystals. It is described in British Pharmacopoeia (BP, 2011) as sparingly soluble in water (the term 'sparingly soluble' describes an approximate solubility between 30 to 100 mL of solvent per gram of solute, i.e. 10 to 33 g of solute/ 1 L of solvent at a temperature between 15 and 25 ˚C). Lustig-Gustafsson et al. (1999) have measured the solubility of caffeine in water to be 16.7 $g.L^{-1}$ at 25 ºC. It is freely soluble in boiling water and slightly soluble in ethanol (96%). It has a molecular weight of 194.19 $g.mol^{-1}$, a melting temperature range of 234 to 239 ˚C and a λ_{max} of 273 nm in water (British Pharmacopoeia, 2011). Caffeine, a weak base (pKa = 0.5), was selected on the basis of its aqueous solubility and lack of ionization over the physiological pH range; it will be mostly unionised in the stomach and even more unionised in the small intestine. Caffeine of 98.5% purity was purchased from Acros Organics (New Jersey, USA) and used as received.

2.2.1.4.2 Paracetamol (PCT)

Paracetamol is N-(4-hydroxyphenyl) acetamide and used as an analgesic and antipyretic. In terms of appearance, it is a white or almost white, crystalline powder. It is described in BP (2011) as sparingly soluble in water. Lustig-Gustafsson et al. (1999) have measured the solubility in water to be 14.3 $g.L^{-1}$ at 25 ºC and 23.7 $g.L^{-1}$ at 37 ºC. It is freely soluble in alcohol. It has a melting temperature range of 168 to 172 ˚C, a λ_{max} of 243 nm in water (British Pharmacopoeia, 2011) and an acidic pK_a of 9.5 at 25 ˚C. Paracetamol was chosen on the basis of its aqueous solubility and lack of ionization over the physiological pH range. Paracetamol was obtained from Alfa Aesar (UK) and was used as received.

2.2.1.5 Other additives

2.2.1.5.1 Poly vinyl pyrrolidone (PVP)

Poly vinyl pyrrolidone (PVP) is an inert, synthetic polymer consisting of a linear chain of 1-vinyl-2-pyrrolidinone groups. It is available in a wide range of molecular weights (2500 to 3 million Daltons) depending on the extent of polymerization. It has the advantage of being soluble in both water and alcohol, but it is very hygroscopic (Cantor et al., 2008). PVP K30 of an average molecular weight of 40,000 was used as a binder solution and added to the drug filler mixture used to prepare the wet granulation and make tablets. It was obtained from Aldrich (UK) and used as received.

2.2.1.5.2 Magnesium Stearate

Magnesium stearate was used as a lubricant. Its function is to ensure that tablet formation and ejection occur with low friction between the tablet and the punches. It was purchased from Aldrich (UK) and used as received.

2.2.1.5.3 Partially Gelatinized Starch (Pre-gelatinised starch)

Partially gelatinized starch was used as a disintegrant due to its swelling properties that causes the tablet to rupture when in contact with a liquid. It was purchased from Colorcon (UK) as the grade Starch 1500 and used as received.

2.2.2 Film Coating Materials

2.2.2.1 Zein

Zein proteins, described in more detail in Chapter 1, are prolamins that are particularly rich in hydrophobic amino acids and are soluble in aqueous alcohol solution but not in water. Commercial zein, as stated by Lawton (2002), is made up of α-zeins which are divided into two types: Z19 and Z22 of approximate weights of 19 and 22 kDa, respectively. The glass transition temperature of dry zein is 165 ˚C and its thermal degradation point is 320 ˚C (Shukla et al., 2001). A single batch of zein was used throughout the study and it was obtained from Sigma-Aldrich (Germany) and used as received.

2.2.2.2 Plasticizers

2.2.2.2.1 Glycerol (GLY)

Glycerol (propane-1, 2, 3-triol) appears as a viscous liquid, colourless or almost colourless, and clear. It is very hygroscopic and miscible with water and with alcohol (British Pharmacopoeia, 2011). It has a density of 1.261 g/cm^3. Glycerol of analytical grade was used as a plasticizer. It was purchased from Fisher Scientific (UK) and used as received.

2.2.2.2.2 Propylene glycol (PG)

Propylene glycol ((*RS*)-propane-1, 2-diol) is a viscous, clear, colourless and hygroscopic liquid. It is miscible with water and with ethanol (96%) (British Pharmacopoeia, 2011). It has a density of 1.036 g/cm^3. Propylene glycol of ≥ 99.5% purity was used for plasticization, obtained from SAFC Supply Solutions (USA) and used as received.

2.2.2.2.3 Polyethylene glycol 400 (PEG400)

Polyethylene glycol 400 is a low molecular weight grade of polyethylene glycol. It is a water-soluble, viscous liquid with a density of 1.128 g/cm^3 (British Pharmacopoeia, 2011). It was used as a plasticizer, obtained from Sigma Aldrich (UK) and used as received.

2.2.2.3 Pore Formers

2.2.2.3.1 Pectin

Pectin is a nontoxic, partly water-soluble, gel-forming polysaccharide extracted from apple pomace or citrus peel (Tho et al., 2001). It is a heterogeneous linear polysaccharide present in the cell wall of most plants, and consists mainly of D-galacturonic acid and its methyl ester linked via α (1-4) glycosidic bonds (Ashford et al., 1993). Pectin is hydrophilic and has numerous sites for hydrogen bonding (See structure in Table 2.1). The carboxylic acid group on the C6 can be either free, methoxylated or amidated, and the degree of substitution and the nature of substituents determine the solubility of pectin; the solubility decreases as the degree of methoxylation increases (Tho et al., 2001). Pectin is digested by colonic bacterial pectinolytic enzymes (Talukder et al., 2008) but it is not susceptible to gastric or intestinal enzymes. Thus, pectin has been used as a carrier for colon-specific

drug delivery in forms such as compression coatings (Ashford et al., 1993) and ethylcellulose-pectin film coatings (Wakerly et al., 1997). In this study, pectin from apple (Pectin USP), a high methoxy pectin, was used. It was obtained from Sigma-Aldrich (Germany) and used as received.

2.2.2.3.2 Acetylated High Amylose Starch (HAS)

Starch is composed of two polysaccharides: amylose and amylopectin. Amylose is a long, essentially linear polysaccharide made up of 1,4-linked-α-D-anyhydroglucose units, and tends to form compacts in starch granules along with the other more prominent and branched polysaccharide, amylopectin. Starch has been used in food, plastics and pharmaceutical industries owing to its natural gelling and film forming properties and its biodegradability. In the pharmaceutical industry, starch has been mainly used as a bulking agent, disintegrant, binder and thickening agent (Freire et al., 2009a). Moreover, native starch has been chemically or physically modified in order to expand its usefulness. Starch is known to be essentially digested and absorbed in the small intestine; however, it was found that a fraction of starch, referred to as resistant starch, is not digested neither absorbed in the small intestine but may, however, be (partially) fermented in the large bowel by the colonic microbiota (Eerlingen et al., 1995). Resistant starch has therefore been investigated for potential uses for colonic-specific drug delivery.

Resistant starch is classified into four types: S1 is physically entrapped starch, which is locked in the plant cell, S2 is granular starch found in uncooked starch, S3 is retrograded starch which forms after certain heat-moisture treatments of the starch and S4 is chemically modified starch such as acetylated amylose starch (Eerlingen et al., 1995; Morita et al., 2005). Starch granules are known for their reversible swelling in cold water; however, starch granules undergo gelatinization at higher temperatures where they swell to many times than their original size until they disrupt and the amylose diffuses out, hence increasing its solubility. This treatment is thought to increase starch digestibility; however, it has been found that upon cooling amylose may have sufficient time to rearrange itself in a more crystalline structure, a phenomenon called retrogradation (Miles et al., 1985).

Chemical modification of starch such as acetylation aims to improve the physicochemical and functional properties of the natural polymer. Acetylation involves esterification of

starch polymers with acetyl groups to form starch acetates. Acetylation resulted in increased hydrophobicity, decreased affinity for water, an increase in the swelling and solubility of starches (Diop et al., 2011), alterations in the granular morphology and a decrease in the initial gelatinization temperature (Freire et al., 2009a). It has been suggested that the introduction of acetyl groups upon acetylation weakens the hydrogen bonding between the starch polymers within the starch granule, thereby increasing the swelling and solubility and decreasing the initial gelatinisation temperature of starch granules (Sodhi et al., 2005; Freire et al., 2009a).

Acetylated high amylose starch (HAS) (Amidon d'amylo mais M-400G) was obtained from Roquette (Italy) and used as received. According to the certificate of analysis, this starch type has an acetyl value of a minimum of 1.6 and a corresponding minimum degree of substitution (DS) of 0.06. In this study, this starch type was studied as a carrier for colonic drug targetting.

2.2.2.4 Solvents

Ethanol absolute is a colourless, clear, volatile, flammable and hygroscopic liquid. It is miscible with water and has a boiling point of about 78 °C (British Pharmacopoeia, 2011). It was obtained from Sigma-Aldrich (UK) and used as received. Distilled water (obtained via a series of steps including filtration, reverse osmosis and UV treatment) was used.

2.2.2.5 Other Additives

2.2.2.5.1 Preservatives

Methyl parabens (Methyl 4-hydroxybenzoate, MP), and propyl parabens (Propyl 4-hydroxybenzoate ≥99%, PP) have been used as preservatives; however, they were found to exhibit some plasticization effect (O'Donnell et al., 1997). They were purchased from Sigma-Aldrich (UK) and used as received.

2.2.2.5.2 Surfactants

Tween 80 (Polysorbate 80) is a non-ionic surfactant and emulsifier derived from polyethoxylated sorbitan and oleic acid. Polysorbate 80 is oily, colourless or brownish-

yellow, clear or slightly opalescent liquid. It is dispersible in water, in anhydrous ethanol, in ethyl acetate and in methanol but practically insoluble in fatty oils and in liquid paraffin. It has a relative density of 1.10 g/cm^3 and a viscosity of circa 400 mPa.s at 25 °C (British Pharmacopoeia, 2011). Tween 80 was used as a surfactant to stabilize the aqueous dispersions of zein. It was purchased from Sigma-Aldrich (USA) and used as received.

Table 2.1 Summary of substrate core and film coating materials, their chemical structure and solubility in water.

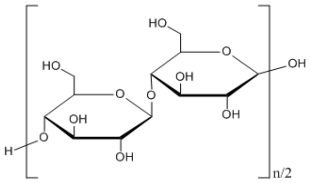
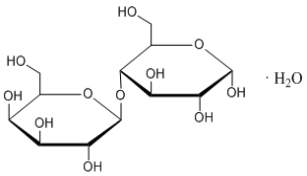
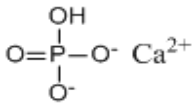
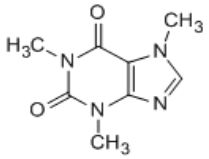
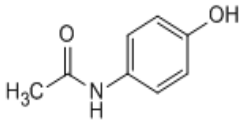
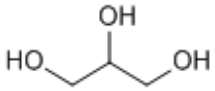
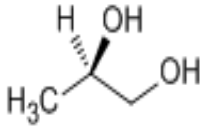
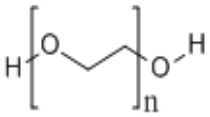
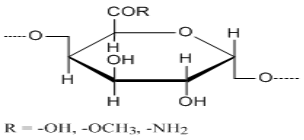
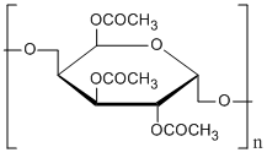
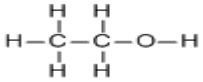
Materials	Structural formula	Water Solubility
Substrate Core Materials		
Microcrystalline cellulose		Insoluble
α-Lactose monohydrate		Freely but slowly soluble
Dibasic calcium phosphate		Practically insoluble
Model drugs		
Caffeine		Sparingly soluble (16.7 g.L^{-1} at 25 ºC)
Paracetamol		Sparingly soluble (14.3 g.L^{-1} at 25 ºC)
Film coating materials		
Zein		Insoluble

Table 2.1 Continued.

Plasticizers		
Glycerol		Miscible
Propylene glycol		Miscible
Polyethylene glycol		Soluble
Pore formers		
Methoxylated pectin		Soluble
Acetylated high amylose starch		Insoluble
Solvents		
Ethanol absolute		Miscible

2.2.3 Dissolution Media Materials

2.2.3.1 Simple and Enzyme-containing Buffers Components

Throughout this study, a wide range of buffers were used. Table 2.2 lists the materials which were used along with their chemical formula and supplier.

Table 2.2 Buffer components of dissolution media.

Buffer component	Chemical formula	Supplier
Hydrochloric acid, 37% purisis	HCl	Sigma Aldrich
Disodium hydrogen orthophosphate dodecahydrate	$Na_2HPO_4.12H_2O$	Sigma Aldrich
Sodium hydroxide	NaOH	Fisher Scientific
Sodium chloride	NaCl	Fisher Scientific
Potassium chloride, 99+%	KCl	Lancaster Synthesis
Potassium dihydrogen orthophosphate	KH_2PO_4	AnalaR
Lecithin (L-α-phosphatidylcholine)	--	Sigma, BioChemika
Taurocholic acid, sodium salt hydrate, 98%	$C_{26}\ H_{44}\ N\ Na\ O_7\ S.\ x\ H_2O$	Acros Organics
Pepsin from porcine gastric mucosa	--	Sigma
Pancreatin from porcine pancreas	--	Sigma

Initial dissolution studies were performed in distilled water, 0.1 M HCl (pH 1), simulated gastric fluid without pepsin (pH 1.2) and phosphate buffer (pH 6.8). Subsequently, the influence of digestive enzymes was assessed using Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF) with pepsin and pancreatin, respectively, based on the British Pharmacopoeia (2011). Further investigations were carried out in some bio-relevant media suggested in literature including Fasting State Simulated Intestinal Fluid (FaSSIF) (Galia et al., 1998), and Simulated Colonic Fluid (SCoF) (Fotaki et al., 2005). Table 2.3 to Table 2.5 list the buffers and their respective compositions.

Table 2.3 The composition of Simulated Gastric Fluid (SGF) with and without Pepsin according to BP 2011.

Buffer component	SGF (without Pepsin)	SGF (with Pepsin)
Sodium chloride	2.0 g	2.0 g
Hydrochloric acid	80 mL of 1 M	80 mL of 1M
Pepsin		3.2 g
Distilled water	To 1000 mL	To 1000 mL
pH	1.2	1.2

Table 2.4 The composition of phosphate buffer (pH 6.8), Simulated Intestinal Fluid (SIF) according to (BP 2011), and Simulated Colonic Fluid (SCoF) according to (Fotaki et al., 2005).

Buffer component	Phosphate buffer	SIF	SCoF
Potassium dihdrogen phosphate	250 mL of 0.2 M	6.8 g	
NaOH	112 mL of 0.2 M	77 mL of 0.2 M	6.28 g
Pancreas powder		10 g	
Acetic acid			10.2 g
Distilled water	To 1000 mL	To 1000 mL	To 1000 mL
pH	6.8	6.8	5.8

Table 2.5 The composition of Fasting State Simulated Intestinal Fluid (FaSSIF) pH 6.5 according to (Galia et al., 1998).

Buffer component	FaSSIF (pH 6.5)
Sodium taurocholate	1.61 g
Lecithin	0.56 g
Potassium dihydrogen phosphate	3.9 g
Potassium chloride	7.7 g
NaOH	Qs to pH 6.5
Distilled water	To 1000 mL

2.2.3.2 Basal Chemostatic Medium Components

The final release studies were performed in a simulated colonic medium using batch fermentation studies and freshly voided faeces. This section lists the components of the basal chemostatic medium (Table 2.6). This medium was used to dilute the faecal sample in batch fermentation studies which is discussed in more detail in Chapter 6.

Table 2.6 Basal chemostatic medium components

Buffer component	Chemical formula	Supplier
Peptone water	--	Oxoid
Yeast extract	--	Oxoid
Sodium chloride	NaCl	Sigma Aldrich
Potassium dihydrogen orthophosphate	KH_2PO_4	Fisher Scientific
Di-potassium hydrogen orthophosphate	K_2HPO_4	Fisher Scientific
Magnesium Sulfate hexahydrate	$MgSO_4.6H_2O$	Riedel-de Haen
Calcium Chloride hexahydrate	$CaCl_2.6H_2O$	Riedel-de Haen
Sodium bicarbonate	$NaHCO_3$	Fisher Scientific
Haemin, minimum 80%	$C_{34}H_{32}ClFeN_4O_4$	Sigma
R-(+)-Cysteine hydrochloride hydrate, 99%	$C_3H_7NO_2S.HCl.\ x\ H_2O$	Aldrich Chemistry
Bile salts	--	Oxoid
Tween 80	$C_{64}H_{124}O_{26}$	Sigma Aldrich
Vitamin K, 0.5%v/v	--	--
Resazurin solution 0.02%	--	--

2.3 Methods

2.3.1 Pellet Production and Characterization

This section describes the process of producing pellets via the extrusion/spheronization technique, described in detail in Chapter 1. In brief, this process starts with the wet granulation of a homogenously mixed powder dispersion to form a wet mass. This is then extruded to form rod-shaped particles of uniform diameter, which will be subsequently

spheronized into spheres or pellets. The individual processes are described below, along with techniques used to characterize the size, flowability and mechanical properties of the pellets.

2.3.1.1 Wet massing and Extrusion/Spheronization

In the preliminary experiments, mixtures of MCC and α- lactose monohydrate powders were used to prepare the wet mass intended for extrusion. Later, MCC-zein mixtures were studied. The model drug (caffeine or paracetamol) was incorporated into MCC-zein mixtures in order to perform the dissolution studies and thus to investigate the release profile of the resulting pellets. The standard procedure used to produce the pellets by extrusion/spheronization was as follows: powders were dry-mixed for 3 to 5 minutes in a mini food chopper (Kenwood Limited, UK). Then, water was added gradually to the powder mass using a 50 mL burette and powders were mixed for a further 3 to 5 minutes in the same equipment along with occasional scraping of the chopper walls. The wet mix was kept in a sealed polyethylene bag overnight and was then extruded through a single-screw extruder (Caleva, Sturminster Newton, UK) fitted with a die of 1.0 mm diameter at a speed of 100 rpm. Fifty grams of the resulting extrudates were spheronized for 20 to 35 minutes at speed of 6 (on a scale of 0 to 9) on a 120 mm diameter cross-hatched plate spheronizer (Caleva, Sturminster Newton, UK). The pellets produced were oven dried at 50 to 60 ˚C for 24 hours until constant weight was achieved. Drug content of pellets was determined by grinding 0.5 g of pellets, dissolving the resulting powder in some water and making the solution up to 100 mL with water using a volumetric flask. A sample was then withdrawn and filtered through a 0.2 μm filter. The absorbance of the filtrate was measured at the λ_{max} corresponding to the drug used and the concentration was determined by reference to a calibration curve.

2.3.1.2 Size and Shape Analysis

2.3.1.2.1 Sieving

Sieving is one of the oldest methods used for particle size characterization. It is a reliable method and allow the measurement of large quantities of particles at the same time; however, particle attrition might occur due to the aggressive motion of the sieving

instrument (Hoag et al., 2008). The particle size distribution of each pellet batch was determined by sieve analysis; however, only one size fraction (710 to 1000 μm) was used for further investigation. A known weight of pellets was placed on the top of a stack of six different sieves (Fischer Scientific UK Ltd) with descending order of mesh size (2 mm, 1.4 mm, 1 mm, 710 μm, 500 μm and 250 μm) and sieved for 10 minutes using a Sieve Shaker (Endecotts EFL2000, UK). The sample is then expected to divide into multiple sieve fractions by the shaking, tapping and vibration of the sieve instrument. Shaking causes the particles to move allowing particles smaller than the aperture size of the sieve to pass but retaining those bigger than the aperture size. The particles retained on each sieve were weighed and the percentage of weight retained on each sieve was then calculated. The particle size was plotted versus the cumulative retained on the sieves to obtain a particle size distribution. The median was determined corresponding to the 50th percentile and the interquartile range was calculated by subtraction of the 25th percentile from the 75th. The yield of pellets between 710 and 1400 μm, based on the criteria of Baert et al. (1992), was calculated and expressed as a percentage of the total weight of pellets used for sieving.

2.3.1.2.2 Light Microscopy

Microscopy is one of the commonly used methods for the characterization of particle size and shape. Microscopy offers the advantage of the simultaneous measurement of individual particle properties such as size and shape; however, it is a very slow and tedious analysis method if manual measurement is done. For spherical particles the diameter or radius is easy to be measured, but an irregularly shaped particle has an almost infinite number of diameters. Equivalent diameters were therefore introduced for defining the particle size of an irregularly shaped particle. These terms assume a hypothetical sphere for the measurement of the diameter and thus represent an approximation to the true size and shape and are therefore called equivalent. For the characterization of the shape of an irregular particle, a number of shape factors are commonly used such as Heywood's elongation ratio and the aspect ratio (AR). The former is defined as the longest dimension from edge to edge of a particle (length, l) divided by the maximum distance between two points that is perpendicular to the length (breadth, b). The aspect ratio (AR) is the inverse of the elongation ratio (Hoag et al., 2008). Other definitions of the AR are also found in literature (Eriksson et al., 1997).

A microscope (Leica DM LS2, Leica Microsystems UK Ltd) connected to a digital camera (JVC colour video camera, TK-C1481BEG, Victor company of Japan Limited, Thailand) was used to obtain images at a magnification of 4x which were further used in shape analysis. Images were taken of a 710 to 1000 μm sieve fraction and the largest (R_1) and the smallest (R_2) diameters of 20 individual spheres were determined. For each sphere the E (= R_1/R_2) value was calculated to determine the roundness of the spheres.

2.3.1.3 Densities and friability

Bulk and tapped density were determined by loading a known weight of pellets into a 100-mL graduated cylinder (Jolting volumeter tapped density powder tester, SMI-LabHut Ltd, UK) and recording the volume before and after tapping the pellets for 600 times. Furthermore, the Hausner ratio and Carr's compressibility index were calculated according to Eq. (2.1) and Eq. (2.2), respectively.

$$Hausner\ Ratio = Tapped\ Density/Bulk\ Density \qquad \text{Eq. (2.1)}$$

$$CompressibilityIndex = \left(\frac{TappedDensity - BulkDensity}{TappedDensity}\right) * 100\% \qquad \text{Eq. (2.2)}$$

where *bulk density* is the sample weight / initial volume and *tapped density*, which is the density after tapping, is the sample weight / final volume. Summers et al. (2007) explained that the Hausner ratio is related to interparticulate friction and could be used to predict powder flow properties. Powders with low interparticulate friction, such as coarse spheres, have ratios of approximately 1.2, whereas more cohesive, less free-flowing powders, such as flakes, have Hausner ratios of greater than 1.6. Carr's compressibility index is a direct measure of the potential powder arch or bridge strength and stability, and each % compressibility range describes a certain pattern of powder flow: for example, a range between 5 and 15 % reflects excellent free-flowing granules.

Friability tests were performed by placing a known amount of pellets in the drum of an Erweka TAR friabilator (Erweka GmbH, Germany), and rotating them for 100 revolutions. Then, the pellets were reweighed and the weight loss was calculated.

2.3.1.4 Mechanical Properties: Texture Analyser

A TA.XT Plus Texture Analyser (Stable Micro Systems Ltd, UK), outfitted with a 50 N load cell, and fitted with cylinder Perspex probe of 20 mm diameter was used to determine fracture force (F). The fracture force was determined by measuring the maximum force required to break the pellets. The latter value was extracted from the force-displacement profile recorded by the machine. A compression mode test was performed and the following parameters were used: a speed of 0.1 mm/s and post-test speed of 0.5 mm/s. Ten pellets from each formulation were tested.

2.3.2 Tablet Production and Specification Tests

2.3.2.1 Introduction

The oral route is the most common way of administering drugs and tablets of various types are the most commonly used oral dosage form. A tablet consists of one or more active ingredients as well as a series of excipients including a filler or a diluent, a disintegrant, a binder, a glidant, a lubricant and an antiadherant. The role of excipients is to ensure that the tableting process runs satisfactorily and that tablets of desired quality are produced.

A variety of tablets exists and includes disintegrating, chewable, effervescent, compressed lozenges, sublingual and buccal, and prolonged-release tablets. The last type can be further subdivided into diffusion-, dissolution-, erosion- and osmosis-controlled release systems. Tablets are used mainly for systemic drug delivery but can be also used for local delivery of drugs in the mouth or gastrointestinal tract.

The process of tableting, as described by Alderborn (2007), involves powder compression which is defined as the reduction in volume of a powder owing to the application of a force. The increased proximity of particle surfaces during compression promotes bond formation between particles which in turn provides coherence to the powder and a compact (a solid unit of defined geometry) is formed. The tableting process can be divided into three stages: die filling, tablet formation and tablet ejection, a sequence of events called a compaction cycle. This cycle occurs in both types of tablet press: the single-punch press and the rotary (multi-station) press. A single-punch press has one die and one pair of punches: lower and upper punch (Figure 2.1). The powder or wet granulation is filled into

a hopper which is connected to a hopper shoe placed at the die table. The hopper shoe can move to and fro over the die and once located over the die, the powder is fed into the die by gravitational powder flow. The position of the lower punch controls the amount of powder filled into the die, and hence the final tablet weight. During the compression phase, the lower punch is stationary and the upper punch descends and enters the die resulting in powder compression into a tablet. The tablet is ejected by the upward movement of the lower punch and then pushed away by the hopper shoe.

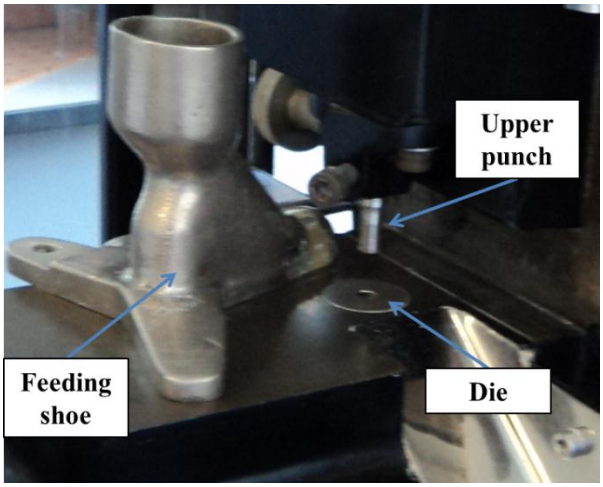


Figure 2.1 A photograph showing the die, feeding shoe and upper punch of the single tablet press used to make the tablets in this work (Manesty E-2 single station tablet press, Manesty Machines Ltd., UK).

Tablets can be produced via wet granulation or direct compaction. The main rationales for granulating the powder mixture (active ingredient and excipients) prior to tableting include: increasing the bulk density of the powder mixture to ensure adequate die filling; improving powder flowability to minimize tablet weight variation; improving mixing homogeneity and reducing segregation; and improving the compactability of the powder by adding a solution binder. On the other hand, direct compaction involves only powder mixing prior to tableting and is therefore known to reduce both production time and cost.

Like all other dosage forms, tablets should fulfil certain tests and specifications, and most importantly, those related to dose content and dose uniformity, tablet disintegration and drug dissolution profile (Alderborn, 2007).

2.3.2.2 Wet Granulation and Tableting

Tablets used in this project were produced via wet granulation. The chemicals shown in Table 2.7 below were mixed with 100 mL of PVP solution (18.75% w/v) and water (as required) using a Kenwood mixer until a suitable granule was formed. The wet granulation was sieved through a 2 mm sieve, and then dried in oven at 60 °C overnight. The residual moisture content was measured by the "loss on drying" (LOD). A predetermined weight of dried granules was heated for 15 minutes at 105 °C and then reweighed. The LOD is the difference in weight and should be between 1 and 2 %. The dried granules were sieved and mixed with 1% w/w of magnesium stearate in a Kenwood mixer. The granules were loaded into a Manesty E-2 single station tablet press (Manesty Machines Ltd., Liverpool, UK) and were compressed using round 7 mm diameter plain, normal concave punches.

Table 2.7 The components of wet granulation.

Chemical	Content (g)/batch	Content (mg)/ lactose-based tablet	Content (mg)/ calcium phosphate-based tablet
L-α-Lactose monohydrate or Dibasic calcium phosphate	423.75	118.60	120.20
Paracetamol	150.00	42.00	42.54
MCC	75.00	21.00	21.30
PVP *	18.75	5.20	5.30
Partially gelatinized starch (Pre-gelatinised starch)	75.00	21.00	21.30
Water	As required	-	-
Magnesium stearate	7.50	2.10	2.10
Total	750.00 g	209.90 mg	212.70 mg

*Added as 100 mL of 18.75 % aqueous solution during granulation.

2.3.2.3 Weight Uniformity, Thickness and Hardness Tests

These specifications apply to all tablets e.g. uncoated, coated, effervescent and chewable tablets. It is critical to check the mass uniformity within a batch to ensure that the tablets have the same weight (within limits), to ensure all tablets contain the same amount of drug (assuming a perfect drug distribution within the tablet), and ultimately to ensure that the patient gets a reproducible dose of drug.

To test weight uniformity, 20 tablets from the same batch were weighed, the mean weight was calculated, and checked if it complies with the following specifications: weight uniformity (mass uniformity) specifications state that not more than two tablets deviate from the mean weight by more than the allowable limit and none by more than twice the limit (British Pharmacopoeia, 2011).

Table 2.8 Specifications for tablet weight uniformity within a batch as described by BP 2011.

Theoretical tablet weight	Allowable deviation of individuals
< 80 mg	10.0 %
80-250 mg	7.5 %
> 250 mg	5.0 %

Thickness and hardness tests are used as in-process tests during compression. Thickness and hardness values of tablets are dependent on their geometry and therefore there are no BP specification values for these two tests. Thickness is usually found not to vary substantially throughout a batch of tablets, whereas hardness can vary considerably. The thickness of 20 tablets was measured with a micrometer (Outside micrometer, 0-25 mm) and the mean value was calculated. The hardness test is essential to ensure that the tablet can withstand handling and further processing without mechanical breakdown, and to ensure that the disintegration and dissolution profiles remain within specification (harder tablets generally exhibit slower disintegration and dissolution profiles). The tablets hardness can be modified by varying the compaction pressure. The hardness of 20 tablets was measured using an Erweka TBH 28 apparatus (Erweka GmbH, Germany) and the mean value was recorded. It should be noted that the term "hardness" is used throughout the pharmaceutical industry and therefore used here, although a more scientifically correct description is "resistance to crushing".

2.3.2.4 Friability Test

The friability test is essential to ensure that the tablet can withstand handling, and ultimately to ensure that the patient receives a reproducible dose of drug. The test was performed by weighing 20 tablets, placing them in the drum of an Erweka TAR friabilator (Erweka GmbH, Germany), and rotating them for 100 revolutions. Then, the tablets were

reweighed and the weight loss was calculated. The British Pharmacopoeia specifies 1% weight loss as the limit; however, 1% loss is regarded to be very high and 0.2% is usually selected as a better limit for product development.

2.3.2.5 Content Uniformity

It is critical to check the content uniformity within a batch to ensure that all individual tablets of a batch contain the stated amount of drug (within limits), and ultimately to ensure that the patient gets a reproducible dose of drug. Stage I involves testing individually 10 tablets from the same batch, calculating the mean drug content, and:

- The batch passes if all tablets are within 85 and 115% of the mean.
- The batch fails if more than one tablet is outside these limits.
- The batch fails if one tablet is outside 75 to 125% of the mean.

Stage II states that if one tablet from stage I was outside 85 to 115% of the mean but within 75 to 125% of the mean, then one should assess individually 20 more tablets from the same batch, calculate the mean drug content of the 30 tablets, and:

- The batch passes if not more than one tablet is outside 85 to 115% of the mean and none is outside 75 to 125% of the mean (British Pharmacopoeia, 2011).

Ten tablets were individually ground, each added to 200 mL of 0.1 M HCl and stirred on a magnetic stirrer plate. The dispersion was then filtered into a 500 mL volumetric flask and the volume was made up to 500 mL using 0.1 M HCl. The solutions were then analysed for the drug concentration using UV-VIS spectrophotometry (Perkin-Elmer Lambda XLS, USA) at a maximum absorbance wavelength of 243 nm. By reference to a standard plot, the drug concentration in the solutions was calculated and hence the actual drug content in the tablets was determined.

2.3.2.6 Disintegration Time Test

It is important to check that all tablets disintegrate within a specific time in order to ensure that the biological effect is reproducible. This test is performed by placing one dosage unit

in each of the six tubes of the basket of a disintegration test apparatus which will be immersed in the specified medium at 37 ± 2 ºC. The time needed for each tablet to disintegrate completely was recorded and the average disintegration time for the six tablets was calculated. In this work, the disintegration time was measured in 0.1 M HCl and in distilled water at 37 ± 0.5 ºC using a Copley DTG 2000 apparatus with disks (n = 6) (Copley Scientific Ltd., Nottingham, UK). The disintegration time is specified in the BP to be 3, 5, 15 and 30 minutes for soluble, effervescent, uncoated and coated tablets, respectively. However, most immediate-release tablets disintegrate in 5 minutes or less.

2.3.2.7 Dissolution Test

Dissolution testing is important to ensure that the dissolution profile of the drug from the tablet is reproducible in order to guarantee a reproducible biological effect and avoid null or toxic effects. The test is performed in a dissolution bath (described in section 2.3.4.7). For immediate-release tablets, > 75% of the drug should be dissolved within 45 minutes (British Pharmacopoeia, 2011). However, the BCS (Biopharmaceutics Classification System) standards specify that no less than 85% of the labelled amount of the drug should be dissolved within 30 minutes (Guidance for Industry, 2000).

2.3.3 Coating Process

The general coating process was described in detail in Chapter 1. This section describes the preparation of coating solutions and dispersions and the optimized coating parameters.

2.3.3.1 Preparation of Organic-based Zein Solution

Ten grams (10 g) of zein were dissolved in 100 mL of 75% aqueous ethanol and stirred on a hotplate magnetic stirrer at 50 ˚C for 10 minutes. Thereafter, 20% w/w glycerol (based on zein weight) was added gradually to the solution. Glycerol was generally used for plasticization at a level of 20% w/w; however, propylene glycol (PG) and polyethylene glycol 400 (PEG 400) were also used, where specified. The solution was covered with aluminium foil to minimize solvent loss and kept on the stirrer for 1 to 2 hours.

2.3.3.2 Preparation of Aqueous Zein Dispersion with Pectin

Aqueous zein dispersions or pseudolatex (10% w/w) were prepared by a precipitation method based on the work of Oshlack et al. (1994). An organic-based zein solution of 10% w/w was prepared by dissolving zein in 75% aqueous ethanol under stirring at 50 ˚C for 30 minutes. The aqueous phase was prepared by dissolving pectin (sufficient to give a final 1:30 w/w pectin/zein ratio) in distilled water and stirring it for 30 minutes at 500 rpm. The zein solution was added drop wise to the aqueous phase while stirring at 800 to 1200 rpm, with the total added volumes of the organic and aqueous phases being equal. The formed dispersion was stirred for a total of 75 minutes, and then the ethanol and part of the water were evaporated under reduced pressure using a rotary evaporator (Buchi rotary evaporator, Switzerland). Propylene glycol (30% w/w, based on zein weight) was added and stirred at 400 rpm for 2 hours; thereafter, methyl parabens and propyl parabens were added in 0.2% and 0.02% w/v (with reference to the final volume of the aqueous dispersion), respectively, and stirred for another 2 hours. Since pectin was incorporated into the aqueous phase, the resulting dispersion could be used directly for coating.

2.3.3.3 Preparation of Aqueous Zein Dispersion with Acetylated High Amylose Maize Starch

Aqueous zein dispersions or pseudolatexes (10% w/w) were prepared by a precipitation method described by Li et al. (2010). An organic-based zein solution of 6% w/w was prepared by dissolving zein in 75% aqueous ethanol with stirring at 50 ˚C for 30 minutes. The aqueous phase was prepared by dissolving Tween 80 (10% w/w based on zein amount) in distilled water and stirring for 30 minutes at 500 rpm. The zein solution was centrifuged at 3000 rpm for 5 minutes, before being added drop wise to the aqueous phase while stirring at 800 to 1200 rpm, with the total added volumes of the organic and aqueous phases being equal. The formed dispersion was stirred for a total of 75 minutes, and then the ethanol and part of the water were evaporated under reduced pressure using a rotary evaporator (Buchi rotary evaporator, Switzerland). The resulting zein concentration (circa 10% w/w) was determined by gravimetry i.e. by weighing samples of defined weight before and after heating up at 110 ˚C for 2 hours. The plasticizer was added to the system prior to dilution and stirred at 400 rpm overnight; 30% w/w (based on zein weight) PEG 400 was used.

Starch occurs as water insoluble granules, so an aqueous dispersion was prepared by dispersing the acetylated high amylose maize starch (HAS) in water in a 14:86 weight ratio (13.04% w/w), and heating it at 80 ± 5 ˚C for 30 minutes based on the method proposed by Freire et al. (2009a). Then, the required amount of HAS was diluted with preheated water (80 ˚C) to give a final concentration of 1.8% w/w. The HAS dispersion was left to cool down to room temperature (approximately 26 ˚C) without stirring prior to being added drop wise to the zein aqueous dispersion. The resulting dispersion was left to stir overnight to achieve complete mixing of the two polymers. Mixing zein and HAS aqueous dispersions reduced the zein concentration to circa 5% w/w. For control pseudolatex systems (no added HAS), zein dispersion was diluted with water to achieve a comparable solid concentration (5% w/w). Figure 2.2 summarizes the sequence of preparation of zein aqueous dispersion, HAS incorporation and various variables that could influence each step.

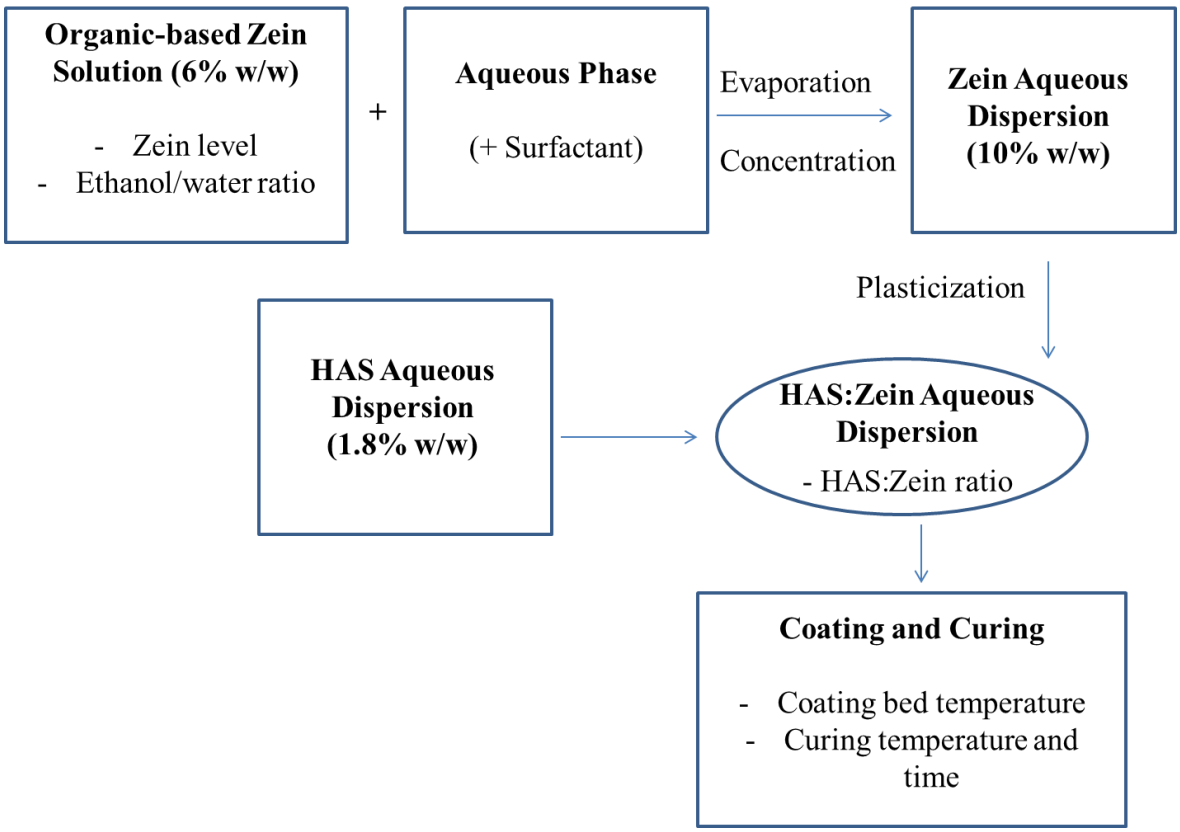


Figure 2.2 Schematic representation of preparation of aqueous HAS/Zein dispersion.

2.3.3.4 Coating Parameters

2.3.3.4.1 Coating Pellets with Organic-based Zein Solution

Pellets were coated with 10% w/v zein solution (See Section 2.3.3.1) in a top-spray fluidized-bed coater (Mini Coater/Drier 2, Caleva Process Solutions Ltd, UK) equipped with a top spray head. Five grams (5 g) of pellets were placed in the vibrating coating chamber. The bed temperature was set at 30 ˚C; meanwhile the zein solution reservoir was being stirred constantly at 50 ˚C. The atomization pressure was 0.7 to 1 bar and the pump rate was 1.09 rpm. The fan speed varied between 6.3 m/s (3%) and 7 m/s (10%) and the agitation was between 11.5 and 13.7 Hz. The height for the spray head was 70 mm from the spray nozzle to the mesh in the base of the cone. After coating, the pellets were left in the coater (bed temperature = 30 ˚C) for 40 minutes to dry. The coating thickness is expressed in terms of the percentage total weight gain (TWG) which is the difference in the weight of tablets prior to and at the end of the coating process divided by the initial weight of tablets. Pellets were stored in sealed amber glass bottles until use.

2.3.3.4.2 Coating Tablets with Organic-based Zein Solution

Tablets were coated with 10% w/v zein solution in the same top-spray fluidized-bed coater described in Section 2.3.3.4.1. The bed temperature was set at 30 ˚C and the zein solution reservoir was stirred constantly at 50 ˚C. Lactose-based (n = 3, 6 or 10) and calcium phosphate-based (n = 10) tablets were placed in the coating chamber. The atomization pressure was 0.7 bar and the pump rate was 1.09 rpm. The fan speed was 12 m/s (60%) with no agitation. The height for the spray head was 70 mm from the spray nozzle to the mesh in the base of the cone. After coating, the tablets were left in the coater (bed temperature = 30 ˚C) for 40 minutes to dry. The coating thickness is expressed in terms of the percentage total weight gain (TWG). Tablets were stored in sealed amber glass bottles until use.

2.3.3.4.3 Coating Tablets with Aqueous Zein Dispersion

Tablets were initially subcoated with an organic-based zein solution (10% w/w plasticized with 20% w/w glycerol, based on zein weight) up to 2 to 3% total weight gain under the same conditions described in section 2.3.3.4.2. Then the tablets were top coated with zein

aqueous dispersion. The bed temperature was set at 45 ˚C and the dispersion reservoir was stirred constantly at room temperature. Ten tablets (calcium phosphate-based tablets) were placed in the coating chamber. The atomization pressure was 0.7 bar and the pump rate was 1.09 rpm. The fan speed was 13 to 13.5 m/s (70 to 75%) with no agitation. The height for the spray head was 65 mm from the spray nozzle to the mesh in the base of the cone. After coating, the tablets were left in the coater (bed temperature = 45 ˚C) for 15 minutes to dry. The coating thickness is expressed in terms of the percentage total weight gain (TWG). Tablets coated with HAS/Zein pseudolatex were cured in an air oven at 45 ˚C for 10 hours, whereas those coated with pectin/zein aqueous dispersion were cured for 24 hours. Tablets were stored in sealed amber glass bottles until use.

2.3.4 Dissolution Studies and Drug Quantification Methods

2.3.4.1 Introduction

Dissolution studies are important during the development of novel oral dosage forms. Although associated with several limitations, dissolution testing, when performed correctly, can provide valuable information regarding the pharmacokinetic behaviour of the drug and can therefore be used as an *in vitro* substitute for *in vivo* performance of drug and dosage form (Wang et al., 2009). It can also be used as a quality control tool to monitor batch-to-batch consistency and to detect manufacturing deviations (Azarmi et al., 2007).

2.3.4.2 The Theory of Dissolution

Dissolution can be defined as the process whereby a solid enters into a solution and is controlled by the relative affinity between the molecules of the solid substance and those of the solvent (Aulton, 2007). The dissolution process of solids consists of two main steps (Figure 2.3). First, the molecules at the solid-liquid interface are solvated and detached from the solid surface. Second, the solvated molecules diffuse away from the interface to the bulk solution. The overall rate of mass transfer that occurs during dissolution will be determined by the rate of the slowest step. It is believed that the first step is much faster than the second, i.e. diffusion is the rate limiting step. Therefore, the concentration of the dissolving substance at the dissolution interface at steady state is assumed to be equal to or

close to its solubility (C_s).

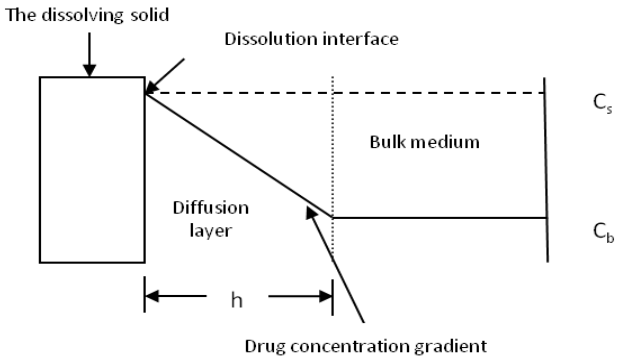


Figure 2.3 Schematic representation of solid dissolution (Wang et al., 2006).

The rate of diffusion (*dC/dt*) can be described by Fick's first law of diffusion which states that the rate of change in the concentration of a dissolved material with time is directly proportional to the concentration difference between the two sides of the diffusion layer, Eq. (2 3):

$$\frac{dC}{dt} = k\Delta C \qquad \text{Eq. (2.3)}$$

where *C* is the concentration of solute in solution at any point and at time *t*, and the constant *k* is the rate constant (s^{-1}). The concentration difference between the two sides of the diffusion layer (ΔC) is the difference in the concentration of solution at the solid surface (C) and the bulk of the solution (C_b). At equilibrium, the solution in contact with the solid (C) will be saturated, i.e. C = Cs. The concentration in the bulk solution (C_b) will determine the direction in which solid molecules will move; the molecules will move from the solid to the bulk if $C_b < C_s$.

The dissolution rate (*dm/dt*) of a solid in a liquid can be described by the Noyes-Whitney equation, Eq. (2.4):

$$\frac{dm}{dt} = \frac{DA(C_s - C_b)}{h} \qquad \text{Eq. (2.4)}$$

where m is the mass, t is the time, A is the surface area of the undissolved solid in contact with the solvent, D is the diffusion coefficient (m^2/s), h is the thickness of diffusion layer, C_s is the solubility, C_b is the concentration in the bulk solution and C_s - C_b is the concentration gradient. Eq. (2.4) assumes that the surface area and the thickness of the hydrodynamic diffusion layer remain constant; therefore, only several simplified cases can be solved by this equation. Other models like the Hixson-Crowell cube-root law have been developed for systems where there is a change in surface area and diameter of particles or tablets (Wang et al., 2006).

Under sink conditions where the volume of the solvent is large, or the solute is removed from the bulk solution by some process at a faster rate than it passes into solution, C_b can be assumed to be close to zero and the term C_s - C_b in Eq. (2.4) can be approximated to C_s (Aulton, 2007). In practice, if the volume of the dissolution medium is so large that C_b does not exceed 10% of the value of C_s, then the same approximation can be made and Eq (2.4) can be adapted to:

$$\frac{dm}{dt} = \frac{DAC_s}{h} \qquad \text{Eq. (2.5)}$$

2.3.4.3 Factors Affecting the Dissolution Rate

The determination of the dissolution rate of a drug from a formulated dosage form such as a tablet or capsule, as well as the rate of dissolution of pure solids, is a concern for pharmaceutical scientists. The various factors that affect the *in vitro* rate of diffusion-controlled dissolution of solids into liquids can be determined by examination of the Noyes-Whitney equation Eq. (2.4) and they include (Aulton, 2007):

1. Surface area of undissolved solid (A)

This term is affected by the size of solid particles as the surface of isodiametric particles is inversely proportional to their particle size. One complication that should be taken into account is the change in particle size during the dissolution process. The surface area available for dissolution is also influenced by the dispersibility of powdered solids in the dissolution medium as the surface area effectively reduces if the particles tend to form coherent masses in the medium. The addition of a wetting agent can improve the

dispersion into primary powder particles. Moreover, the porosity of solid particles can change the surface area available for dissolution. Pores tend to increase the available surface area, and in some materials, particularly granulated ones, may be large enough to allow inward access of the dissolution medium and outward diffusion of dissolved solute molecules.

2. Solubility of solid in the dissolution medium (C_s)

Factors such as the temperature, the nature of the dissolution medium, the molecular structure and crystalline form of the solute, and the presence of other compounds that can induce complex formation or solubilisation can all influence the solubility of solid and hence its dissolution rate.

3. Concentration of solute in the bulk solution at time t (C_b)

Solute concentration in the bulk solution is affected by the volume of dissolution medium; if the volume is small, C_b can build up to an extent that it approaches C_s, and the overall dissolution rate will be zero according to Eq. (2.4). C_b is usually negligible with respect to C_s in large volumes of dissolution medium and thus sink conditions are valid in this case. In addition, C_b can be changed by any process that removes dissolved solute from the dissolution medium, such as adsorption to an insoluble adsorbent, partition into a second liquid that is immiscible with the dissolution medium, removal of solute by dialysis or by continuous replacement of solution by fresh dissolution medium.

4. Thickness of diffusion layer (h)

This is affected by the degree of agitation, which depends, in turn, on the speed of stirring or shaking; the shape, size and position of the stirrer; the volume of the dissolution medium; the shape and size of the container; and the viscosity of the dissolution medium.

5. Diffusion coefficient of solute in the dissolution medium (D)

For a dissolved molecule, the diffusion coefficient (D) of a solute is influenced by the temperature (T) and the viscosity of the dissolution medium (η) and the radius (r) of the diffusing molecules, according to the Stokes equation, Eq (2.6):

$$D = kT/(6\pi\eta r) \qquad \text{Eq (2.6)}$$

where *k* is the Boltzman constant.

2.3.4.4 Preparation of Dissolution Media

Dissolution media were prepared by weighing the individual buffer components into plastic weighing boats prior to their transfer into a clean, 5 L volumetric flask. Distilled water was added to the flask and the contents were stirred until the solid material had fully dissolved. Gentle stirring was applied when enzymes were added. The final pH was checked using a calibrated pH meter, and when necessary adjusted by the addition of small quantities of HCl or NaOH. A further amount of distilled water was added in order to obtain a final volume of 5 L.

2.3.4.5 Ultraviolet-Visible Spectrophotometer (UV-VIS)

The interaction of light with different compounds can be used to perform quantitative and qualitative measurements. The Beer-Lambert law is one of the most widely used relationships in analytical chemistry and is the base for UV and visible quantitative analysis (Higson, 2004). The Beer-Lambert law Eq. (2.6) relates the light absorbance (*A*) of molecules to their concentration (*c*), the path length (*l*) and the molar absorptivity, also known as the extinction coefficient (ε).

$$A = \varepsilon \cdot c \cdot l \qquad \text{Eq. (2.6)}$$

A path length of 1 cm is usually used to simplify the calculation of absorbance and molar absorptivity. Absorbance is unitless whereas the molar absorptivity has a unit of $dm^3\ mol^{-1}\ cm^{-1}$. The Beer-Lambert law applies, i.e. the absorbance of the sample increases linearly with the concentration of the analyte, to the majority of compounds over a wide range of experimental conditions; however, the absorbance of light by individual compounds is highly wavelength specific. Most UV-VIS spectrophotometers are able to measure the absorbance across a range of wavelengths.

A Perkin-Elmer Lambda XLS spectrophotometer (USA) was used to quantify the model drug in the various dissolution media used at the wavelength of maximum absorbance, λ_{max} specified for each drug.

2.3.4.6 Construction of Beer Lambert Plots

It is essential to prepare calibration standards in order to prove that a linear relationship exists between absorbance and drug concentration over the required range, and to determine the drug loading in a dosage form that has an absorbance within the machine detection limits.

Beer-Lambert plots were constructed for each individual buffer and drug combination. Approximately 50 mg of drug was accurately weighed out into a tared plastic weighing boat then transferred into a clean, dry 0.5 L volumetric flask. The drug was dissolved (by stirring or inverting the flask several times) prior to the solution being made up to volume with the appropriate medium. Once a suitable solution of the drug had been prepared, 0.1, 0.2, 0.3, 0.5, 1.0, and 1.5 mL aliquots of the stock solution (0.01% w/v) were transferred into separate 10 mL volumetric flasks and made up to volume with buffer to produce concentrations of 0.1, 0.2, 0.3, 0.5, 1.0, and 1.5×10^{-3} % w/v, respectively. Three calibration sets were prepared for each buffer solution and the average absorbance value was plotted against the corresponding drug concentration. Measurements were made using a Perkin-Elmer Lambda XLS spectrophotometer (USA). The wavelength of maximum absorbance, λ_{max} was 243 and 270 nm for paracetamol and caffeine, respectively.

2.3.4.7 Dissolution Method

Dissolution was carried out using a BP Apparatus I (D8000, Copley Scientific Ltd., Nottingham, UK, Figure 2.4); 0.5 g of pellets or one tablet was added into each basket. Each vessel was filled with 900 mL of the medium. The temperature was set at 37.0 ± 0.5 ˚C and the rotation speed at 50 ± 1 rpm. At predetermined sampling points, 10 mL sample was collected from each vessel using a syringe and filtered through a 0.2 μm membrane filter (Minisart High-Flow single use syringe filter, Sartorius Stedim Biotech GmbH, Germany). The first few millilitres were discarded and the absorbance of the filtrate was measured at the wavelength of maximum absorbance (λ_{max}) specified for each model drug. Samples that contain the pancreatic digestive enzymes were centrifuged at 13,000 rpm for 30 minutes prior to filtration and UV measurements. An equivalent volume of fresh medium was added to each vessel to replace the volume of the withdrawn sample. The dissolution studies were conducted in triplicate under sink conditions and the average drug release ± SD was calculated. The results are expressed as cumulative percentage drug

release versus time profiles.

Figure 2.4 A photograph showing the dissolution bath used to conduct the dissolution tests presented in this work (D8000, Copley Scientific Ltd.).

2.3.4.8 High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) is one of the most widely used forms of chromatography. Separation occurs by partitioning of the sample components between the mobile phase and the stationary phase according to their relative solubilities or affinities towards the two phases. HPLC is a highly selective separation technique and achieves high-quality separation in the minimum of time by passing a mobile liquid phase through a finely divided stationary phase under high pressure. It is a sensitive and selective analytical technique used for the identification and quantification of analytes within complex mixtures (Higson, 2004).

The use of the UV-VIS spectrophotometer was not valid for the more complex colonic medium as the medium showed some absorbance over a wide range including the λ_{max} of the drug of interest. Therefore, an HPLC method for quantifying paracetamol was developed. HPLC analysis was carried out with a Dionex (Leeds, UK) high performance liquid chromatography having a P 580 HPLC isocratic pump, equipped with a Dionex ASI-100 automated sample injector and a Dionex PDA-100 photodiode array detector. Chromeleon (Dionex, Leeds, UK) software was used to collect, integrate and analyse the chromatographic data. UV detection was carried out at 245 nm. A C18, 5 µm, 250 x 4.6

mm column (Phenomenex, Macclesfield, Cheshire, UK) was used. For the analysis, the mobile phase consisted of acetonitrile (MeCN)-water (25:75 v/v) adjusted to pH 2.5 with phosphoric acid, at an injection volume of 10 µL and a flow rate of 1.7 mL min^{-1}. The retention time of paracetamol was approximately 2.38 minutes with this system. Linearity between absorbance and concentration was tested using external standards at various concentrations and the response was linear with a correlation coefficient $r^2 = 0.9988$, over the whole range of used concentrations. A standard stock solution of paracetamol (0.05% w/v) was prepared in the supernatant of the colonic medium. The latter consists of chemostatic basal medium (Table 2.6) inoculated with faecal material. The following standard solutions were prepared in triplicate in the 0.05 to 0.0005% w/v range (i.e. 0.05%, 0.045%, 0.0375%, 0.025%, 0.0125%, 0.005%, 0.002%, 0.00125%, 0.001%, 0.00075% and 0.0005% w/v) by appropriate dilution of the stock solution with the same supernatant of the colonic medium.

2.3.5 Characterization Techniques

2.3.5.1 Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) is an electron microscopy technique that is used to achieve three-dimensional visualization of the sample surface at high resolution (Kaminskyj et al., 2008). SEM images are therefore useful to assess the structure of the sample surface. In this work, the surface morphology of coated tablets and coated pellets, before and after dissolution tests, and of pure zein and HAS/Zein free films was observed using SEM. Tablets or pellets used for dissolution studies were left to dry at room temperature before being tested. The samples were mounted onto stubs using double-sided tape and were gold coated by a Polaron SC7640 sputter gold coater manufactured by Quorum Technologies. The thickness of the gold coating was about 15 nm. The imaging process was performed in a high vacuum environment. Imaging process was performed with a JEOL JSM5900 LV SEM (Japan), mounted with a tungsten filament with an acceleration voltage of 5–20 kV.

SEM images were kindly taken with the assistance of Bertrand Leze, the geotechnical microanalysis laboratory chief technician in School of Environmental Sciences at the University of East Anglia.

2.3.5.2 Attenuated Total Reflectance-Fourier Transform Infra-Red (ATR-FTIR) Spectroscopy

Infra-Red (IR) analytical spectroscopy is based on the molecular absorption of radiation; energy is imparted from the radiation to the molecule and absorption occurs when the wavelength of the incident IR radiation matches the frequency of oscillation of a molecular bond (Higson, 2006). Molecular oscillations or vibrations involve stretching, bending, and other motions. Since each motion involves oscillation at a specific frequency (wavelength-wavenumber), absorption can be used to determine the type of molecular oscillation and the functional groups present in a molecule. An IR spectrum is normally plotted in terms of percentage transmittance, rather than absorbance, versus wavenumbers. Wavenumbers are the reciprocal of the wavelength and have the units of cm^{-1}. Fourier Transform Infra-Red (FTIR) spectrometers offer high speed, sensitivity and wavelength precision. Attenuated total reflectance (ATR) allows direct analysis of solid samples as well as liquids and solutions. It can be used to identify the structure of the material used and subsequently to detect any structural changes upon various processing steps. Duodu et al. (2001) used the Fourier transform infrared (FTIR) to study the structural changes in maize and sorghum proteins on wet (wet cooking) and dry (popping) thermal treatments. It can also be used to detect interactions between mixed polymers. Freire et al. (2009b) used this technique to investigate the miscibility and the occurrence of interactions between a polymeric mix of ethylcellulose and high amylose starch.

Infrared spectra of zein powder, HAS samples, zein alone free films and HAS/Zein mixed films were recorded in the wave number range of 4000 to 550 cm^{-1} using an IFS-60/S Fourier transform infrared (Bruker Optics, Coventry, UK) with an attenuated total reflectance (ATR) accessory (SPECAC, Orpington, UK). For each sample a total of 16 scans were determined at a resolution of 4 cm^{-1}, respectively. Spectra were analysed using OPUS software (version 6.0).

2.3.5.3 Modulated Temperature Differential Scanning Calorimetry (MTDSC)

Conventional differential scanning calorimetry (DSC) is the most widely used thermo-analytical method in the pharmaceutical industry. The process starts with applying a linear heating or cooling signal to a sample and then measuring the temperature and energy associated with the relevant thermal events, such as melting, crystallization, glass

transitions and decomposition reactions of the materials (Reading et al., 2007a). There are two types of DSC instruments: heat flux and power compensation. Power compensation DSC has two separate furnaces for the reference and the sample. The same programmed temperature profile is applied to both reference and sample and the electrical power supplied to the sample in order to maintain a zero temperature difference between the sample and the reference is measured. On the other hand, heat flux DSC has only one furnace but two crucibles for the reference and the sample. Both of them are heated from the same source with symmetrical heat paths and the temperature difference between the sample and the reference as a result of a thermal event is measured. The temperature difference is then converted to heat flow measurements and the results are typically expressed in terms of the heat flow as a function of temperature.

Modulated temperature differential scanning calorimetry (MTDSC) is based on the conventional DSC; it however applies two simultaneous heating rates (linear and sinusoidal) to the sample, allowing the separation of overlapping processes or weak thermal events. The linear or average heating rate provides the total heat flow signal as obtained by the conventional DSC, whereas the sinusoidal (or modulated) heating rate is used to determine the fraction of the total heat flow contributed by the heat capacity change. The latter fraction is referred to as reversing heat flow signal. A third signal, named non-reversing heat flow signal, is obtained by subtracting the reversing signal from the total heat flow. Thermal events associated with changes in heat capacity, such as the glass transition, can be detected from the reversing signal, whereas those which are kinetically hindered, such as degradation, are seen in non-reversing signals. MTDSC is used to characterize the glass transition temperature, to determine the miscibility and to detect phase separation of polymeric materials (Reading et al., 2007b).

Thermal behaviour of various samples including zein powder, HAS samples, zein alone free films and HAS/Zein mixed films was studied by means of MTDSC using a Differential Scanning Calorimeter (Q2000, TA Instruments, Newcastle, USA). Samples (n = 3) were accurately weighed (1 to 3 mg) into pinhole aluminium pans (Perkin Elmer) in order to minimize the influence of water on the sample's behaviour. Samples were scanned between -20 ^{o}C and 250 ^{o}C with optimized modulation temperature amplitude of ±1.0 ^{o}C, modulation time of 60 s and a ramp rate of 2 ^{o}C/minute. Temperature and cell constant of

DSC instrument was calibrated with indium, n-octadecane, and tin by using their melting point, whereas heat capacity was calibrated with aluminium oxide in modulated mode. Free films of zein alone or mixed zein-starch were stored on P_2O_5 for a minimum of 24 hours before being tested.

2.3.5.4 Thermogravimetric Analysis (TGA)

Thermogravimetric analysis (TGA) measures changes in mass, as a result of chemical or less commonly physical processes, as a function of temperature or isothermally as a function of time (Galwey et al., 2007). It is a simple and rapid method to measure weight loss. The most basic pharmaceutical use of TGA is the measurement of the water content; it is able to determine the temperature range over which dehydration and water loss from solid drug or excipient systems occur. TGA is also used to identify the degradation temperature of compounds.

Thermogravimetric analysis was performed using Hi-Res TGA 2950 (TA Instruments, Newcastle, USA) in order to measure the water content various samples. To identify water content as measured by weight loss due to sample dehydration, samples were heated from room temperature to 100 ^{o}C at a heating rate of 20 ^{o}C/minute, and then were held (isothermally) at 100 ^{o}C for 10 minutes.

2.3.5.5 Laser Diffraction Analysis (LD)

Laser diffraction (LD) has become one of the most widely used and reliable techniques for measuring the particle size of different physical forms such as dry powder, suspensions, spray dispersions and emulsions. Laser diffraction measurements are based on the principle that particles will scatter light at different angles with different intensities, i.e. large particles scatter light at smaller angles with higher intensities, while small particles scatter light at wider angles with lower intensities. The intensity of the light scattered at different angles determines the relative amounts of different size particles. A typical laser diffraction instrument is made of a laser, a sample presentation system and a series of detectors and uses an optical model based on several theories. LD is fast and adequately precise over a wide particle size range; however, it can not determine the particle shape and assumes all particles to be spherical (Tinke et al., 2005). Based on this assumption, the reported

diameter is an equivalent volume diameter. This might therefore result in discrepancy in particle size values of any non-spherical shaped particle measured by LD and those measured by other particle size analysis methods (Hoag et al., 2008). It is generally only used to detect particles larger than 1 µm as, below this size, light scattering would occur at an angle that cannot be detected by the machine design.

The particle size distribution of zein alone or HAS/Zein aqueous dispersions was screened using a Sympatec HELOS/BF (HI146, TYPE: cuv-50 mL/US, Germany) laser diffraction particle size analyzer. A droplet of the dispersion was added to the quartz cuvette and each sample was scanned 4 times using a small lens of size detection range of 0.25/0.45 to 87.5 µm. Measurements were performed in triplicates and the average density distribution (y-axis) was then plotted against the particle size on a log scale (x-axis).

2.3.5.6 X-Ray Powder Diffraction (XRPD)

X-rays are electromagnetic radiations of very short wavelength. X-ray diffraction can provide details of internal structure of the order of angstrom (Å) in size. X-ray powder diffraction (XRPD) has been used in the pharmaceutical field for a range of applications. These applications include the determination of the crystal structure and degree of crystallinity of a material, and the recognition and quantification of amorphous content in partially crystalline mixtures (Du et al., 2011). The technique can also be used to investigate the changes in structure of the material upon treatment such as heating.

X-Ray Powder Diffraction analysis of HAS samples was performed with a XRPD, Thermo ARL Xtra model (Switzerland) equipped with a copper X-ray tube (1.540562 Å). Samples were exposed to an X-ray beam with a voltage of 45 kV and a current of 40 mA. Other test conditions were: scanning range at 2θ of 5 to 60°, step size 0.01°, and time per step: 0.5 seconds.

XRPD experiments were kindly carried out by Bertrand Leze, the geotechnical microanalysis laboratory chief technician in School of Environmental Sciences at the University of East Anglia.

2.3.5.7 Nano-Thermal Analysis (n-TA)

Nano-thermal analysis (n-TA) (and previously microthermal analysis (μ-TA)), are recently introduced techniques that combine thermal analysis with scanning probe microscopy (SPM) and allow site-specific thermal characterization, compared to bulk techniques such as DSC and MTDSC (Dai et al., 2009). The instrumentation is based on atomic force microscopy (AFM); however, the conventional SPM ultra-sharp tip is replaced by a thermal probe. The temperature at the tip of these probes can be adjusted by supplying a controlled voltage. In μ-TA, micro-scale resolution only is achievable, as the standard AFM probe is replaced by the physically larger Wollaston wire probe (Craig et al., 2002). More recently, the development of nanoprobes for n-TA has resulted in an order of magnitude improvement in resolution compared to that obtained in μ-TA. This technique can be used to attain topographic images and local thermomechanical analysis can be carried out once a topographic image is obtained. The latter involves placing the probe on the area of interest in a topographic image and applying a linear temperature ramp, inducing changes in the mechanical properties of the material on heating. These changes involve thermal expansion of the sample forcing the cantilever upwards, softening at the glass-rubber transitions or melting causing the probe tip to penetrate into the sample.

Another application includes tapping mode where the probe is oscillated at a constant frequency, providing intermittent contact with the sample, while maintaining a constant temperature of the probe tip. Tapping mode can be used to distinguish materials based on their mechanical properties as the intermittent contact can provide information on adhesion and stiffness. Changes in the phase of the probe in relation to the drive oscillation are sensitive to changes in the mechanical properties of the surface which are independent of topography. By heating the probe to the softening temperature of a particular material in a multi-component sample, it is possible to deliberately alter the mechanical properties of a particular material and provide greater differences in the phase of the probe.

Nano-thermal analysis (n-TA) was performed using an Anasys Instruments Nano TA2 Controller with Veeco Calibre AFM head. Thermal nanoprobes (AN-2 probes, Anasys Instruments, Santa Barbara, CA) were used. The scan area for all AFM images was 50 μm x 50 μm with a resolution of 512 pixels and a scan rate of 0.5 Hz. The nanoprobes were heated from room temperature to 200 °C at a heating rate of 10 °C/s. Tapping mode was

operated by oscillating the probe at its resonant frequency and scanning with the same parameters as mentioned previously; however, the probes were held at constant temperature during the run using the nano TA2 controller.

3 Chapter 3 The Potential Use of Zein as a Carrier in a Matrix Drug Delivery Device Prepared Using Extrusion/Spheronization

3.1 Introduction

The overall aim of this project is to investigate the potential utilization of zein biopolymers to control the rate of drug release from pharmaceutical formulations. Pellets (uniformly-sized spherical multiparticulates) produced by extrusion/spheronization are the main drug delivery devices used in this section of the study. Extrusion/spheronization is a multi-step process used to produce pellets or spheres (Erkoboni, 2003; Summers et al., 2007). This process involves dry mixing of powders into a homogenous dispersion, which is then wet granulated to produce a sufficiently plastic wet mass. The wet mass is then extruded to form rod-shaped particles of uniform diameter that will be spheronized and rounded off into spheres. The spheres are subsequently dried to achieve the desired moisture content and may then be screened to attain a selected size distribution. Pellets provide some advantages over larger solid dosage forms: they may improve the bioavailability of the drug, decrease the local irritation of the GIT (Thommes et al., 2007), reduce the risk of dose dumping, result in less variable transit times through the GIT and offer a way to separate incompatible drugs by mixing different types of pellets in the same macroscopic dosage form (Lustig-Gustafsson et al., 1999). In addition, they can be utilised to give a constant level of drug release through a mix of immediate release pellet fractions and sustained release pellet fractions (Zhang et al., 2009). Mixing different types of pellets can also provide an alternative option if blending polymers within same pellet matrix or film coating is not possible.

The objective of this chapter is to prepare pellets using extrusion/spheronization with zein proteins and to study the drug release characteristics of these devices. The chapter presents the pellet production and characterization of zein- and non-zein containing pellets and discusses the dissolution test results of such dosage form in different media, along with the various attempts that were carried out to manipulate the drug release.

3.2 Materials

Zein and α-lactose monohydrate (Lact) were purchased from Sigma-Aldrich (USA). Microcrystalline cellulose (MCC) was obtained from FMC BioPolymer (Brussels Belgium, and distributed by IMCD UK limited). Caffeine (CAFF) was obtained from Acros Organics (USA). Paracetamol (PCT) was purchased from Alfa Aesar (UK). Hydrochloric acid, potassium dihdrogen phosphate and sodium hydroxide were obtained from Sigma Aldrich, AnalaR and Fisher Scientific, respectively. All materials were used as received.

3.3 Methods

This section briefly describes the methods involved in preparation of pellets and determination of pellet properties such as size and size distribution, shape, densities and flowability, friability and drug release. A more detailed description of these methods is provided in Chapter 2.

3.3.1 Preparation of Pellets

Pellets were prepared using zein, MCC (Avicel PH 301 grade) and α-lactose monohydrate. The drug (caffeine or paracetamol) concentration in all the formulations was kept constant, unless otherwise specified, at 10% w/w on a dry basis. The amount of granulation fluid (water unless otherwise specified) used in the formulations was variable. In Section 3.4.3.4.3, the required amount of paracetamol was dissolved at room temperature in a solution of zein, prepared by dissolving the required amount of zein in 60% aqueous ethanol at 50 ˚C and plasticized with 30% w/w (based on the amount of zein) propylene glycol, and the resulting solution was used as the granulation fluid. Powders were dry mixed and wet granulated in a mini food chopper (Kenwood Limited, UK). The wet mass was extruded through a Caleva single screw extruder (Caleva, Sturminster Newton, UK), fitted with a die of 1.0 mm diameter, at a speed of 100 rpm. Fifty grams (50 g) of the resulting extrudates were spheronized for 20 to 35 minutes at speed of 6 (on a scale of 0 to 9) on a 120 mm diameter cross-hatched plate spheronizer (Caleva, Sturminster Newton, UK). The pellets were oven dried at 50 to 60 ˚C for 24 hours.

Two types of pellets were prepared: one consists of MCC, α-lactose monohydrate and a model drug (control pellets). Alpha-lactose monohydrate was substituted by zein in the

second type of pellets. The two types were referred to in the text as MCC-Lact-(CAFF or PCT) and MCC-Zein-(CAFF or PCT), respectively. Table 3.1 presents the different zein- and α-lactose monohydrate-based formulations prepared as part of this work.

Table 3.1 A list of the prepared zein- and α-lactose monohydrate-based formulations.

Formulation	MCC	Zein	α-Lactose monohydrate	Caffeine	Paracetamol
1	75%	15%		10%	
2	75%		15%	10%	
3	75%	15%			10%
4	65%	25%		10%	
5	65%		25%	10%	
6	55%	35%		10%	
7	55%	35%			10%
8	30%	65%		5%	

3.3.2 Size and Shape Analysis

Size analysis of the pellets was performed using sieve analysis. A sieve shaker (Endecotts EFL2000, UK) was used for 10 minutes to separate the pellets into various size fractions. The percentage of weight retained on each sieve was calculated and cumulative percentage undersize curve was obtained, from which the median (50^{th} percentile) and the interquartile range (difference between 75^{th} and 25^{th} percentiles) of pellet diameter was determined. The median and interquartile range were employed to characterize the pellet size and size distribution, respectively. The fraction between 710 and 1400 μm, based on the criteria of Baert et al. (1992), was denoted as the yield and was expressed as a percentage of the total weight of pellets used for sieving.

The shape of pellets was determined by the roundness of the spheres (E value). Images of pellets of a 710 to 1000 μm sieve fraction were taken using an optical microscope (Leica DM LS2, Leica Microsystems UK Ltd) connected to a digital camera (JVC colour video camera, TK-C1481BEG, Victor Company of Japan Limited, Thailand). The largest (R_1)

and the smallest (R_2) diameters of 20 randomly selected individual spheres of each batch were determined. For each sphere the E (= R_1/R_2) value was calculated and an average E value per batch was then determined.

3.3.3 Densities and Friability

Densities (bulk and tapped) were determined using a graduated cylinder (Jolting volumeter tapped density powder tester, SMI-LabHut Ltd, UK). The Hausner ratio and Carr's compressibility index were also calculated. Friability was measured using an Erweka TAR friabilator (Erweka GmbH, Germany).

3.3.4 Fracture Force (F)

The mechanical characteristics of pellets were investigated using a texture analyser (TA.XT Plus Texture Analyser, Stable Micro Systems Ltd, UK). The fracture force (*F*) of 10 pellets per batch of 710 to 1000 μm sieve fraction at a loading rate of 0.1 mm/s was determined by measuring the maximum force required to break the pellets. The latter value was extracted from the force-displacement profile recorded by the instrument. The tensile strength (σ) was calculated according to Eq. (3.1) (Thommes et al., 2007):

$$\sigma = \frac{1.6 * F}{\pi * d^2} \qquad \text{Eq. (3.1)}$$

where *d* is the diameter of pellet in the crushing direction.

3.3.5 Dissolution Studies

A BP Apparatus I (D8000, Copley Scientific Ltd., Nottingham, UK) was used for all dissolution tests. Tests were performed in 900 mL of distilled water, 0.1 M HCl or phosphate buffer (pH 6.8) at 37.0 ± 0.5 ˚C, with a rotation speed of 50 ± 1 rpm. Fifty milligrams (50 mg) of pellets of 710 to 1000 μm sieve fraction were used for the drug release studies. A constant sieve fraction of the pellets was used for all batches so as to minimize the influence of the change in surface area of the pellets on the dissolution rate and hence to facilitate the comparison of dissolution profiles. At predetermined sampling

points, 10 mL sample was collected from each vessel using a syringe, filtered through a 0.2 μm membrane filter and replaced with an equivalent volume of fresh medium. Measurements of drug release were made using a UV-VIS spectrophotometer (Perkin-Elmer Lambda XLS, USA). The wavelength of maximum absorbance (λ_{max}) was 243 nm and 270 nm for paracetamol and caffeine, respectively. A placebo (no drug) batch of zein-based pellets of 710 to 1000 μm sieve fraction was produced and tested in the aforementioned dissolution media under the same conditions. The UV-visible spectra of samples withdrawn upon testing the placebo pellets showed negligible absorbance over the wavelength of maximum absorbance of both drugs under study. The dissolution studies were conducted in triplicate under sink conditions and the average drug release ± SD was calculated. The end point was taken as 100% drug release and earlier time points were scaled to that for easier inter batch comparability. The results are expressed as cumulative percentage drug release versus time profiles.

Dissolution profiles were compared over the first 30 minutes (first 3 points) of drug release because maximal drug release had been obtained at this point, based on a model independent approach that uses a similarity factor (f_2) to compare the dissolution profiles of immediate release solid oral dosage forms. The similarity factor (f_2) is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percent (%) dissolution between the two curves (Guidance for Industry, 1997). The similarity factor (f_2) is calculated according to Eq. (3.2):

$$f_2 = 50 \bullet \log \{[1 + (1/n) \Sigma_{t=1}^{n} (R_t - T_t)^2]^{-0.5} \bullet 100\} \qquad \text{Eq. (3.2)}$$

where n is the number of time points, R_t is the dissolution value of a reference batch at time t, and T_t is the dissolution value of a second batch at time t. Generally, f_2 values greater than 50 (i.e. 50-100) are taken to mean the sameness or equivalence of the two dissolution curves and, thus, of the performance of the test and reference products. The f_2 equation is most commonly used to compare products before and after minor changes in formulation and/or processing, but using the same dissolution testing method; here, it has been used to assess differences in the profiles acquired from the same batch but under different testing conditions or from different batches under the same testing conditions.

3.3.6 Swelling Test

The diameter of ten individual pellets randomly selected from the sample used in the dissolution test was measured via microscopic imaging and averaged to obtain the mean diameter before dissolution. The mean diameter after dissolution was measured in the same way. The swelling % was calculated according to the following equation, Eq. (3.3):

$$Swelling\ \% = ((d2 - d1)/d1) * 100\% \qquad \text{Eq. (3.3)}$$

where d_1 is the mean diameter before the dissolution test and d_2 is the mean diameter after dissolution.

3.4 Results and Discussion

3.4.1 Mechanism of Pellet Formation

Baert et al. (1993a) suggested that spheronization starts with rope-folding of the extrudates followed by twisting and formation of dumb-bell shaped units which finally break into two parts; each has a round and a flat side, as shown in Figure 3.1. The frictional forces cause the edges of the flat side to fold together like a flower forming the cavity observed in certain pellets. This mechanism suggests that some type of folding might occur during spheronization resulting in formation of a central cavity.

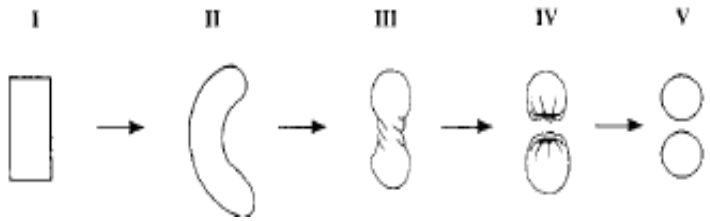


Figure 3.1 A model of sphere forming mechanism using plasticine as a model compound: (I) extrudates (starting material); (II) rope; (III) dumb-bell; (IV) sphere with a cavity outside; (V) sphere (Baert et al., 1993a).

A quite different mechanism to that described in the work of Baert et al. (1993a) was observed during the spheronization step of this part of the study. Nevertheless, some type of folding might have also occurred during pellet formation. The suggested mechanism starts with the folding of rod-shaped extrudates and formation of doughnut-shaped units

which ultimately formed spheres when subjected to higher attrition and frictional forces on the spheronizer plate, Figure 3.2 (a to c). A cavity was observed in the cross-section of these pellets (Figure 3.2, d). It is worth noting that this mechanism and the formation of cavities were observed in both pellet types i.e. MCC-Lact-Drug and MCC-Zein-Drug pellets.

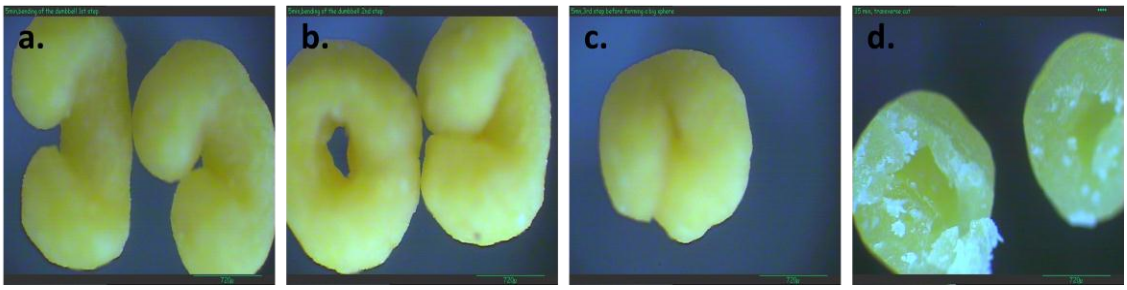


Figure 3.2 Microscopic images (4x) that represent the suggested mechanism of sphere formation during the spheronization step. The mechanism starts with: (a) partially folded extrudates, (b) doughnut-shaped units, (c) sphere, and (d) cross-section of a pellet showing a cavity.

3.4.2 Characterization of Pellets

Pellets were characterized based on the work of Baert et al. (1992). The spheres were evaluated based on the following criteria: yield of spheres between 710 and 1400 µm and roundness of the spheres (represented by E value). Spheres were considered of acceptable quality if ≥ 90% of the spheres had a particle size between 710 and 1400 µm, a friability value lower than 0.2%, and an E value between 1 and 1.20. The characterization tests are described in detail in sections 3.3.2 to 3.3.4. The results of these tests are shown in sections 3.4.2.1 to 3.4.2.3. Only selected results of certain batches are shown here to avoid repetition.

3.4.2.1 Size and Shape Analysis

Table 3.2 presents the size and shape analysis of pellets obtained from different formulations (formulations 1, 2, 4, 5 and 6 in Table 3.1). The size and size distribution of the pellets obtained from different formulations expressed as median diameter and interquartile range, respectively, were similar, and the interquartile range values suggested a narrow particle size distribution. The cumulative percent undersize distributions (data not shown) were comparable for all the formulations, indicating essentially similar spread or

range of diameters. The median pellet diameter was less than 1.0 mm (i.e. the die diameter) in all cases, which can be explained by the shrinkage of pellets during drying. A similar observation was reported by Bashaiwoldu et al. (2004). A spherical shape is the most characteristic property of pellets and is evaluated by E value. The shape of the zein and lactose-based pellets as described by their E values was sufficiently round, as the E values were between 1 and 1.2. E values of all formulations were essentially the same. The yield of pellets between 710 and 1400 μm of all the formulations was more than 90%. Accordingly, the selected batches were found to comply with the criteria of Baert et al. (1992).

Table 3.2 Size and shape analysis of pellets obtained from different formulations.

Formulation	Median Diameter (mm)	Interquartile Range (mm)	Yield between 710 and 1400 μm (%)	E value ± SD (n = 20)
MCC-Lact (15%)-CAFF	0.89	0.18	90.40	1.10 ± 0.04
MCC-Lact (25%)-CAFF	0.88	0.19	98.00	1.14 ± 0.05
MCC-Zein (15%)-CAFF	0.84	0.14	96.20	1.14 ± 0.07
MCC-Zein (25%)-CAFF	0.86	0.17	97.90	1.18 ± 0.08
MCC-Zein (35%)-CAFF	0.94	0.30	95.40	1.13 ± 0.05

Figure 3.3 shows some microscopic images of pellets obtained from the different formulations listed in Table 3.2. The images suggest that the pellets are not perfectly spherical and their contour is slightly irregular.

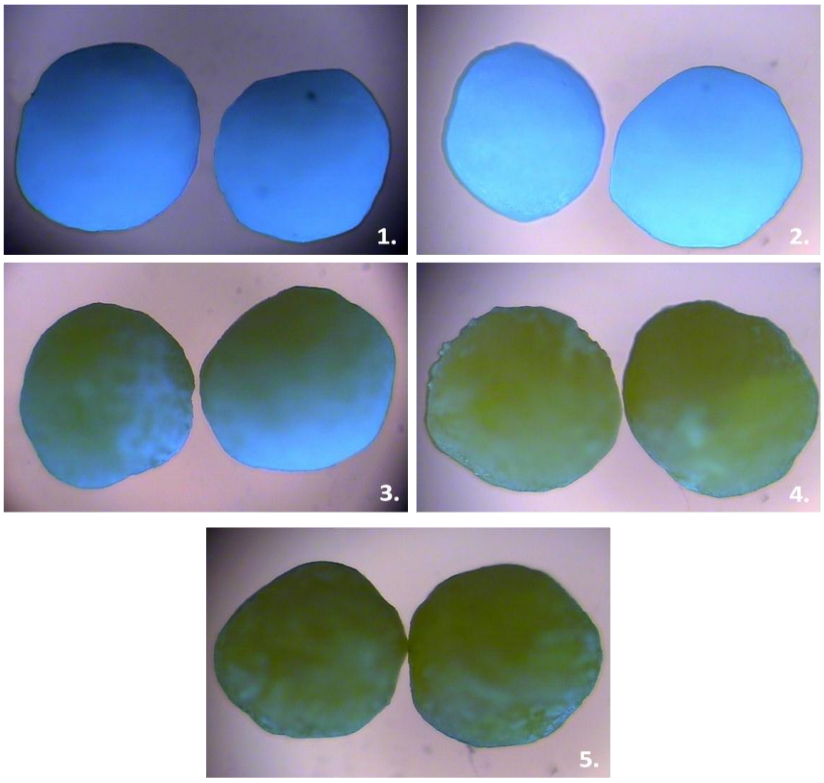


Figure 3.3 Microscopic images (4x) of all formulations: (1) MCC-Lact (15%)-CAFF, (2) MCC-Lact (25%)-CAFF, (3) MCC-Zein (15%)-CAFF, (4) MCC-Zein (25%)-CAFF and (5) MCC-Zein (35%)-CAFF.

3.4.2.2 Densities and Friability

The densities (bulk and tapped density), the Hausner ratio and Carr's compressibility index of pellets obtained from different formulations are shown in Table 3.3. Little difference in densities was found among pellets obtained from different formulations (formulations 1, 2, 4, 5 and 6 in Table 3.1). These results can be possibly explained by the similar size and size distribution and thus the similar packing properties of the pellets obtained from different formulations (Table 3.2). The Hausner ratio and Carr's compressibility index were almost identical for all the batches. These values suggested that these pellets are free flowing with low interparticulate friction. Friability values could not be determined due to the generation of electrostatic forces and the loss of some pellets upon emptying the friability disc.

Table 3.3 Densities, the Hausner ratio and Carr's compressibility index of pellets obtained from different batches.

Formulation	Bulk Density (g/mL)	Tapped Density (g/mL)	Hausner Ratio	Carr's compressibility index
MCC-Lact (15%)-CAFF	0.80	0.85	1.06	5.88
MCC-Lact (25%)-CAFF	0.76	0.79	1.04	4.04
MCC-Zein (15%)-CAFF	0.69	0.74	1.07	6.76
MCC-Zein (25%)-CAFF	0.78	0.83	1.06	6.02
MCC-Zein (35%)-CAFF	0.77	0.82	1.06	6.10

3.4.2.3 Fracture Force (F)

Pellets are required to possess a high mechanical stability in order to withstand further processing and handling steps such as drying, coating and tableting. The mechanical properties of pellets were determined by the fracture force (F) and tensile strength (σ). The diameter of 10 individual pellets per batch was measured by a micrometer (Outside micrometer, 0-25 mm) and the corresponding fracture force of these pellets was measured. The tensile strength was subsequently calculated according to Eq. (3.1). Pellets were randomly selected from the MCC-zein (1:0.5) batch with no loaded drug and compared to those selected from the MCC-Lact (1:0.5) batch with no loaded drug. Zein-based pellets had an average diameter of 1.22 ± 0.1 mm, an average fracture force of 29.8 ± 8.8 N and an average tensile strength of 10.2 ± 2.8 MPa, whereas lactose-based pellets had an average diameter of 1.02 ± 0.06 mm, an average fracture force of 18.04 ± 3.6 N and an average tensile strength of 8.9 ± 1.9 MPa. The fracture force and tensile strength of zein-based pellets show a trend of being higher than those of lactose-based pellets, i.e. greater force is needed to break zein-based pellets than that needed to break the lactose-based pellets. This result suggests that zein-based pellets exhibit higher mechanical strength and stability. The difference in the tensile strength values of zein-based and lactose-based pellets, unlike the difference in the fracture force values, was insignificant ($p > 0.05$). This could possibly be related to the larger mean diameter of zein-based pellets which might have counterbalanced the higher values of the fracture force according to Eq. (3.1).

Fracture force values were, however, variable within a batch and force-displacement curves on individual pellets showed different patterns. Figure 3.4 shows the force-displacement curves of two different pellets of MCC-zein (1:0.5) batch with no loaded drug using the same machine settings. The graphs reflected different displacement patterns suggesting that the probe came into contact with two different surfaces in the second pellet (blue line). This result can be linked to the previously observed cavity in the pellet structure (Section 3.4.1, Figure 3.2; d). It may be explained by the probe contacting the pellet's outside surface first, subsequently coming into contact with the air in the cavity within the pellet, then meeting the internal surface at the bottom of the cavity. Thus, the presence of such cavities might have led to these inconsistent displacement patterns. The red line would represent the probe penetrating a solid pellet, i.e. one without an internal cavity.

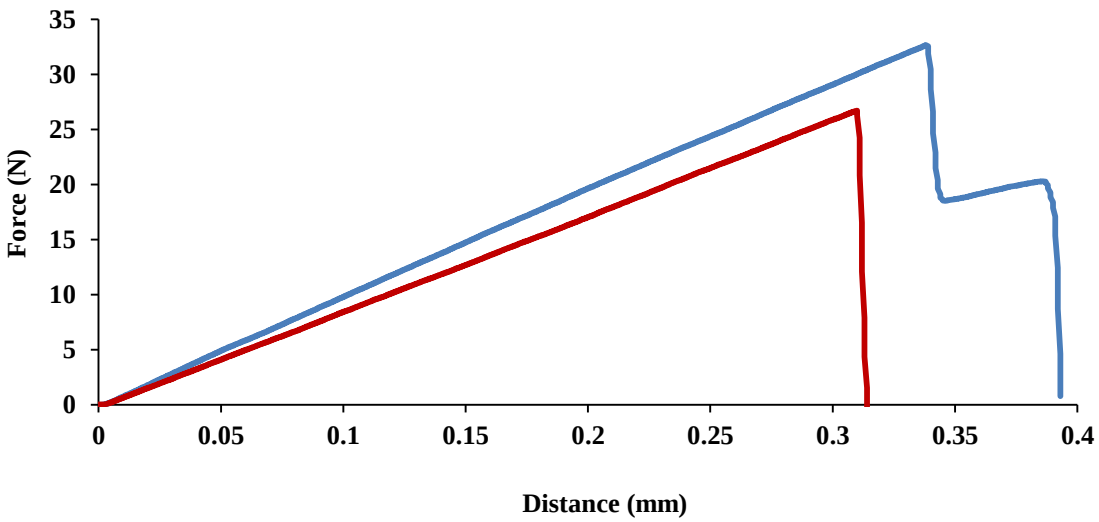


Figure 3.4 Two examples of force-displacement curves of unloaded (no drug) MCC-zein (1:0.5) pellets as obtained via a compression test performed using TA.XT Plus texture analyser.

3.4.3 Dissolution Results

The study of the release characteristics of drug from pellets made of zein via extrusion/spheronization is essential to define the ability of these hydrophobic proteins to control the release of the drug into the external medium. Sections 3.4.3.1 to 3.4.3.4 present the dissolution studies carried out on zein- and lactose-based (control) pellets.

3.4.3.1 Influence of Zein/MCC ratio on Drug Release from Pellet Matrix and on Swelling of Pellets

Figure 3.5 presents the dissolution profiles of MCC-Lact (15%)-CAFF (control experiment) and MCC-Zein (15%)-CAFF pellets in distilled water. Figure 3.6 presents the dissolution profiles of the same formulations in 0.1 M HCl. Zein-based and lactose-based pellets both showed characteristics of immediate release profiles in distilled water, essentially releasing all the drug within 30 minutes. However, the drug release from zein-based spheres was lower than that released from the lactose-based pellets over the first 30 minutes with the f_2 value being less than 50% (f_2 = 40%). The same was seen in 0.1 M HCl (f_2 = 41%).

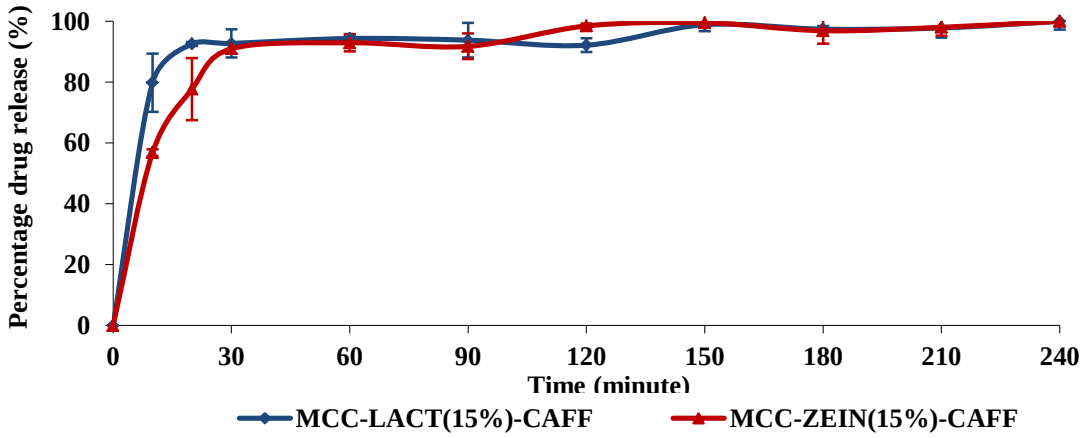


Figure 3.5 Dissolution profiles of different pellet cores: MCC-Lact (15%)-CAFF and MCC-Zein (15%)-CAFF, in distilled water. Each point represents the mean ± SD (n = 3).

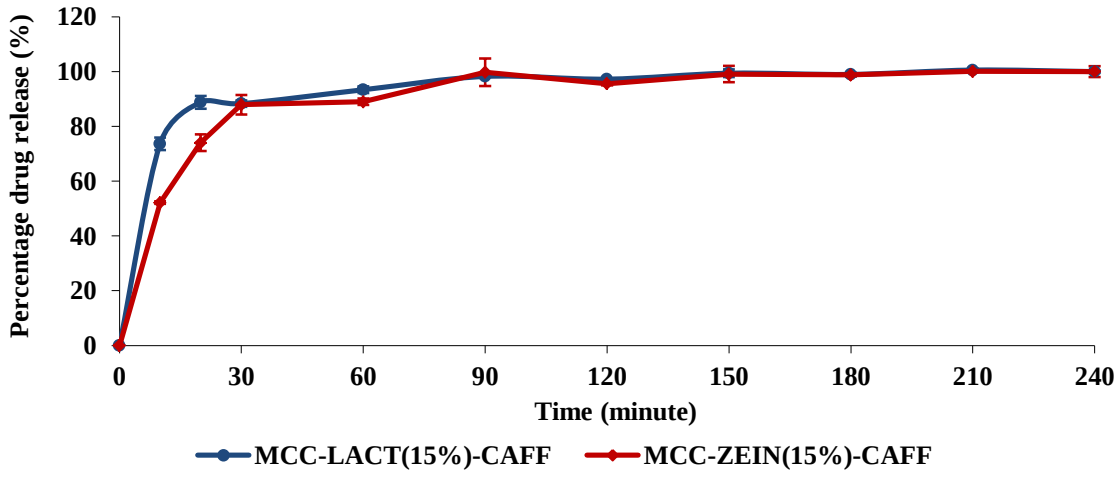


Figure 3.6 Dissolution profiles of different pellet cores: MCC-Lact (15%)-CAFF and MCC-Zein (15%)-CAFF, in 0.1 M HCl. Each point represents the mean ± SD (n = 3).

Figure 3.7 compares the dissolution profiles of MCC-Zein (15%)-CAFF pellets in distilled water and 0.1 M HCl. The drug release from this formulation was essentially the same in both media with the f_2 value being greater than 50% (f_2 = 70%). Caffeine was quickly released from zein-based pellets in both media, with circa 88% being released over the first 30 minutes. However, it can be noticed that the release rate slowed down after the first 10 minutes as indicated by the slope of the two curves. This release pattern can be explained by the faster release of the drug molecules existing at or near the surface of the pellet followed by the longer diffusion route of the drug molecules entrapped deeper in the zein matrix.

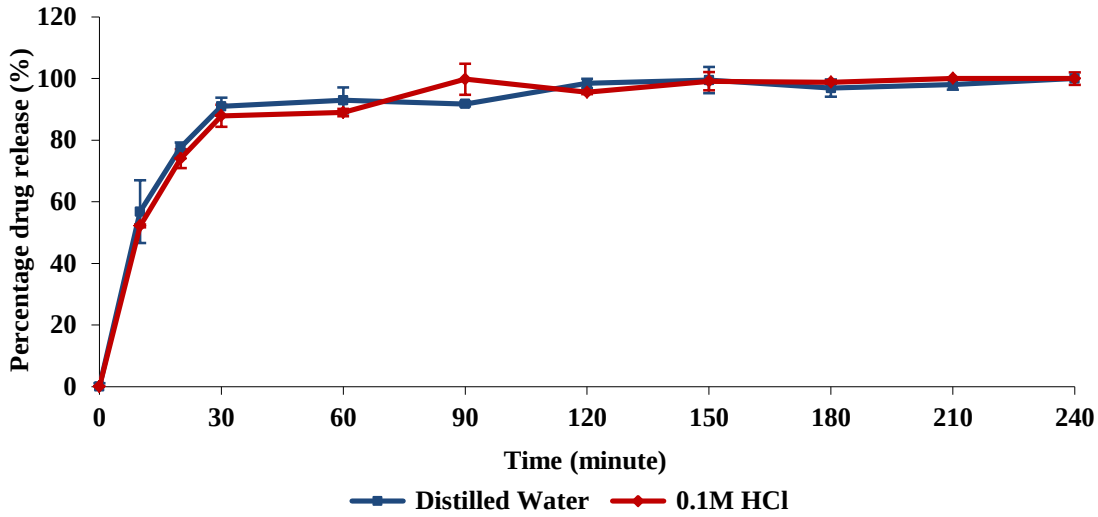


Figure 3.7 Dissolution profiles of MCC-Zein (15%)-CAFF pellets in distilled water and 0.1 M HCl. Each point represents the mean ± SD (n = 3).

The influence of increasing the zein level in pellet cores was investigated and is shown in Figure 3.8 and Figure 3.9. Figure 3.8 shows the drug release profile of MCC-Lact (25%)-CAFF (control experiment) and MCC-Zein (25%)-CAFF in 0.1 M HCl. Lactose-based pellets showed faster drug release than the zein-based pellets up to 60 minutes, with the f_2 being less than 50% (f_2 = 28%), however, at this point (60 minutes) essentially all the of the drug has been released from both formulations.

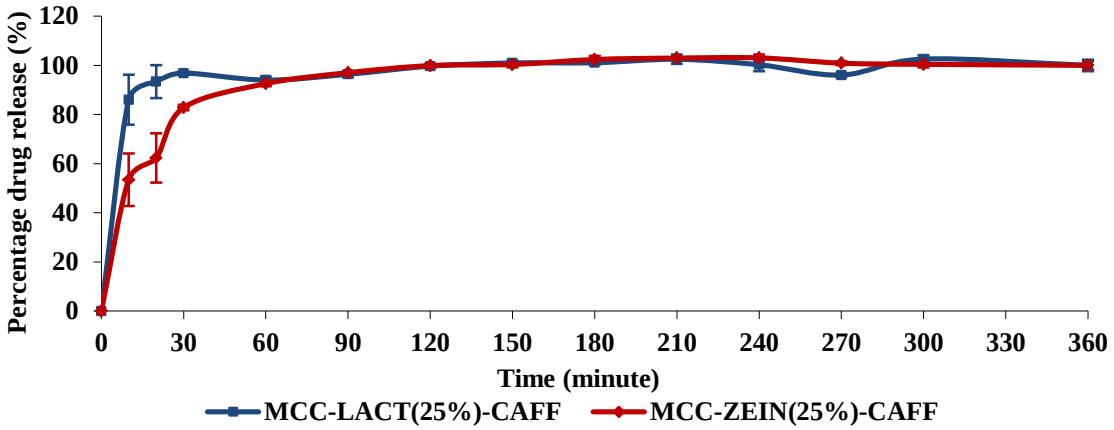


Figure 3.8 Dissolution profiles of different pellet cores: MCC-Lact (25%)-CAFF and MCC-Zein (25%)-CAFF, in 0.1 M HCl. Each point represents the mean ± SD (n = 3).

The drug release data for these two formulations were fitted to various kinetic models such as zero order, first order and Higuchi model (Higuchi, 1963) and the quality of fit was assessed on the basis of correlation coefficient (r^2). The data could not however be fitted to the Korsmeyer-Peppas model (Korsmeyer et al., 1983) due to insufficient time points where the drug release is ≤ 60%. The drug release from MCC-Zein (25%)-CAFF pellets exhibited the best linearity when fitted to the first-order equation (r^2 = 0.9792), whereas an r^2 value of 0.8029 was obtained when the data was fitted to the Higuchi model which describes a linear relationship between the drug release and the square root of time based on Fickian diffusion process. On the other hand, the drug release from MCC-Lact (25%)-CAFF pellets in comparison with that from the corresponding zein-based formulation showed a poor fit to all the models. These results suggest different release kinetics between the two formulations and can be possibly explained by the fact that zein-based pellets remained intact during the test duration whereas lactose-based pellets completely disintegrate, so the controlled-release model are not appropriate here.

Figure 3.9 compares the release of caffeine from pellets containing 15%, 25%, and 35% zein in 0.1 M HCl. The data show a trend of decreasing the drug release with increasing zein content in the first 30 minutes. The value of f_2 obtained upon comparing pellets containing either 25% or 35% zein against pellets of the lowest zein content, i.e. 15% zein content, was greater than 50% (f_2 = 56% and 52%, respectively). The values of the similarity factor (f_2) suggest that the drug release from all the formulations is essentially the same, indicating that increasing the level of zein polymer within the pellets matrix did not result in an actual decrease in drug release rate and/or extent.

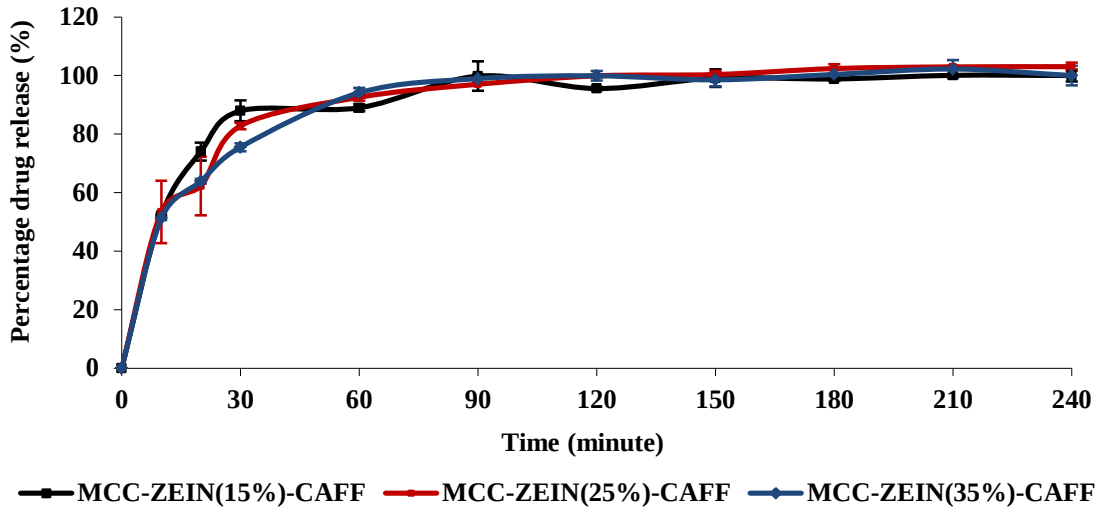


Figure 3.9 Dissolution profiles of different pellet cores: MCC-Zein (15%)-CAFF, MCC-Zein (25%)-CAFF, and MCC-Zein (35%)-CAFF in 0.1 M HCl. Each point represents the mean ± SD (n = 3).

Although the drug release from zein-based pellets reached a plateau within circa 1 hour, the pellets had not disintegrated at the end of the 4 hours dissolution test. However, zein pellets did swell and crack to some extent, as shown in Figure 3.10. Cracking can be related to the presence of MCC, an excipient that swells and possesses some disintegration properties. On the other hand, lactose-based pellets became soft and formed a paste at the end of the test. Comparing the behaviour of the two pellet types at the end of dissolution test highlights the importance of zein as a water-insoluble polymer in maintaining the integrity of the matrix.

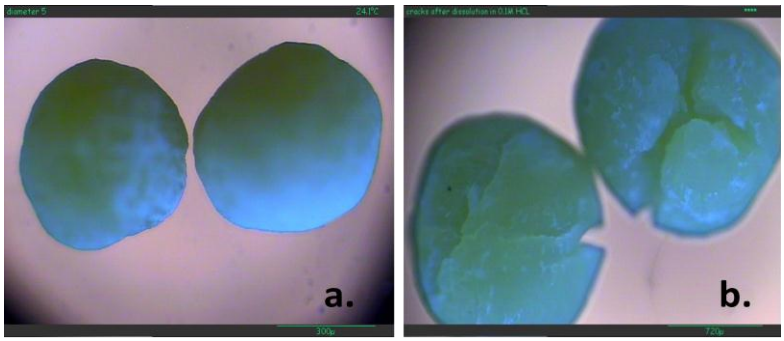


Figure 3.10 An microscopic image (4x) of zein-containing pellets (MCC-Zein (25%)-CAFF: prior to (a) and after (b) the dissolution test in 0.1 M HCl.

Pellets with different zein:MCC ratios (0.2, 0.4, and 0.6) swelled to different extents (25, 11, and 13%, respectively). Figure 3.11 shows the influence of the zein:MCC ratio on the

swelling of the pellets at the end of 4-hour dissolution test in 0.1 M HCl. It can be noted that increasing the zein:MCC ratio from 0.2 to 0.4 resulted in circa 2-fold decrease in the swelling of pellets; however, no further decrease in swelling was associated with the higher zein:MCC ratio (i.e. 0.6). These results suggest that increasing zein level in a mixture of excipients (MCC and drug) helped in restraining the swelling and maintaining the integrity of the matrix, possibly due to the fact that zein is a water-insoluble and hydrophobic protein (Shukla et al., 2001) with low water uptake properties (Li et al., 2010). The drop in swelling might be also due to the lower amounts of MCC used at higher levels of zein to keep a constant total amount of excipients.

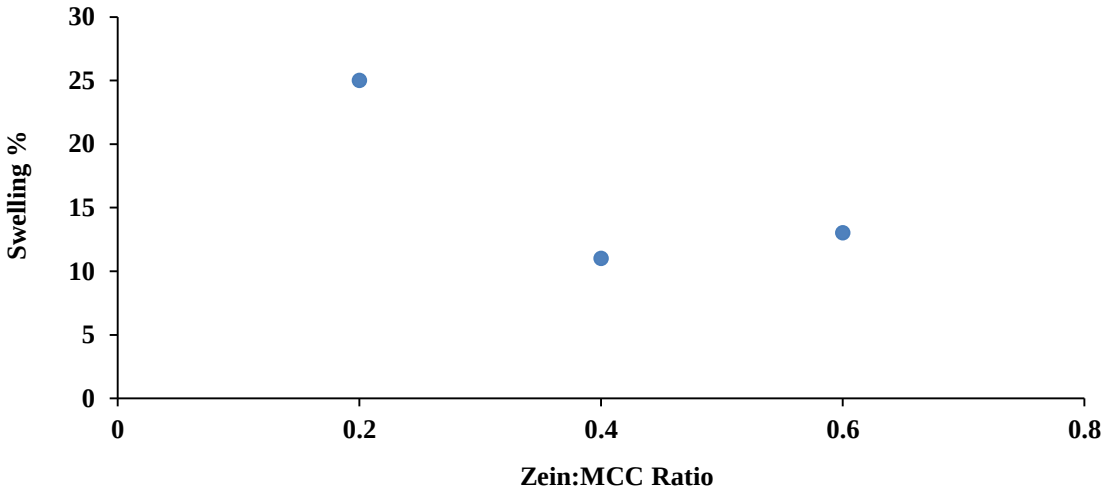


Figure 3.11 A plot showing the influence of zein:MCC ratio on the swelling % of the pellets at the end of 4-hours dissolution test in 0.1 M HCl.

3.4.3.2 Influence of pH of Dissolution Medium on Drug Release from Pellet Matrix

Figure 3.12 shows the influence of the pH value of the medium on the drug release from MCC-Zein (25%)-CAFF. Figure 3.13 similarly presents the drug release from MCC-Zein (35%)-CAFF pellets in 0.1 M HCl (pH 1) and phosphate buffer (pH 6.8). The drug release from MCC-Zein (25%)-CAFF pellets showed characteristics of immediate drug release profiles with circa 80% of the drug being released within the first 30 minutes in both 0.1 M HCl (pH 1) and phosphate buffer (pH 6.8). The drug release was essentially similar in both media, i.e. the f_2 value was greater than 50% (f_2 = 63%), indicating that the pH value of the release medium had no influence on the drug release from zein-based pellets. The same was seen when the MCC-Zein (35%)-CAFF pellets were tested (f_2 = 68%).

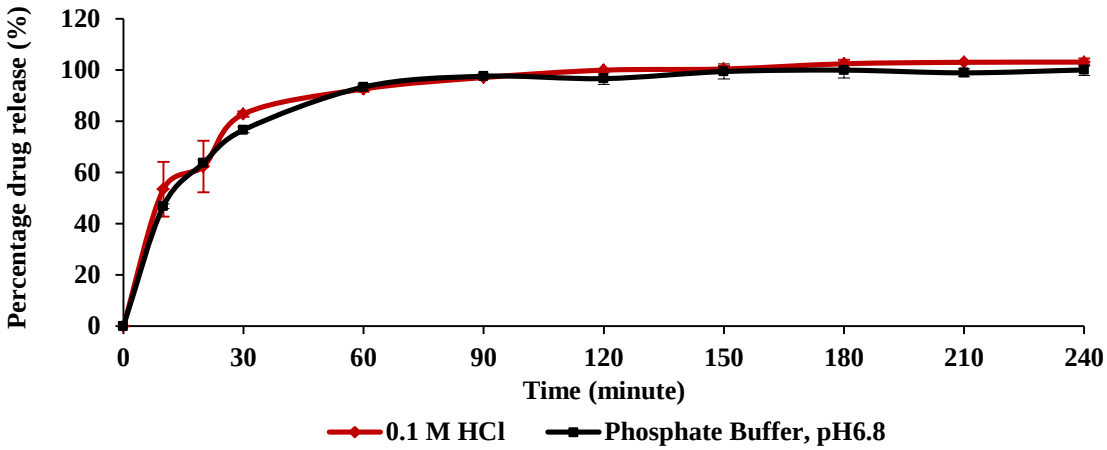


Figure 3.12 Dissolution profiles of MCC-Zein (25%)-CAFF pellets in 0.1 M HCl (pH 1) and phosphate buffer (pH 6.8). Each point represents the mean ± SD (n = 3).

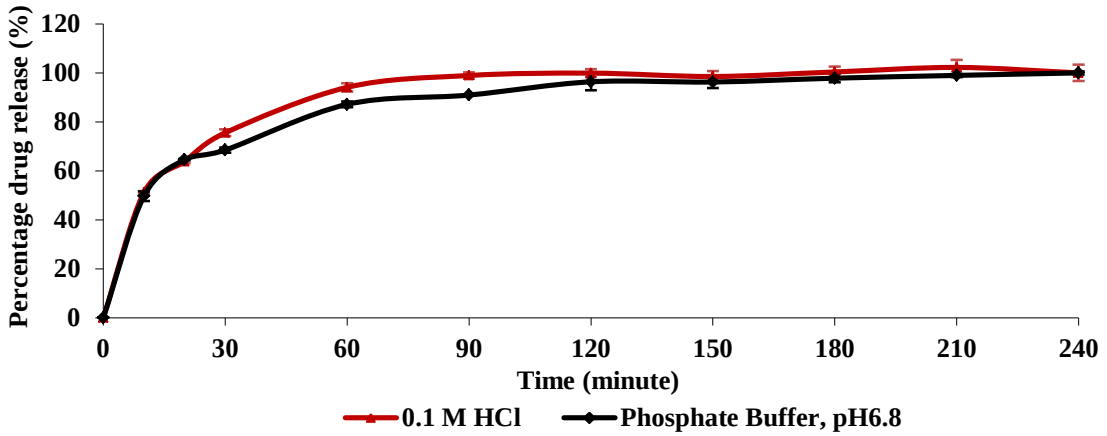


Figure 3.13 Dissolution profiles of MCC-Zein (35%)-CAFF pellets in 0.1 M HCl (pH 1) and phosphate buffer (pH 6.8). Each point represents the mean ± SD (n = 3).

3.4.3.3 Influence of Drug Type and Drug Migration on Release Rate

Drug migration was observed visually and thought to be the cause of the immediate drug release profile seen with the zein-based pellets. Optical microscopy was used to determine the extent of this phenomenon**.** Figure 3.14 shows microscopic images of oven-dried caffeine-loaded zein-based pellets at a magnification of 4x and 20x. The images showed white needle-shaped crystals on the yellow surface of zein-based pellets, which is consistent with caffeine's crystal habit.

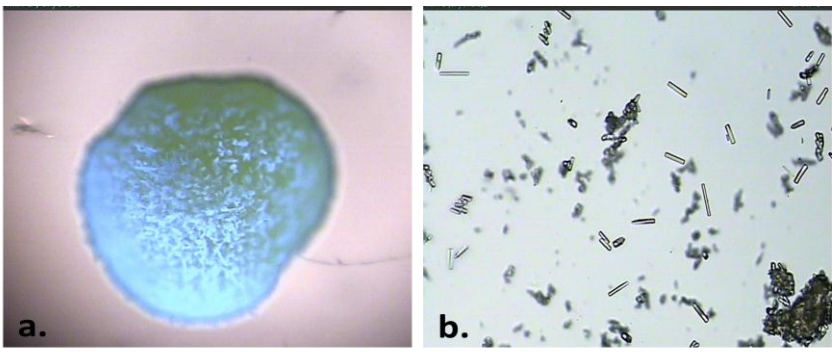


Figure 3.14 Optical microscopic images of caffeine-loaded zein-based pellets showing: a) white needle-shaped crystals on their surface (4x) and b) a sample of the crystals scraped from the surface of pellets (20x).

Therefore, caffeine (CAFF) was replaced with paracetamol (PCT) to check if the migration process was drug-dependent. Figure 3.15 shows some microscopic images of MCC-Zein (15%)-PCT (a) and MCC-Zein (15%)-CAFF (b) pellets surface showing migration and crystallization of soluble drugs during drying. On a visual basis, it can be concluded that migration of paracetamol to the surface of pellets was less pronounced than that of caffeine. The difference in partition coefficient (log P) values, -0.07 (Khan et al., 2005) and 0.89 (Isariebel et al., 2009) for caffeine and paracetamol, respectively, might explain the drug-related migration process. Due to the fact that caffeine is more hydrophilic than paracetamol, caffeine is expected to favour and partition into water within pellets and later would be carried up to the surface upon the drying step in oven.

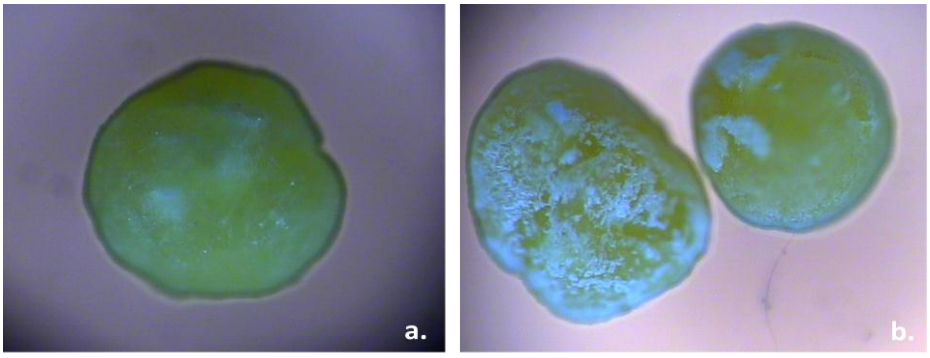


Figure 3.15 Microscopic images (4x) of MCC-Zein (15%)-PCT (a) and MCC-Zein (15%)-CAFF (b) pellet surface showing migration and crystallization of soluble drugs during drying.

Figure 3.16 represents the release of paracetamol from pellets containing 15 and 35% zein into 0.1 M HCl. Paracetamol showed an immediate-release profile from zein pellets, similar to that of caffeine from zein containing spheres. The value of the similarity factor (f_2 = 51%) suggests that the drug release from both formulations is essentially the same, indicating that increasing the level of zein polymer within the pellets matrix did not influence the rate and/or extent of drug release.

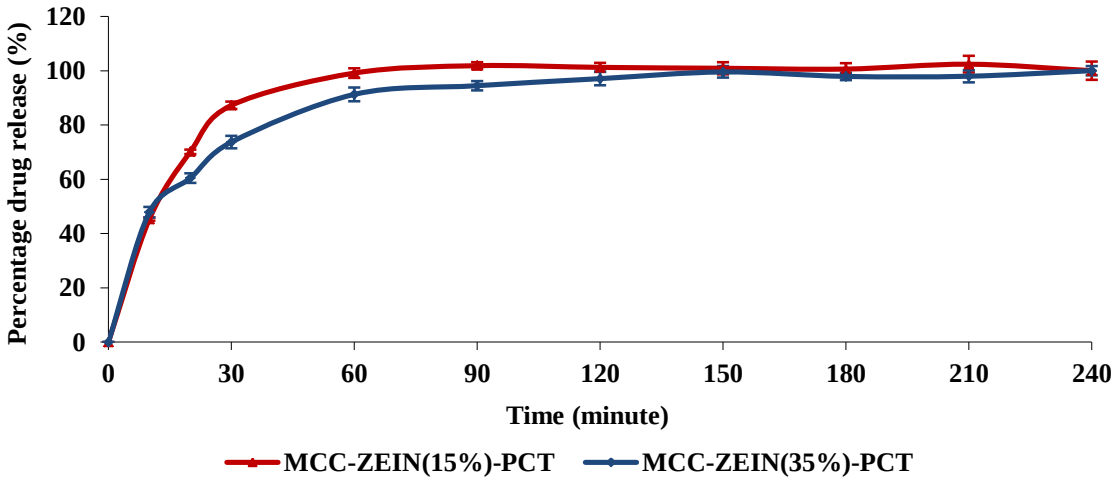


Figure 3.16 Dissolution profiles of MCC-Zein (15%)-PCT and MCC-Zein (35%)-PCT pellets in 0.1 M HCl. Each point represents the mean ± SD (n = 3).

Figure 3.17 and Figure 3.18 compare the release of paracetamol and caffeine from pellets containing 15% and 35% zein, respectively, in 0.1 M HCl. Both figures suggest that the release of caffeine and paracetamol from zein-based pellets at these two zein contents, i.e. 15% and 35%, was essentially the same in 0.1 M HCl, with the f_2 value being greater than 50% (f_2 = 66% and 75%, respectively). Although, the migration of paracetamol and caffeine was observed to different extents, the release rate of both drugs was comparable (f_2 > 50%), suggesting that drug migration is not the main cause of the fast drug release obtained in these dissolution studies.

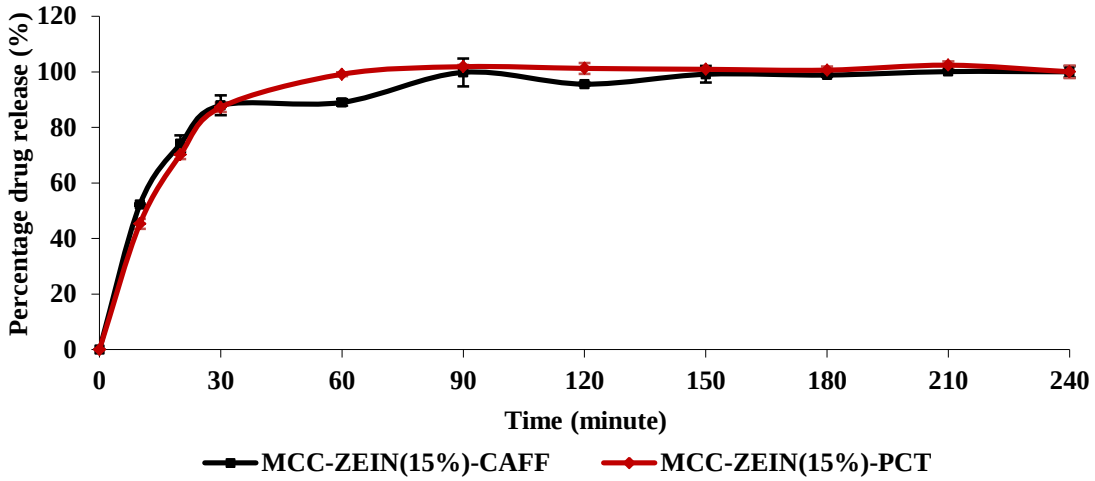


Figure 3.17 Dissolution profiles of MCC-Zein (15%)-CAFF and MCC-Zein (15%)-PCT pellets in 0.1 M HCl. Each point represents the mean ± SD (n = 3).

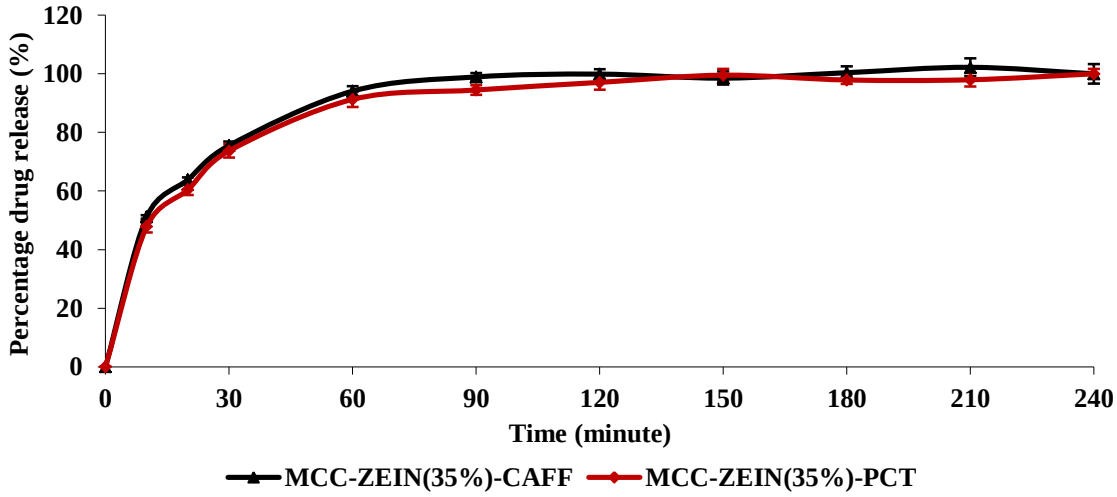


Figure 3.18 Dissolution profiles of MCC-Zein (35%)-CAFF and MCC-Zein (35%)-PCT pellets in 0.1 M HCl. Each point represents the mean ± SD (n = 3).

3.4.3.4 Attempts to Further Decrease Drug Release Rate from MCC/Zein pellets

3.4.3.4.1 Reduction of Zein Powder Particle Size by Grinding

Zein was ground using a mortar and pestle before being mixed with the other powders, in an attempt to enhance the contact between zein and the drug and hence to further control the drug release. Grinding of zein powder resulted in an approximately 13 times decrease in the mean particle size compared to the non-ground zein, as measured by laser diffraction, with the X_{50} values of unground and ground zein being 773.9 and 57.3 µm,

respectively. Figure 3.19 compares the drug release from MCC-ground Zein (35%)-CAFF and MCC-Zein (35%)-CAFF pellets in 0.1 M HCl. The drug release from both formulations was technically different (f_2 = 49%), suggesting that the grinding of zein did result in a reduction in the release of caffeine from zein-based pellets to a certain extent in 0.1 M HCl.

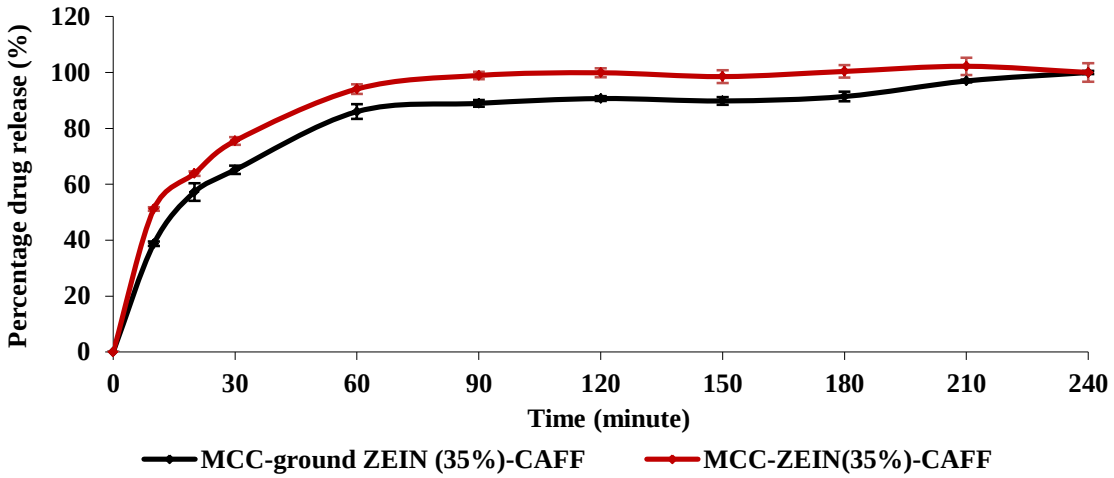


Figure 3.19 Dissolution profiles of MCC-ground Zein (35%)-CAFF and MCC-Zein (35%)-CAFF pellets in 0.1 M HCl. Each point represents the mean ± SD (n = 3).

3.4.3.4.2 Increasing Zein/MCC Ratio and Decreasing Drug Loading

Microcrystalline cellulose (MCC) is the excipient used in almost all the formulations processed via extrusion/spheronization, mostly at a level of 50%. In the previous experiments, a maximum of 35% zein content was used. Therefore, increasing the zein level up to 65% along with decreasing MCC and caffeine level down to 30% and 5%, respectively, was a reasonable step. Figure 3.20 compares the drug release from zein-based pellets of different zein contents, i.e. 15% 25%, 35% and 65%, loaded with 10% caffeine, with the exception of those pellets with the highest zein level (i.e. 65%) being loaded with 5% caffeine, in 0.1 M HCl. The figure shows a trend of decreasing the drug release over the first 60 minutes with increasing the zein content. As mentioned in Section 3.4.3.1, the value of f_2 obtained upon comparing pellets containing either 25% or 35% zein against pellets of the lowest zein content, i.e. 15% zein content, was greater than 50% indicating that the drug release from the aforementioned formulations is essentially similar, thus increasing the level of zein polymer within the pellets matrix did not result in an actual

decrease in the release rate and/or extent of the drug. However, increasing the level of zein from 15% to 65% and decreasing the drug loading from 10% to 5% resulted in a further decrease in the drug release and the dissolution profiles of MCC (30%)-Zein (65%)-CAFF (5%) and MCC (75%)-Zein (15%)-CAFF (10%) were essentially different with the f_2 value being less than 50% (f_2 = 38%).

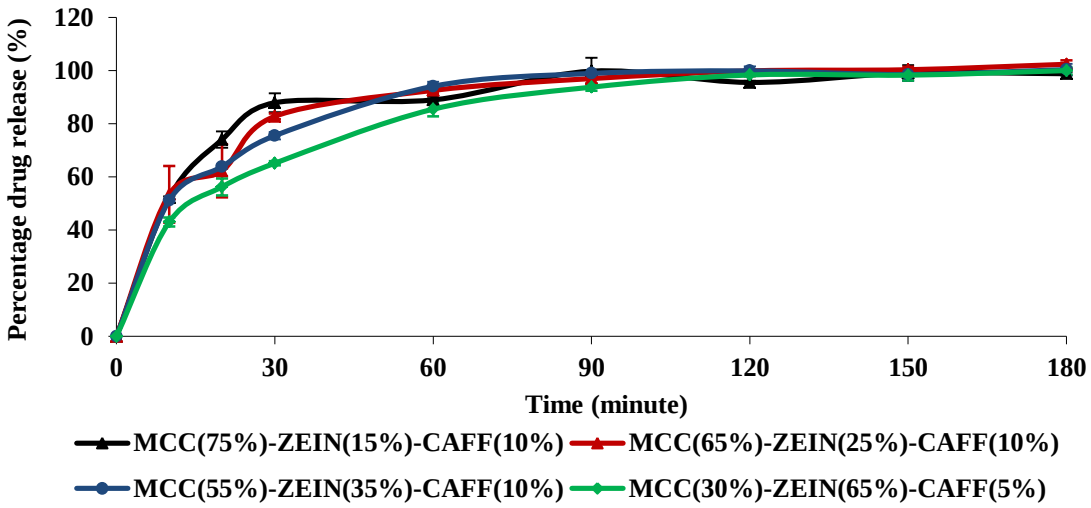


Figure 3.20 Dissolution profiles of MCC (75%)-Zein (15%)-CAFF (10%), MCC (65%)-Zein (25%)-CAFF (10%), MCC (65%)-Zein (25%)-CAFF (10%), MCC (55%)-Zein (35%)-CAFF (10%) and MCC (30%)-Zein (65%)-CAFF (5%) pellets in 0.1 M HCl. Each point represents the mean ± SD (n = 3).

As explained earlier, the slower release rate after the first sample time point, as indicated by the slope of the dissolution curve, probably represents the longer diffusion pathway of the more deeply entrapped drug molecules to the dissolution medium. Increasing the zein/MCC ratio would result firstly in an increase in the hydrophobic character of the pellet matrix and hence a delay of water penetration into the pellet structure and ultimately retardation in the diffusion of drug into the release medium. Secondly, less swelling and cracking would be expected at lower MCC content, hence maintaining the matrix integrity and consequently providing less surface area available for drug diffusion.

3.4.3.4.3 Changing the Granulation Solvent

Wet granulation involves the massing of a mix of dry primary powder particles using a granulating fluid, resulting in an initial particle aggregation (Summers et al., 2007). The granulation fluid consists of a solvent alone or, more usually, in combination with a

binding agent to ensure particle adhesion upon drying. The solvent must be volatile, in order to be removed by drying, and non-toxic, such as water, ethanol, and isopropanol either alone or in combination. Water is commonly used for economical and ecological reasons; however, organic solvents are used as an alternative to dry granulation in the case of water-sensitive drugs or when rapid drying is needed.

As mentioned above, the intimacy of contact between zein and drug may have an effect in terms of prolongation of drug release from zein-based pellets. To maximize the chance of intimate contact between zein and drug, the three components (paracetamol, zein and plasticizer) were all dissolved in a 60% aqueous ethanol and this combined solution was used to prepare pellets with MCC. Aqueous ethanol (60%) was used to minimize the reduction in pellet mechanical strength associated with the use of organic solvents. A plasticizer was added along with the polymer to help improve the ability of the latter to interact with hydrophilic excipients and hence further retard the drug release. This possible role of the plasticizer was suggested by Mulye et al. (1994). Accordingly, the addition of water to granulate a mixture of zein, MCC and paracetamol powders was compared to the wet granulation of MCC using a zein/drug solution (paracetamol was dissolved in a solution of zein, prepared in 60% aqueous ethanol and plasticized with 30% w/w propylene glycol based on zein amount, See Section 3.3.1). These pellets were more irregular in shape compared to water formulated pellets.

Figure 3.21 shows the influence of the granulation solvent on the drug release from MCC (75%)-Zein (15%)-PCT (10%) pellets in 0.1 M HCl. The drug release was slower from pellets prepared by wet granulation using water as the solvent, with an f_2 value of less than 50% (f_2 = 36%). This result suggests that changing the granulation solvent led to differences in the drug release from zein-based pellets which can be explained by the fact that the use of organic solvents negatively affects the hardness of pellets and increases their porosity (Baert et al., 1993a). Similarly, Millili et al. (1990) found that increasing the fraction of water in the granulating solvent results in more dense, less friable and less porous pellets, and postulated that the drug release is therefore slower due to the stronger bonding in water granulated pellets.

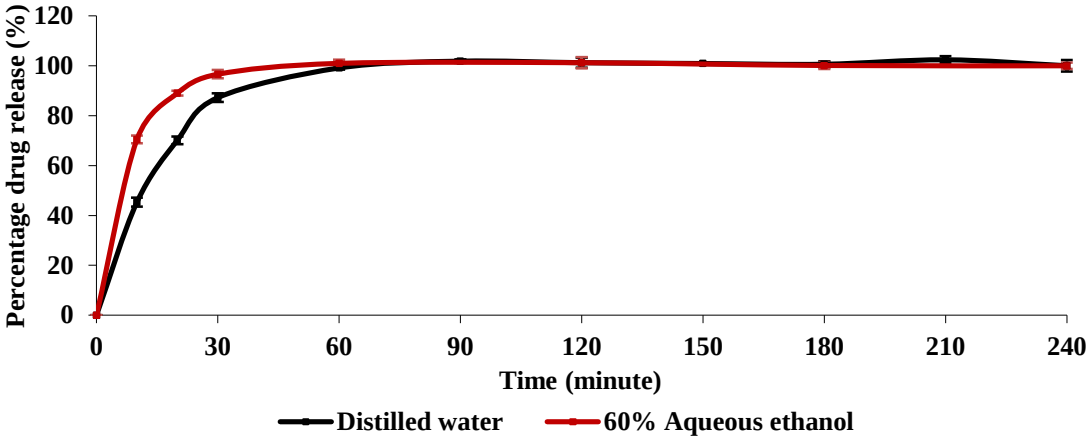


Figure 3.21 Dissolution profiles of MCC (75%)-Zein (15%)-PCT (10%) pellets using: distilled water and 60% aqueous ethanol as granulation fluid, in 0.1 M HCl. Each point represents the mean ± SD (n = 3). (Error bars are very small).

3.5 Discussion and Conclusion

Zein-based pellets were successfully produced and were of adequate physical and mechanical quality even at high zein content (as high as 65% w/w on a dry basis). A cavity was detected within the final pellet structure. It may be expected that the presence of such cavities would have an influence on drug distribution within the pellet and hence on the drug release. Zein pellets remained intact at the end of the dissolution tests, whereas lactose-based pellets had completely disintegrated. The rate and extent of drug release from zein-based pellets was essentially different (f_2 < 50%) from those obtained from the corresponding lactose-based (control) pellets over the first 30 minutes under the same testing conditions. Immediate drug release profiles were seen with the zein-based pellets. The drug release kinetics from zein-based pellets loaded with 10% caffeine seemed to be insensitive (f_2 > 50%) to the zein content in the concentration range studied, i.e. 15%, 25% and 35%. However, increasing the zein content up to 65% and reducing the drug loading to 5% resulted in a further decrease (f_2 < 50%) in the drug release as compared to pellets of 15% zein content. Reducing zein particle size by grinding did result in a reduction in the release of caffeine from zein-based pellets in 0.1 M HCl. The use of organic-based granulation fluid increased (f_2 < 50%) the total drug release from zein-based pellets as compared to those granulated using water as the granulation solvent. Drug migration to the surface of pellets occurred during the drying step and it was found to be drug dependent; i.e. the two drugs used in this work migrated to different extents. However, both drugs were released at comparable rates (f_2 > 50%) from zein-based pellets.

There were no literature reports on zein pellets at the time of this work, but data from zein-based tablets prepared by direct compression or wet granulation (Georget et al., 2008) suggested that zein can be successfully used as a matrix in monolithic controlled release tablets. Similarly, directly compressed tablets of spray-dried particles of a mixture of a model drug and zein exhibited a retarded drug release compared to the drug release from tablets prepared from the physical mixture (Katayama et al., 1992). Pure zein and zein blends such as zein:MCC or zein:starch were used for dry coating of tablets and the drug was released in zero-order kinetics from these systems (Guo et al., 2009). Lastly, the drug release from zein microspheres prepared by phase separation followed a sustained-release pattern when tested as free or tabletted microspheres (Liu et al., 2005), or as films (Wang et al., 2005). However, the results obtained in this work clearly disagree with the aforementioned reports in literature; the model drugs were immediately released from zein pellets prepared by extrusion/spheronization.

The immediate release profiles obtained with zein-based pellets prepared by extrusion/ spheronization might be related to the distribution of drug in the pellet matrix and the nature of molecular interaction. Unlike the aforementioned preparation methods based on solutions of zein and the drug, such as spray-drying and phase separation, extrusion/spheronization might have not offered such an intimate mixing between the zein polymer and the drug, leaving zein unable to retard the drug release. Furthermore, microcrystalline cellulose (MCC), the most important extrusion aid, is known to control the movement of water through the wet powder mass during extrusion (Kleinebudde, 1997), and to work as a molecular sponge for the added water forming a plastic mass (Law et al., 1998). Therefore, model drugs are expected to have favoured and partitioned into water and become entrapped within MCC resulting in poor mixing of the model drug and zein. Additionally, MCC, mostly used at a level of 50% (Lustig-Gustafsson et al., 1999), swells and possesses some disintegration properties which might have disrupted the integrity of the pellet structure providing larger surface area available for drug diffusion. Multiparticulates such as pellets are known to have a higher specific surface area in comparison with single unit dosage forms. The larger surface area available for drug release from pellets might therefore have contributed to the obtained fast drug release. No information is available on the quality of pellets surface and on pellets porosity which could also have influenced the drug release kinetics.

In conclusion, zein pellets of good quality were produced over a range of polymer content (15 to 65% w/w on a dry basis) suggesting that zein could be used to reduce the level of the commonly used MCC which has been reported not to be the excipient of choice in several cases such as chemical incompatibility (Dukic-Ott et al., 2009). Excipients intended for the production of pellets via extrusion/spheronization should exhibit large water absorption and retention capacity. Therefore, zein might not be able to completely replace MCC due to its low water uptake properties. The drug release from zein-based pellets followed first order kinetics but the release did not comply with the Biopharmaceutics Classification System (BCS) guidelines for immediate release products. At the same time, they showed no real evidence of a sustained-release profile. Taken along with the cracking issues, zein-based pellets were not considered for further development. It seemed that extrusion/ spheronization did not permit an intimate mixing of the drug and polymer to occur. Other reasons could be related to the high specific surface area provided by multiparticulates and to drug migration during the drying step. However, further studies would be necessary to fully understand the reasons for the fast release obtained with these devices and to possibly manipulate the formulation and/or process parameters that could ultimately retard the drug release. It might be interesting to investigate the use of zein pellets to enhance the drug release of poorly water-soluble drugs.

Alternatively, zein proteins were investigated for their potential use as polymeric coating materials. The application of organic-based zein solutions and aqueous zein dispersions for coating dosage forms is presented and discussed in Chapter 4 and 5, respectively.

4 Chapter 4 The Potential Use of Zein as a Film Coating Material: a) applied as an organic solution

4.1 Introduction

Zein proteins are soluble in aqueous alcohol solutions, but not in pure water and can form tough, glossy and hydrophobic coatings (Shukla et al., 2001). Zein films have low permeability to oxygen and carbon dioxide, a property particularly important for coating and packaging applications in the food and pharmaceutical industries. Zein has been used as a food coating in order to increase the gloss and to prevent oxidation and development of off-odours (Bai et al., 2003). It was also used to coat pharmaceutical tablets based on its film-forming properties and microbial resistance (Winters et al., 1958). Thus, in this part of the work, organic solutions of zein have been investigated as a potential film-coating material for controlled drug release. The conventional top-spray fluidized-bed coater (Mini Coater/Drier 2, Caleva Process Solutions Ltd) is used here.

Fluidized-bed coating is a complex process that starts with droplet formation, contact, spreading, coalescence and evaporation; particles have to pass through this sequence many times before a complete single layer of coating can be formed on the surface (Dewettinck et al., 1999). Numerous variables are involved in film coating and only trial and error together with experience can be used to determine the optimum operating conditions (Link et al., 1997). Top-spray coating can, however, be associated with coating imperfections due to the possibility of premature droplet evaporation as the coating liquid is sprayed countercurrently onto the fluidized particles making it impossible to control the distance the droplets travel before contacting the substrate (Dewettinck et al., 1999).

In this chapter, organic-based zein solutions were used to coat pellets produced via an extrusion/spheronization technique and tablets produced using a single station tablet press. The release of the model drug from the substrates coated with this polymeric system was investigated under different conditions in an attempt to verify zein's potential as a pharmaceutical coating material. In addition, the process of producing, testing and coating the tablets along with the associated coating imperfections including morphology and mass coating variability are discussed.

4.2 Methods

This section briefly describes the methods involved in the preparation of pellets and tablets and the determination of tablet properties such as weight uniformity, thickness, hardness, content uniformity and disintegration time. It also describes the coating process of tablets and pellets and the dissolution tests performed on these coated substrates. A more detailed description of these methods is provided in Chapter 2.

4.2.1 Preparation of Pellets

Pellets were prepared using MCC (Avicel PH 301 grade) and α-lactose monohydrate. The drug (paracetamol) concentration in all the formulations was kept constant at 10% w/w on a dry basis. Caffeine was not used here as extensive migration had been observed in pellets produced and discussed in Chapter 3. Paracetamol did not show such an extensive migration, therefore it was chosen for further studies. The amount of granulation fluid (water) used in the formulations was variable. Powders were dry mixed and wet granulated in a mini food chopper (Kenwood Limited, UK). The wet mass was extruded through a Caleva single screw extruder (Caleva, Sturminster Newton, UK), fitted with a die of 1.0 mm diameter, at a speed of 100 rpm. Fifty grams (50 g) of the resulting extrudates were spheronized for 20 to 35 minutes at speed of 6 (range 0 to 9) on a 120 mm diameter cross-hatched plate spheronizer (Caleva, Sturminster Newton, UK). The pellets were oven dried at 50 to 60 ˚C for 24 hours.

4.2.2 Wet Granulation and Tableting

In this project, two formulations were produced via wet granulation with either α-lactose monohydrate or dibasic calcium phosphate (DCP) being used as fillers. In this context, tablets are thereafter named as α-lactose monohydrate- or dibasic calcium phosphate-based tablets. The chemicals shown in Table 4.1 below were mixed with 100 mL of 18.75% w/v PVP solution and water (as required) using a Kenwood mixer until a suitable granule was formed. The wet granulation was sieved through a 2 mm sieve and then dried in oven at 60 °C overnight. The dried granules were sieved and mixed with 1% w/w of magnesium stearate in a Kenwood mixer. The granules were loaded onto a Manesty E-2 single station tablet press (Manesty Machines Ltd., Liverpool, UK) and were compressed using round 7 mm diameter plain, normal concave punches.

Table 4.1 The components of wet granulation.

Chemical	Content (g)/batch	Content (mg)/ lactose-based tablet	Content (mg)/ calcium phosphate-based tablet
L-α-Lactose monohydrate or Dibasic calcium phosphate	423.75	118.60	120.20
Paracetamol	150.00	42.00	42.54
MCC	75.00	21.00	21.30
PVP *	18.75	5.20	5.30
Partially gelatinized starch (Pre-gelatinised starch)	75.00	21.00	21.30
Water	As required	-	-
Magnesium stearate	7.50	2.10	2.10
Total	750.00 g	209.90 mg	212.70 mg

*Added as 100 mL of 18.75% aqueous solution during granulation.

4.2.3 Uncoated Tablet Specification Tests

4.2.3.1 Weight Uniformity, Thickness and Hardness Tests

To test weight uniformity, 20 tablets from the same batch were weighed and the mean weight was calculated. The thickness of the same tablets batch (n = 20) was measured with a micrometer (Outside micrometer, 0-25 mm) and the mean value was calculated. Their hardness values were measured using an Erweka TBH 28 apparatus (Erweka GmbH, Germany) and the mean value was recorded.

4.2.3.2 Friability Test

The test was performed by weighing 20 tablets, placing them in the drum of an Erweka TAR friabilator (Erweka GmbH, Germany) and rotating them for 100 revolutions. Then, the tablets were reweighed and the weight loss was calculated.

4.2.3.3 Content Uniformity

Ten tablets were individually ground and each added to 200 mL of 0.1 M HCl. The dispersion was then filtered into a 500 mL volumetric flask and the volume was made up to 500 mL using 0.1 M HCl. The solutions were then analysed for the drug concentration

using UV-VIS spectrophotometry (Perkin-Elmer Lambda XLS, USA) at a maximum absorbance wavelength of 243 nm. By reference to a standard plot, the drug concentration in the solutions was calculated and hence the actual drug content in the tablets was determined.

4.2.3.4 Disintegration Time

This test is performed by placing one dosage unit in each of the six tubes of the basket of a disintegration test apparatus which will be immersed in the specified medium at 37 ± 2 C. The time needed for each tablet to disintegrate completely was recorded and the average disintegration time for the six tablets was calculated. In this work, the disintegration time was measured in 0.1 M HCl and in distilled water at 37 ± 0.5 C using a Copley DTG 2000 apparatus with disks (n = 6) (Copley Scientific Ltd., Nottingham, UK).

4.2.4 Coating of Pellets and Tablets

A 10 % w/v solution of zein in 75% aqueous ethanol was prepared and plasticized with glycerol (20% or 30% w/w, based on the amount of zein), as described in Chapter 2. Glycerol was generally used for plasticization at a level of 20% w/w; however, propylene glycol (PG) and polyethylene glycol 400 (PEG 400) were also used, where specified. Tablets and pellets were coated with the 10 % w/v zein solution in a top-spray fluidized-bed coater (Mini Coater/Drier 2, Caleva Process Solutions Ltd, UK) equipped with a top spray head. Five grams (5 g) of pellets were placed in the vibrating coating chamber. The bed temperature was set at 30 ˚C; meanwhile the zein solution reservoir was being stirred constantly at 50 ˚C. The atomization pressure was 0.7 to 1 bar and the pump rate was 1.09 rpm. The fan speed varied between 6.3 m/s (3%) and 7 m/s (10%) and the agitation was between 11.5 and 13.7 Hz.

α-Lactose monohydrate-based (n = 3, 6 or 10) and dibasic calcium phosphate-based (n = 10) tablets were placed in the coating chamber. The atomization pressure was 0.7 bar and the pump rate was 1.09 rpm. The fan speed was 12 m/s (60%) with no agitation. The height for the spray head was 70 mm from the spray nozzle to the mesh in the base of the cone. The coating thickness is expressed in terms of the percentage total weight gain (TWG). Different batches were coated to different weight gains. After coating, tablets or pellets

were further fluidized in the coater (bed temperature = 30 ˚C) for 40 minutes to dry. The coated substrates were stored in sealed amber glass bottles until use.

4.2.5 Dissolution Studies

A BP Apparatus I (D8000, Copley Scientific Ltd., Nottingham, UK) was used for all dissolution tests. Tests were performed in 900 mL of the dissolution medium at 37.0 ˚C ± 0.5 and a rotation speed of 50 ± 1 rpm. One tablet or 0.5 g of pellets was added into each basket. At predetermined sampling points, 10 mL sample was collected from each vessel using a syringe, filtered through a 0.2 μm membrane filter and replaced with an equivalent volume of fresh medium. Measurements of drug release were made using UV-VIS spectrophotometry (Perkin-Elmer Lambda XLS, USA). The wavelength of maximum absorbance (λ_{max}) of paracetamol is 243 nm. A placebo (no drug) batch of calcium phosphate-based tablets was produced, coated and tested in the same dissolution media under the same conditions of those used to test drug-containing tablets. The UV-visible spectra of samples withdrawn upon testing the placebo formulations showed negligible absorbance over the wavelength of maximum absorbance of the model drug under study. The dissolution studies were conducted in triplicate under sink conditions and the average drug release ± SD was calculated. The results are expressed as cumulative percentage drug release versus time profiles. The drug release profiles were compared at a single time point at the end of the experiment (i.e. the last sampling point) using a 2-tailed T-test (2 samples). The lag time was taken to be the last experimental dissolution time point at which zero percentage drug release was observed. However, the sampling was conducted intermittently, thus the actual point of onset of dissolution would be some short time after the lag time as defined above.

4.2.6 Characterization Techniques

4.2.6.1 Scanning Electron Microscopy (SEM)

The surface morphology of coated tablets and pellets before and after dissolution tests was observed using SEM. In addition, SEM images were used to measure the thickness of the film coatings. For this purpose, the coated tablets were cut into two halves and mounted onto stubs using double-sided tape and were gold coated by a Polaron SC7640 sputter gold

coater manufactured by Quorum Technologies. The thickness of the gold coating was about 15 nm. The imaging process was performed in a high vacuum environment. Imaging process was performed with a JEOL JSM5900 LV SEM (Japan), mounted with a tungsten filament with an acceleration voltage of 5–20 kV.

4.2.6.2 Optical Microscopy

Images of the coated substrates under study were captured using a light microscope (Leica Mz75, Switzerland) connected to a digital camera (IC D model).

4.3 Results and Discussion

4.3.1 Tablet Specification Tests

The specification tests of the tablets included weight and content uniformity, thickness, hardness, friability, disintegration time and dissolution tests. Table 4.2 summarizes the weight and content uniformity, tablet thickness, hardness, and disintegration time values for the two formulations (α-lactose monohydrate and dibasic calcium phosphate based tablets). The weight and content uniformity, the friability and the disintegration time values were within the limits of the British Pharmacopeia. The dissolution test was performed in distilled water and both formulations met the BCS standards where > 85% of the drug was dissolved within 30 minutes (Figure 4.1). The hardness values were acceptable for tablets with these dimensions and both tablet types had a friability value < 0.2%. Dibasic calcium phosphate-based tablets were harder and thinner than the α-lactose monohydrate-based tablets, but the former tablet type disintegrated faster than the latter. The fast disintegration of the dibasic calcium phosphate-based tablets contradicts the retarding effect of DCP on the drug release proposed by Lin et al. (1995) and usually observed. They suggested that the retarding effect observed in their studies was induced by the compact structure and slow disintegration behaviour of tablets based on this excipient.

Table 4.2 Specification tests data for α-lactose monohydrate- and dibasic calcium phosphate-based tablets (mean ± SD).

Tablet formulation	Weight uniformity (mg, n = 20)	Content uniformity (mg, n = 10)	Thickness (mm, n = 20)	Hardness (kp, n = 20)	Disintegration time (minutes, n = 6)	
					0.1 M HCl	Distilled water
α-Lactose Monohydrate	209.9 ± 2.8	45.4 ± 4.2	4.7 ± 0.04	7.6 ± 0.6	4.2 ± 1.0	5.7 ± 1.3
Dibasic Calcium Phosphate	212.7 ± 2.5	43.3 ± 0.9	3.9 ± 0.03	12.0 ± 1.0	0.8 ± 0.3	1.78 ± 0.4

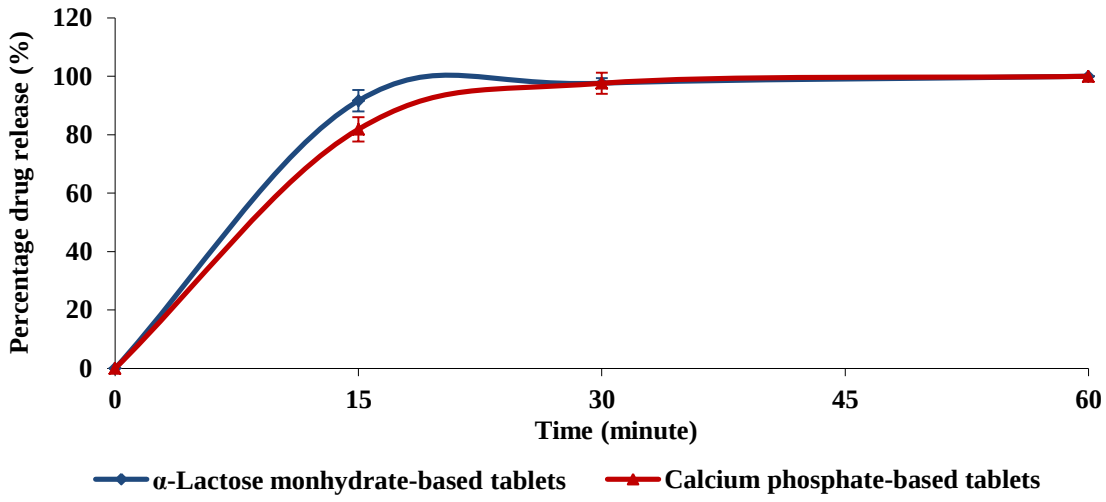


Figure 4.1 Dissolution profiles of uncoated α-lactose monohydrate and dibasic calcium phosphate based tablets in distilled water. Each point represents the mean ± SD (n = 6).

4.3.2 Coating Uniformity

The degree of coating uniformity that is required depends on the functionality of the coat along with whether the coated substrates are to be used as the final dosage form. For example, it is more important to ensure coating uniformity when coating is used for enteric or controlled drug release compared to its use for taste masking. The term coating uniformity involves both mass coating uniformity and coating morphology uniformity. Mass coating uniformity was examined using the SEM imaging technique. Images of the cross-sections of α-lactose monohydrate- (TWG = 10%) and dibasic calcium phosphate-based (TWG = 20%) tablets were captured under the same magnification (250x) using SEM and the corresponding thickness was measured using Leica Application Suite version

V 2.5.0 software. Table 4.3 displays the average thickness values of the four dimensions of the tablet cross-section: top surface (S_1), bottom surface (S_2), right waist (W_1) and left waist (W_2). It is noted that the thickness values are quite uniform at the two coating levels; however, the coating is thinner on the tablet waists compared to the top and bottom surfaces. This is thought to be due to the tablet motion during the coating cycle that makes it more probable for the tablet surfaces to be coated compared to the tablet waists. On average, a TWG of 10% is equivalent to a 106 µm film thickness, whereas a TWG of 20% is equivalent to 203 µm thickness. To further confirm the coating mass uniformity, α-lactose monohydrate-based tablets were engraved manually with numbers from 1 to 10, coated up to 10% TWG, weighed before and after coating and the individual tablet weight gain was calculated. The average weight gain ± SD was 10.6% ± 0.25. The small standard deviation reflects the uniform weight gain of the tablets coated within the same batch.

Table 4.3 The average thickness values of the four dimensions of the tablet cross-section, top surface (S_1), bottom surface (S_2), right waist (W_1) and left waist (W_2) in µm (mean ± SD).

Coating level	**Average thickness (µm)**			
TWG	**Top surface (S_1)**	**Bottom surface (S_2)**	**Right waist (W_1)**	**Left waist (W_2)**
10% (n = 10)	117 ± 2	122 ± 4	93 ± 3	92 ± 4
20% (n = 8)	231 ± 12	237 ± 10	169 ± 6	175 ± 11

On the other hand, coating imperfections and morphology variability whether within the coating surface or cross-sections can lead to significant variability in the desired performance of a coat that may not be related to mass coating variability (Turton, 2008). Coating defects, such as the probability of forming continuous water-filled channels through which the drug release can occur, are more likely expected at lower coating thickness. Coatings of higher thickness are composed of a higher number of polymeric layers and are therefore less prone to coating defects and their performance is thus more reproducible (Siepmann et al., 2007).

4.3.3 Dissolution Studies on Coated Pellets

As explained in Chapter 3, the initial aim was to investigate the potential role of zein in obtaining a controlled drug release profile when used as a matrix in spheres (pellets) produced via extrusion/spheronization. Matrix formulation was investigated to eliminate the coating step; however, the drug release was not significantly retarded compared to the control formula where zein was substituted with α-lactose monohydrate. The conventional polymeric coating using an organic-based zein solution was therefore used in an attempt to achieve controlled drug release from pellets.

4.3.3.1 Influence of Pellet Core

Figure 4.2 compares the drug release profile from uncoated zein-based pellets [MCC (55%)-Zein (35%)-PCT (10%)] vs. the release from α-lactose monohydrate-based pellets (MCC (70%)-Lact (20%)-PCT (10%) and MCC (45%)-Lact (45%)-PCT (10%)), both coated with organic-based zein solution (TWG = 25%) in distilled water. Zein-based pellets are an example of matrix devices whereas the lactose-based pellets coated with zein films are reservoir systems. The release of paracetamol from the uncoated zein-based pellets followed an immediate-release pattern, with complete drug release seen by 60 minutes, whereas the drug release from the coated pellets approached zero-order kinetics after an initial lag period of circa 10 minutes. The lag period is thought to be the time needed for the aqueous medium to hydrate and penetrate into the coating, hydrate the core and begin to dissolve the drug and for the drug to start to diffuse out. The formation of aqueous channels within the film coating, either by the dissolution of the water-soluble components of the coating membrane such as the plasticizer (i.e. glycerol) or by the hydration and swelling of the polymeric coat, is thought to allow the penetration of water into the core. It has been suggested by Oh et al. (2010) that zein membranes hydrate and swell, forming aqueous channels through which drug diffusion may occur.

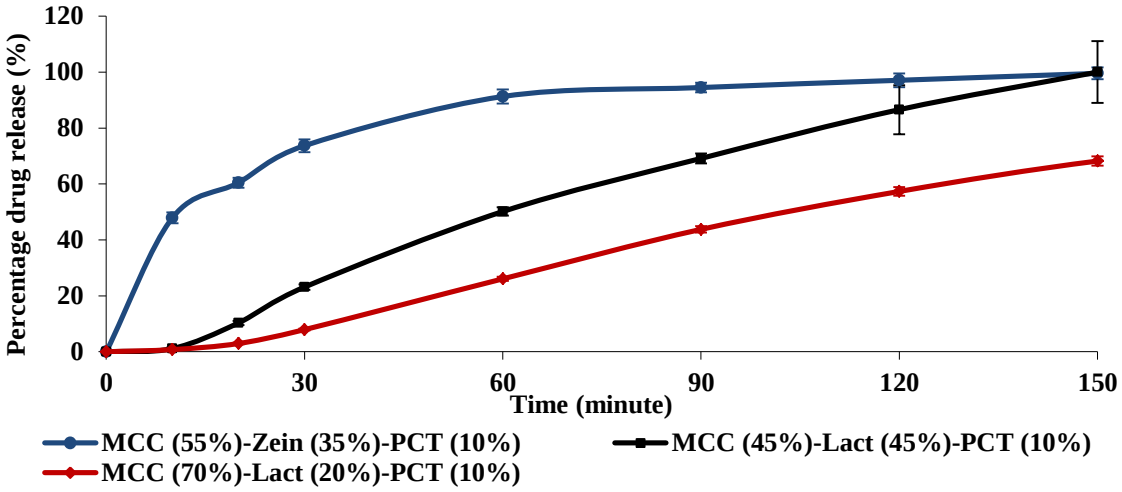


Figure 4.2 Dissolution profiles in distilled water of α-lactose monohydrate-based pellets of different core composition: MCC (45%)-Lact (45%)-PCT (10%) and MCC (70%)-Lact (20%)-PCT (10%) coated with organic-based zein solution (TWG = 25%) and uncoated zein-based pellets (MCC (55%)-Zein (35%)-PCT (10%)). Each point represents the mean ± SD (n = 3).

It can be clearly noted that the release of paracetamol from the coated pellets of (MCC (45%)-Lact (45%)-PCT (10%)) core type was much slower than that from the uncoated zein-based pellets, with approximately 23% and 73% being released, respectively, after 30 minutes. The graph also shows the influence of the pellet core (i.e. the α-lactose monohydrate level) on the drug release from these coated spheres. Comparing the dissolution profiles of α-lactose-monohydrate pellets (lactose level = 20% or 45%), coated (TWG = 25%) with organic-based zein solution plasticized with 20% w/w glycerol, clearly shows that decreasing the amount of α-lactose monohydrate significantly ($p < 0.05$) decreased the extent of drug release from these coated pellets. This can be mainly attributed to the osmotic activity of lactose which will imbibe more water into the pellet core generating an osmotic pressure. The high pressure inside the coat membrane can induce swelling of the pellet core and can introduce cracks into the film coating particularly if the films are mechanically weak and hence result in a faster drug release. Accordingly, increasing the lactose content from 20% to 45% will give increased osmotic pressure. An essentially similar lag period of circa 10 minutes, however, preceded the drug release from both pellet cores. As mentioned above, the lag period is the time needed to form aqueous channels within the coating. Thus, the lag period is expected to be the same upon the dissolution of approximately the same level of glycerol within the coating membranes of the two pellet cores, i.e. MCC (45%)-Lact (45%)-PCT (10%) and MCC (70%)-Lact (20%)-PCT (10%).

4.3.3.2 Influence of Plasticizer level

Figure 4.3 shows the dissolution profiles of pellets of (MCC (70%)-Lact (20%)-PCT (10%)) core type coated (TWG = 25%) with organic-based zein solution plasticized with 20% and 30% glycerol in distilled water. The drug release was faster from pellets coated with the zein solution of higher plasticizer content. Glycerol is a water soluble ingredient that would potentially form pores once it dissolves in the dissolution medium. Therefore, although a higher plasticization level improves the film forming properties it increases the hydrophilic content in the water insoluble film coat able to hydrate and dissolve, creating aqueous channels through which drug release can occur. Moreover, the uptake of water by these hydrophilic additives is expected to increase the mobility of the macromolecules of the polymeric film and thus increase the volume available for drug diffusion, as explained by Siepmann et al. (2007).

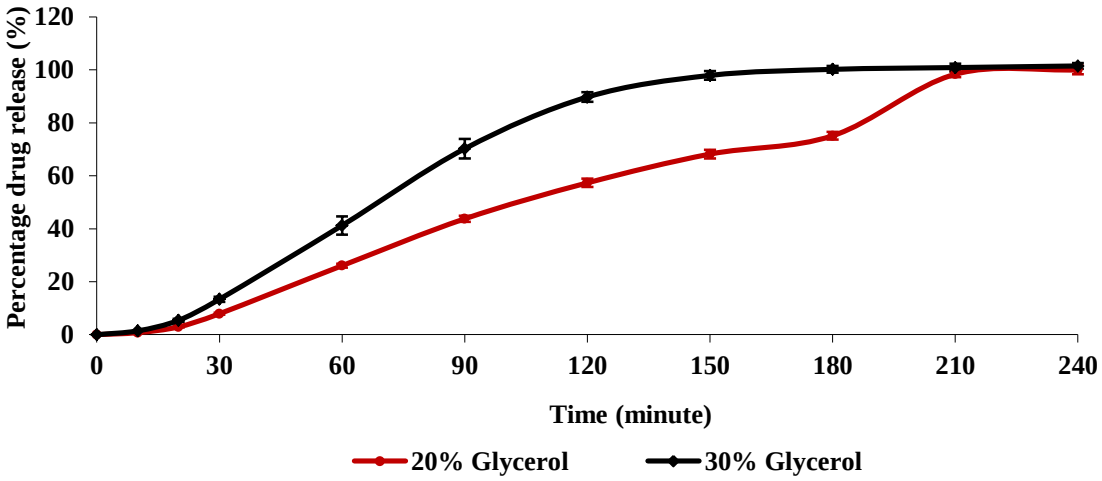


Figure 4.3 Dissolution profiles in distilled water of pellets (MCC (70%)-Lact (20%)-PCT (10%)) coated with organic zein solution (TWG = 25%) plasticized with 20% and 30% glycerol. Each point represents the mean ± SD (n = 3).

SEM images of the spheres coated with zein solution plasticized at these two levels, shown in Figure 4.4, revealed a smoother and more complete film formation at the higher plasticization level (30%). It is worth noting that the pellets had a quite rough surface prior to coating which might have negatively influenced the coating process and the quality and continuity of the forming film.

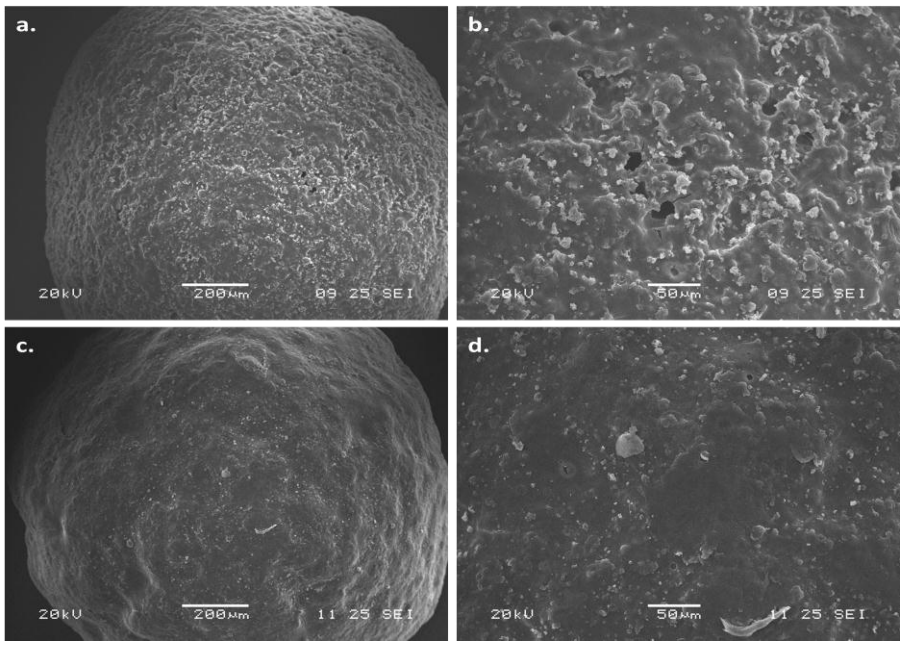


Figure 4.4 SEM images of pellets (MCC (70%)-Lact (20%)- PCT (10%)) coated with organic zein solution plasticized with 20% (a and b) and 30% (c and d) w/w glycerol (a and c, 95x) (b and d, 300x).

One complication that was observed during and at the end of the dissolution test of the coated pellets was the adherence of the pellets to each other upon contact with the aqueous dissolution medium. Figure 4.5 presents optical microscopic images (a and b) and SEM images (c and d) that show the network-like arrangement of the zein-coated pellets after the dissolution test in distilled water. The structure of zein protein (Z19) is rich in hydrophobic amino acids, particularly aliphatic amino acids, yielding high aliphatic indexes and high surface hydrophobicity which count for its water insolubility and its tendency to aggregate (Cabra et al., 2007). The adherence is therefore thought to be promoted by the hydrophobic character of zein and its adhesive properties in order to minimize the contact with the aqueous medium. Water as well as the incorporated glycerol is an effective plasticizer of zein and the action of these plasticizers is thought to facilitate the adhesion of pellets to each other in a network-like order. This observation is expected to exert mechanical force on the adjacent coatings which might influence the mechanical stability of the coating and hence the drug release from these coated pellets.

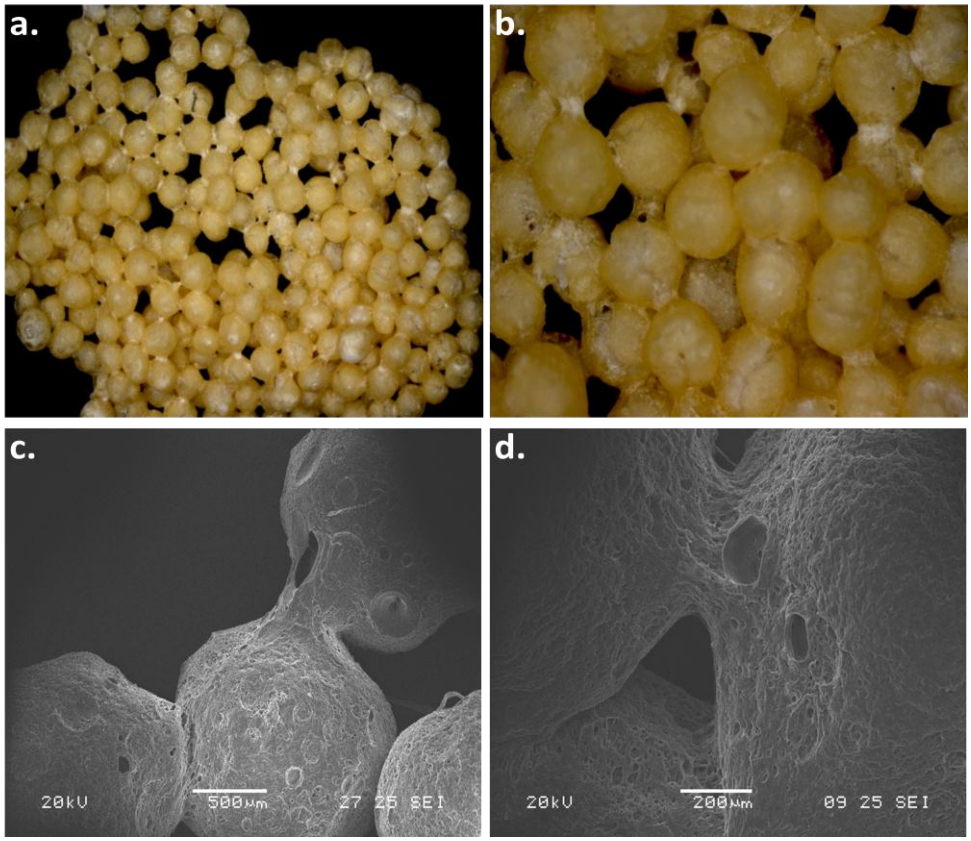


Figure 4.5 Optical microscopy images (a (4x) and b (10x)) and SEM (c (40x) and d (95x)) images of bench-dried pellets after 4 hours dissolution test showing pellets networking.

4.3.4 Dissolution Studies on Coated Tablets

In order to better understand the influence of different media on the performance of zein coatings, individual dosage forms, i.e. tablets, were used instead of pellets and coated with the organic-based zein solution. The α-lactose monohydrate-based and dibasic calcium phosphate-based tablets described earlier were used.

4.3.4.1 Influence of Tablet Core Material and Geometry

Figure 4.6 compares the drug release profiles of α-lactose monohydrate-based tablets coated up to 10% TWG in distilled water, 0.1 M HCl and phosphate buffer (pH 6.8). The drug release profiles showed two phases: the lag phase and the burst release phase. The lag period is thought to be the time needed for the aqueous medium to hydrate and penetrate into the coating, hydrate the core and begin to dissolve the drug and for the drug to start to diffuse out. The formation of aqueous channels within the film coating, either by the

dissolution of the water-soluble components of the coating membrane such as the plasticizer (i.e. glycerol) or by the hydration and swelling of the polymeric coat, is thought to allow the penetration of water into the core. The drug release was close to zero during this lag time. The burst release might, however, be related to rupture of the film coat. The rupture might be a function of the osmotic pressure generated inside the coat membrane by the dissolution of the tablet core and the mechanical stability of the film coating. The latter depends on the thickness of the coat and its continuity, i.e. thicker coats are able to withstand higher pressures before the surrounding membrane ruptures. The poorly reproducible dissolution profiles can possibly be explained by the fact that rupture can occur at different time points and/or different spots on the tablet surface.

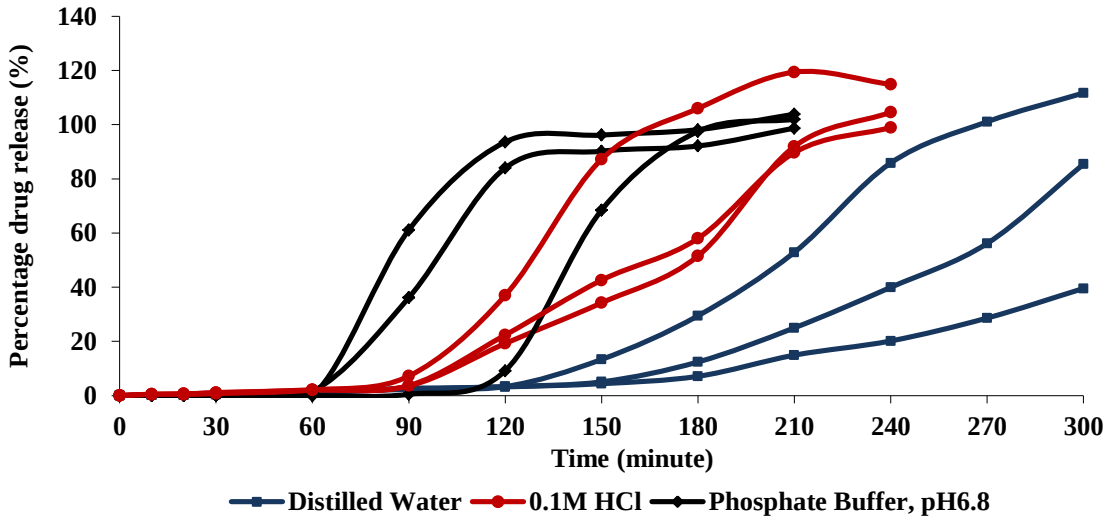


Figure 4.6 Dissolution profiles of α-lactose monohydrate-based paracetamol tablets coated with organic-based zein solution (TWG = 10%) in distilled water, 0.1 M HCl and phosphate buffer pH 6.8 (n = 3). Each profile represents a single experiment.

α-Lactose monohydrate was replaced in the core tablet formulation with dibasic calcium phosphate, which is a water insoluble and directly compressible excipient. Figure 4.7 presents the dissolution profiles of dibasic calcium phosphate-based tablets coated up to 10% TWG in the same aforementioned conditions. The dissolution profiles also showed a two-phase drug release, as observed for the α-lactose monohydrate-based tablets, with the lag phase being essentially the same for both formulations. However, the dibasic calcium phosphate-based tablets showed evidence of a burst release only in 0.1 M HCl, with ruptures in the film coat also being observed only after exposure to this medium. Drug release was less abrupt in distilled water and phosphate buffer pH 6.8, and the profiles

being more reproducible and showing more of a zero-order character. The variation in release profiles can possibly be explained by the influence of the core material and the generated osmotic pressure upon the dissolution of the tablet core. Dibasic calcium phosphate is water insoluble but it is soluble in dilute acids and therefore it would be expected to dissolve and build an osmotic pressure within the tablet core on exposure to 0.1 M HCl, causing the film coating to rupture. This would not be observed to the same extent in distilled water or phosphate buffer (pH 6.8).

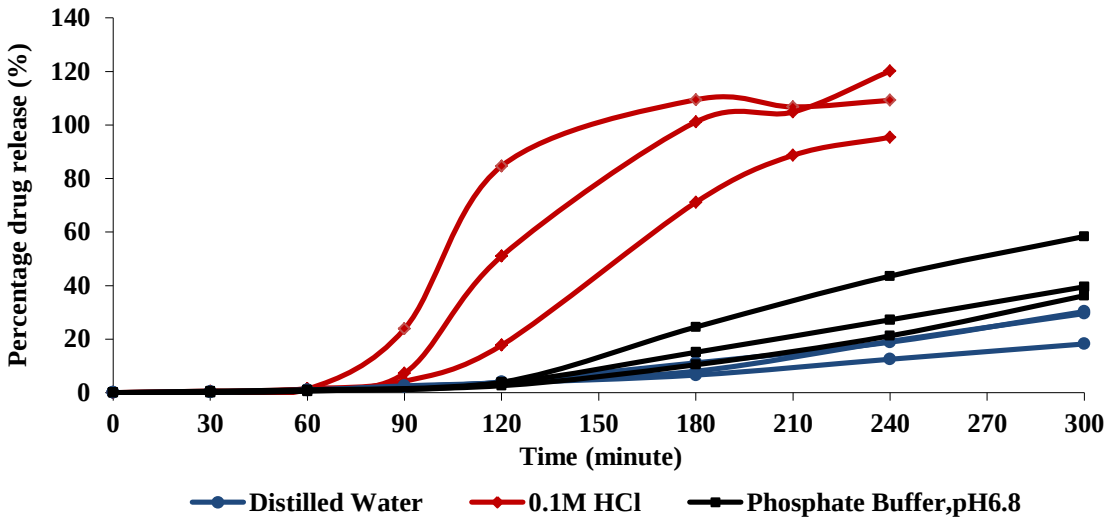


Figure 4.7 Dissolution profiles of dibasic calcium phosphate-based paracetamol tablets coated with organic-based zein solution (TWG = 10%) in distilled water, 0.1 M HCl and phosphate buffer pH 6.8 (n = 3). Each profile represents a single experiment.

Based on the results of coating mass uniformity (Section 4.3.2), it is unlikely that the poor reproducibility of the drug release profiles associated with the lower coating levels is caused by a variation in the coating thickness. The poor reproducibility can however be explained by the higher probability of coating defects at lower coating levels along with the osmotic gradient formed across the membrane which can be beyond the mechanical stability of the film coat or can aggravate any existing coating defect and potentially induce coating rupture. Therefore, thicker coatings were applied onto the tablets in order to minimize the coating imperfections, offer higher mechanical strength, greater ability to restrain the swelling of tablets upon contact with the aqueous media and ultimately to enhance the reproducibility. Both tablet types (α-lactose monohydrate- and dibasic calcium phosphate-based tablets) were coated with the same coating solution up to the same coating level (TWG = 20%). The two tablet types, however, behaved differently when

tested in 0.1 M HCl. The α-lactose monohydrate-based coated tablets, unlike the dibasic calcium phosphate-based tablets, showed poor reproducibility of the dissolution results (Figure 4.8). Figure 4.8 presents the dissolution profiles in 0.1 M HCl of nine α-lactose monohydrate-based paracetamol tablets of the same batch, coated up to 20% TWG with organic-based zein solution. The figure shows that within the same coated batch of α-lactose monohydrate-based tablets variable dissolution profiles in 0.1 M HCl were obtained. Rupture of the film coating of some of the tablets under test was also observed (Figure 4.9).

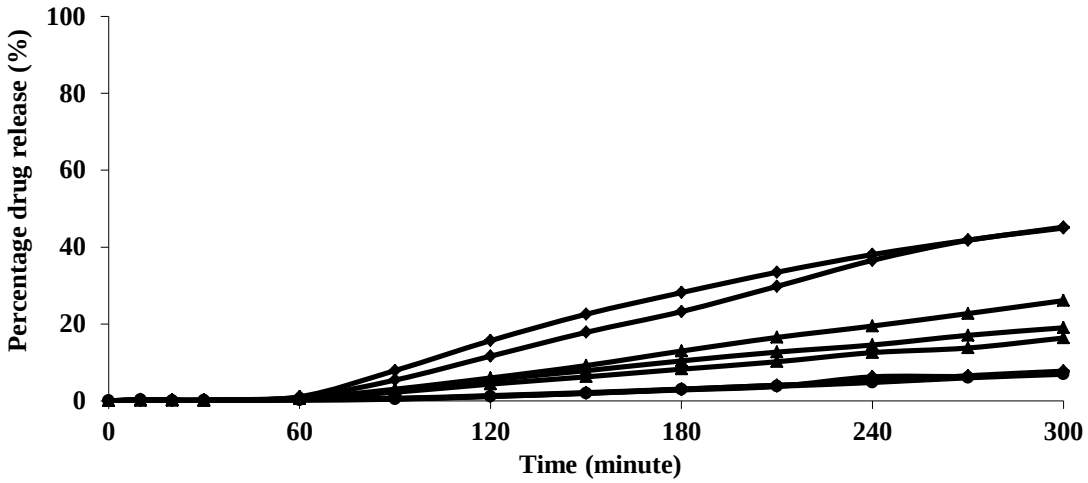


Figure 4.8 Dissolution profiles in 0.1 M HCl of α-lactose monohydrate-based paracetamol tablets coated (TWG = 20%) with organic-based zein solution (n = 9). Each profile represents a single experiment.

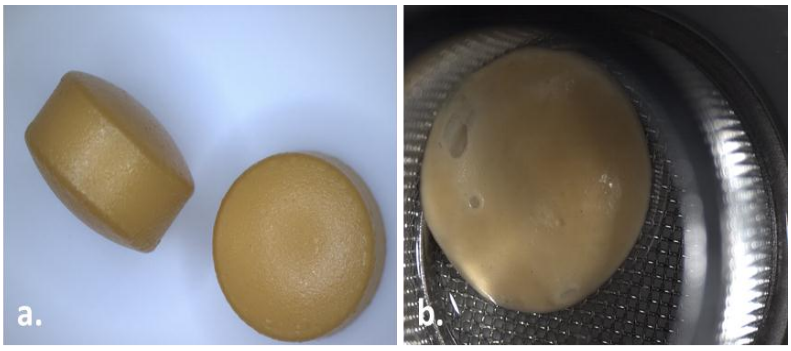


Figure 4.9 Optical microscopy images showing: a) coated (TWG = 20%) α-lactose monohydrate-based tablets prior to dissolution and b) coating rupture after 5 hours dissolution test in 0.1 M HCl.

This can be attributed to the difference in the geometry of the uncoated tablets, i.e. the α-lactose monohydrate-based tablets were thicker than the dibasic calcium phosphate-based tablets, which can influence the coating efficiency. This variability in the performance of α-lactose monohydrate-based tablets coated up to 20% TWG in 0.1 M HCl may also help to explain the variability seen in the dissolution profiles of the same tablet type at the lower coating level of 10% TWG. Thus, the apparent general trend in Figure 4.6 of a different dissolution rate with pH may not actually be a true reflection of the behaviour of the formulation, but more likely due to variability within the product.

SEM imaging of the coating surface and the cross-section of the two tablet types (α-lactose monohydrate- and dibasic calcium phosphate-based tablets) was carried out to further investigate this variation. Both tablet types had a fairly smooth coating surface with signs of incomplete droplet spreading and pores of circa 2 µm in diameter (as pointed by the arrows in Figure 4.10, a and b); however, the cross-section of the former tablet type was more porous (network-like structure) compared to compact cross-section of the dibasic calcium phosphate-based ones (Figure 4.10, c and d), which might be due to some surface dissolution of the lactose into the coating material. Thus, dibasic calcium-phosphate-based tablets were used hereafter.

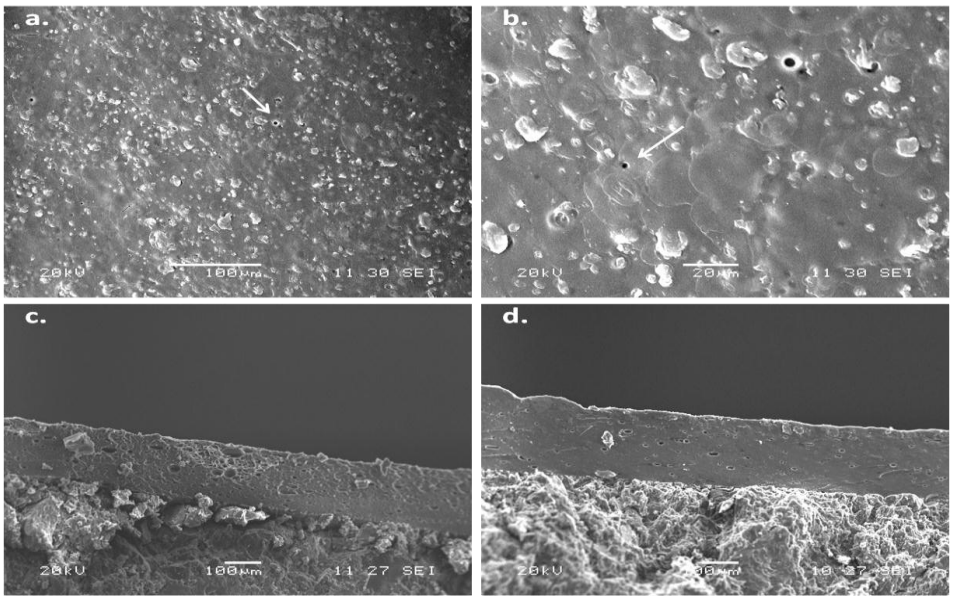


Figure 4.10 SEM images of the surface of coated tablets showing incomplete droplet spreading (a (250x) and b (750x), as pointed by the arrows) and cross-section of α-lactose monohydrate (c, 100x) or calcium phosphate dibasic (d, 100x) based tablets coated with organic-based zein solution (TWG = 20%).

4.3.4.2 Influence of Plasticizer Type and Coating Level

Zein films are known to be brittle on their own (O'Donnell et al., 1997), so a plasticizer is required in order to improve the flexibility of the protein-based films. Glycerol, polyethylene glycol 400 (PEG 400), and propylene glycol (PG) were used to plasticize the organic-based zein solution used for coating at a concentration of 20% w/w (based on the amount of zein). The three plasticizers were all of GRAS (Generally Recognized As Safe) status and previously used in oral pharmaceutical formulations. Figure 4.11 demonstrates the influence of the plasticizer type on the drug release from dibasic calcium phosphate-based tablets coated with organic-based zein solution up to 10% TWG in Simulated Gastric Fluid without pepsin (SGF without pepsin) pH 1.2. The test was carried out under acidic conditions in order to maximally challenge the formulation, as the dibasic calcium phosphate within the tablet core is soluble in dilute acids, potentially exerting an osmotic pressure against the coating after dissolution of (at least part of) the tablet core. The dissolution profiles exhibited a lag period of approximately 2 hours prior to the fast release phase, with the maximum release at 5 hours (when the experiment was terminated) being 55%, 76% and 100% from coatings plasticized with PEG 400, PG and glycerol, respectively, at a plasticization level of 20% w/w (based on zein amount). Coatings plasticized with glycerol exhibited the greatest drug release rate and extent whereas those plasticized with propylene glycol and polyethylene glycol exhibited more comparable drug release rates and extents, but lower than those of glycerol-plasticized coatings. All of these formulations exhibited relatively high variability in performance, as indicated by the large error bars, when tested in SGF (without pepsin) of pH 1.2.

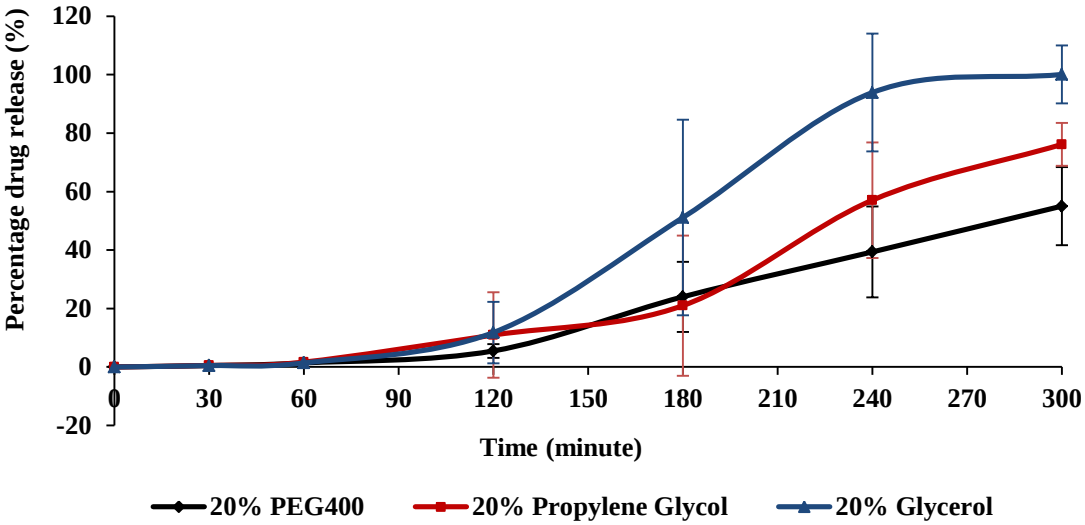


Figure 4.11 Dissolution profiles of paracetamol tablets coated with organic-based zein solution (TWG = 10%) plasticized with 20% w/w (based on zein amount) PEG 400, PG and glycerol in SGF (without pepsin) pH 1.2. Each point represents the mean ± SD (n = 3).

To evaluate the influence of the coating level on the drug release, dibasic calcium phosphate-based tablets were coated to a TWG of 20% with organic-based zein solution plasticized with 20 % w/w of PEG 400, PG or glycerol and tested in SGF (without pepsin) pH 1.2 for up to 5 hours (Figure 4.12). The drug release from tablets with higher coating thickness (TWG = 20%) showed an initial lag phase of circa 2 hours similar to that obtained at lower TWG (10%), followed by constant drug release (zero-order kinetics), irrespective of the plasticizer type. Thus, increasing the coating thickness from 10% to 20% did not influence the lag phase duration; however, thicker coatings were able to prevent the coating rupture. These results are in line with the work of Marucci et al. (2007) who explained that thicker coats are able to reduce the water influx and to withstand high pressure before coating rupture occurs . The influence of the plasticizer type on the drug release was negligible at higher coating thickness (TWG = 20%). The drug release was slow with an average drug release at 5 hours of 5% from coatings plasticized with PG and 8% from coatings plasticized either with glycerol or PEG 400. All results were reproducible.

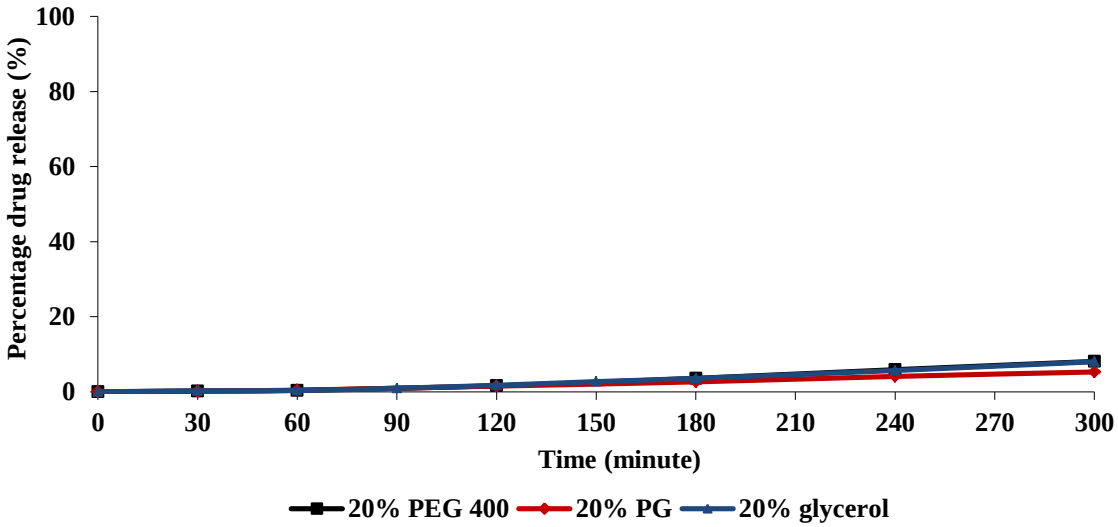


Figure 4.12 Dissolution profiles of paracetamol tablets coated with organic-based zein solution (TWG=20%) plasticized with 20% w/w (based on zein amount) PEG 400, propylene glycol and glycerol in SGF (without pepsin) pH 1.2. Each point represents the mean ± SD (n = 3). (Error bars are very small).

As the results were more reproducible at higher coating levels and the plasticizer type showed no difference on the drug release at higher coating thickness (TWG = 20%), tablets coated with organic-based zein solution plasticized with 20% w/w glycerol up to 20% TWG were used in the next studies. The choice of the plasticizer was based on the physical properties of the final product. Calcium phosphate-based tablets coated with zein films plasticized with PG showed cracking upon storage (Figure 4.13). Cracking of zein-based coatings on tablets when plasticized with PG was also reported by O'Donnell et al. (1997). Zein-based coatings plasticized with either glycerol or PEG 400 did not crack. Glycerol was, however, used hereafter.

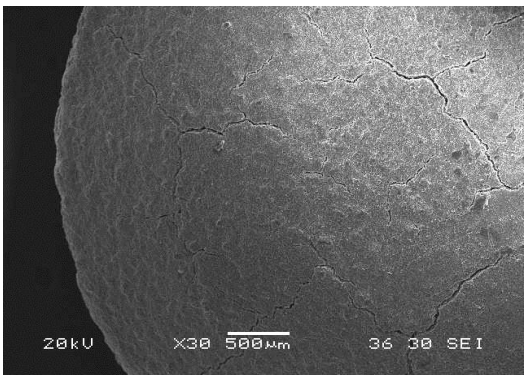


Figure 4.13 An SEM image (30x) of the cracking of zein-based coatings plasticized with PG observed on the surface of calcium phosphate-based tablets.

4.3.4.3 Influence of Dissolution Model and Rotation Speed

Dissolution testing is generally assumed to be straight-forward and relatively simple to perform. However, poor control of the hydrodynamics of the system has been reported in the literature as causing poor stirring and mixing in the dissolution vessel which in turn could lead to high variability and unpredictability of the dissolution results (Qureshi, 2004). In order to select the proper dissolution apparatus (i.e. baskets or paddles) and the rotation speed (50 and 100 rpm) for this study, dissolution tests using tablets coated within the same batch were performed using the two models running at the two speeds. Figure 4.14 and Figure 4.15 assess the influence of the dissolution model (basket vs. paddle) on the drug release from zein-coated tablets in 0.1 M HCl at a rotation speed of 50 and 100 rpm, respectively. Both models showed a similar release pattern with an average release of 7% at both rotation speeds over the 5 hours test duration. The basket model was thereafter used at a rotation speed of 50 rpm.

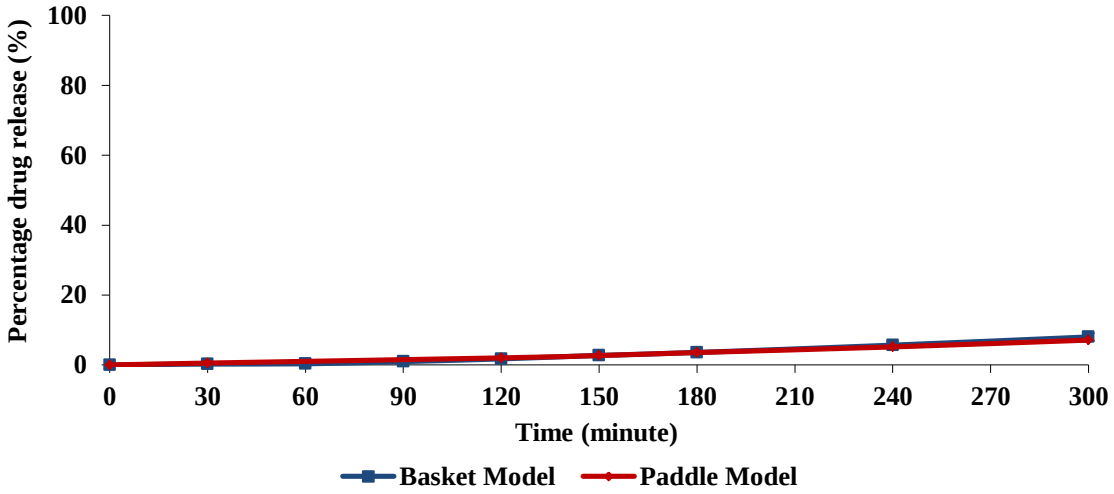


Figure 4.14 Dissolution profiles of paracetamol tablets coated with organic-based zein solution (TWG = 20%) in 0.1 M HCl using the basket model and the paddle model at a rotation speed of 50 rpm. Each point represents the mean ± SD (n = 3). (Error bars are very small).

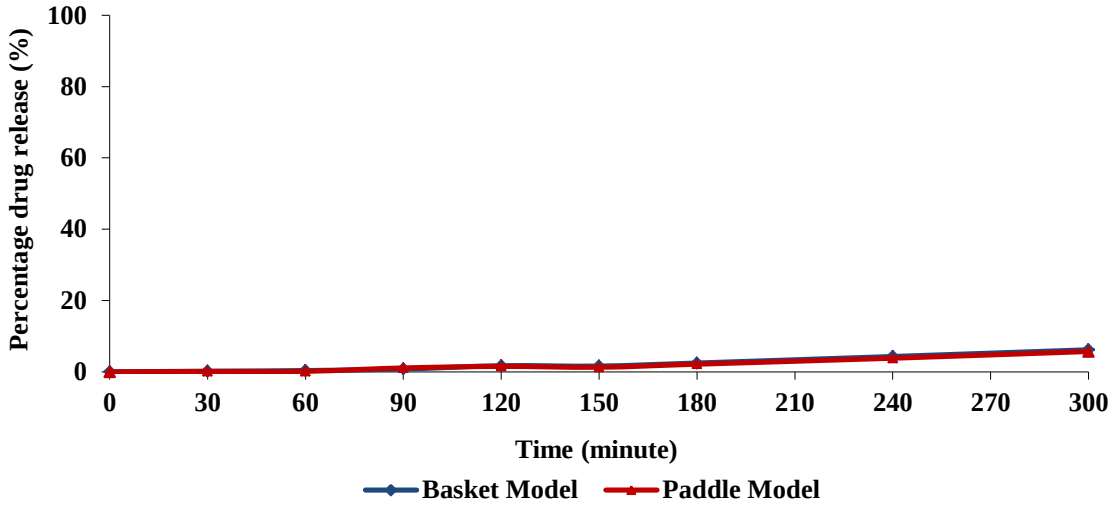


Figure 4.15 Dissolution profiles of paracetamol tablets coated with organic-based zein solution (TWG = 20%) in 0.1 M HCl using the basket model and the paddle model at a rotation speed of 100 rpm. Each point represents the mean ± SD (n = 3). (Error bars are very small).

4.3.4.4 Influence of the Dissolution medium pH value

The susceptibility of zein coatings to acidic and basic conditions was investigated in order to determine any particular behaviour of zein that could point out potential uses of this protein as a film former in the pharmaceutical field. Figure 4.16 shows the dissolution profiles of dibasic calcium phosphate-based paracetamol tablets coated with organic-based zein solution up to 20% TWG in 0.1 M HCl (pH 1), distilled water and phosphate buffer (pH 6.8). The drug release was very slow with the maximum release being 8% at pH 1 and 5% in both distilled water and phosphate buffer (pH 6.8) over 5 hours test duration. The drug release profiles exhibited a linear relationship between the cumulative percentage drug release and time suggesting zero-order kinetics, irrespective of the pH of the release medium.

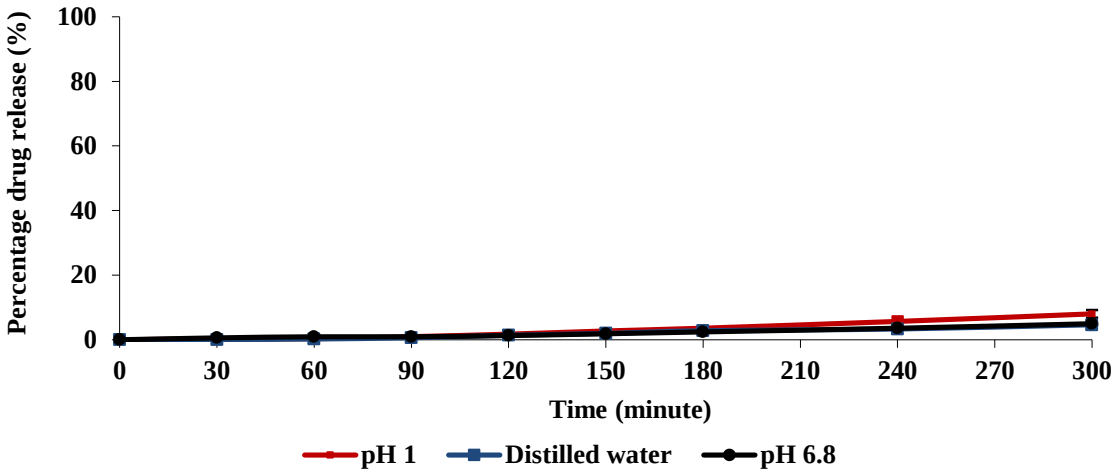


Figure 4.16 Dissolution profiles of paracetamol tablets coated with organic-based zein solution (TWG = 20%) in pH 1, distilled water and phosphate buffer pH 6.8. Each point represents the mean ± SD (n = 3). (Error bars are very small).

SEM images before and after the release studies from zein coated tablets in 0.1 M HCl and phosphate buffer (pH 6.8) are shown in Figure 4.17. The tablet surface was fairly smooth before the release (Figure 4.17, a) whereas some pores appeared after the drug release in 0.1 M HCl (Figure 4.17, b) and the tablet surface exhibited rimmed craters spread over a smooth surface after the test in phosphate buffer (Figure 4.17, c).

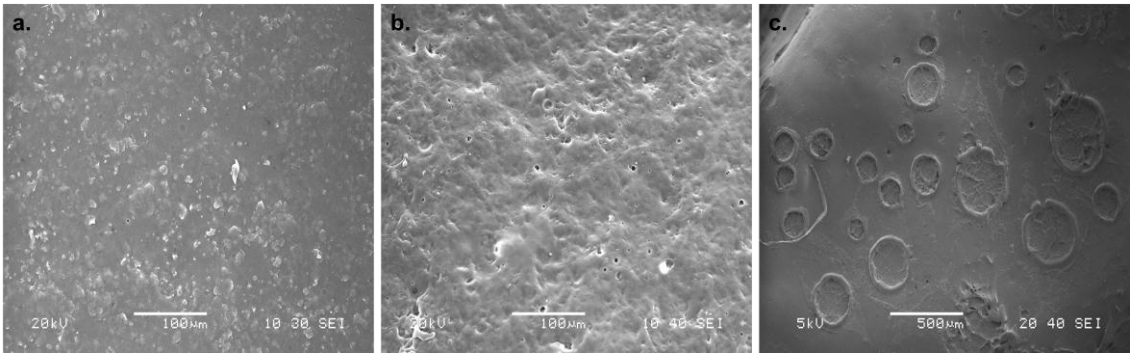


Figure 4.17 SEM images of paracetamol tablets coated with organic-based zein solution prior to any treatment (a, 250x), and after 5 hours test in 0.1M HCl (b, 250x) and phosphate buffer pH 6.8 (c, 50x).

This result suggests that zein does not show pH-dependent drug release behaviour at this particular coating thickness (TWG = 20%). There is a wide debate in the literature regarding zein solubility. Georget et al. (2008) reported an increase in drug dissolution from a zein tablet matrix under acidic conditions (0.1 M HCl) which might be explained by the acidic deamidation of the glutamine and aspargine present in the zein protein.

Similarly, O'Donnell et al. (1997) found that the drug release from tablets coated with zein pseudolatex occurred at a faster rate in 0.1 M HCl and the rate decreased with increasing the pH suggesting a greater solubility of the zein in an acidic environment. On the other hand, the pH of the dissolution media had a negligible effect on the release of paracetamol from tablets coated with a 4.39% (of the tablet weight) zein pseudolatex, as stated by Oshlack et al. (1994).

To further confirm the results obtained in Figure 4.16, the pH of the dissolution medium was changed to test whether the drug release from the tablets coated with zein film could be influenced by the pH value of the dissolution medium. A pH change method was used involving dissolution in Simulated Gastric Fluid (SGF) (pH 1.2, both with and without pepsin) for 2 hours followed by 6 hours in phosphate buffer (pH 6.8). The release medium was changed by complete substitution of the dissolution vessels. Figure 4.18 shows the drug release from paracetamol tablets coated with organic-based zein solution (TWG = 20%) tested in SGF (without pepsin) for 2 hours followed by 6 hours in phosphate buffer in comparison to those tested in SGF (with pepsin) for 2 hours followed by 6 hours at pH 6.8. The release pattern was found to remain constant regardless of the pH of the medium with the maximum release being 14% and 25% after 8 hours in SGF (without pepsin) - pH 6.8 and SGF (with pepsin) - pH 6.8 media, respectively. The higher release ($p < 0.05$) obtained upon the addition of pepsin in to the dissolution medium in the first 2 hours suggests that the film integrity was compromised by the digestive effect of the enzyme.

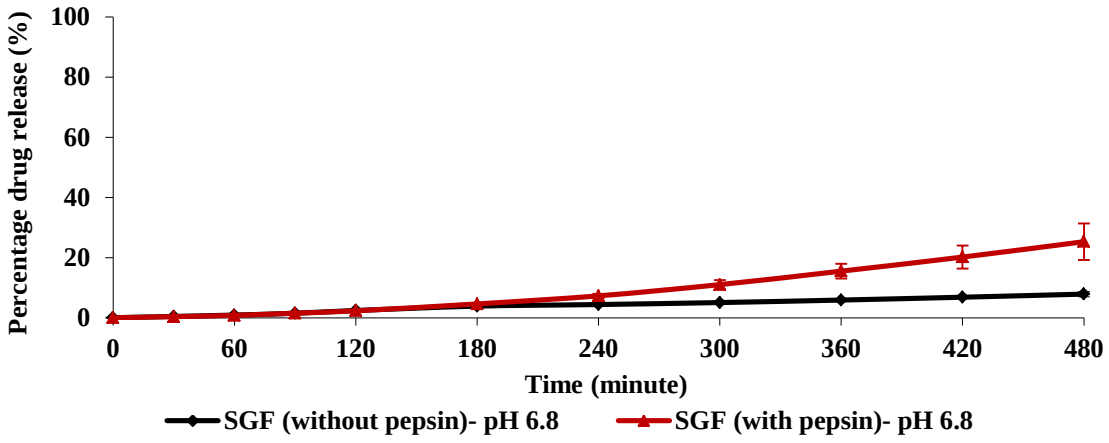


Figure 4.18 Dissolution profiles of paracetamol tablets coated with organic-based zein solution (TWG = 20%) in SGF (without pepsin) or SGF (with pepsin) for 2hours followed by 6 hours in phosphate buffer (pH 6.8). Each point represents the mean ± SD (n = 3).

SEM images before and after the dissolution study are shown in Figure 4.19. The tablet had a fairly smooth surface before the test (Figure 4.19, a), whereas the surface of tablets tested in SGF (without pepsin) - pH 6.8 medium showed porous craters (Figure 4.19, b) that were larger in diameter and less in number compared to those observed on the surface of the tablets tested in phosphate buffer alone of pH 6.8 (Figure 4.17, c). Tablets tested in SGF (with pepsin) - pH 6.8 showed an open cell (foam structure) with coating material between the cells (Figure 4.19, c).

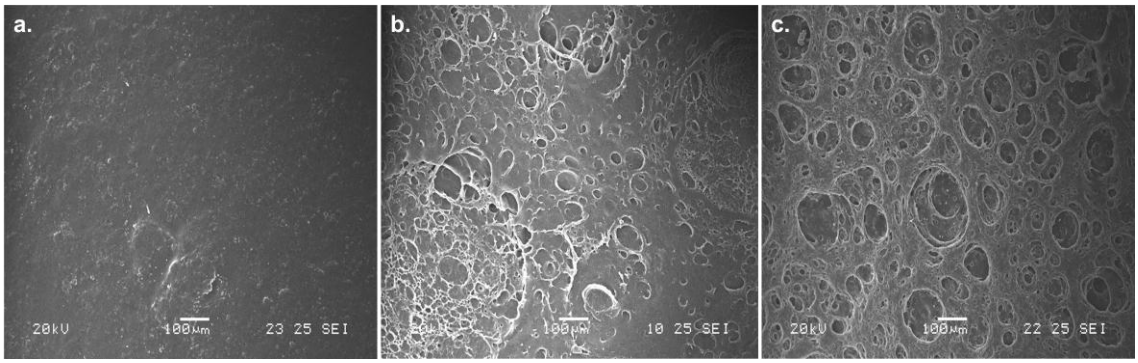


Figure 4.19 SEM images (100x) of paracetamol tablets coated with organic-based zein solution prior to any treatment (a), and after 8 hours test in SGF (without pepsin) - pH 6.8 (b) and SGF (with pepsin) - pH 6.8 (c).

4.3.4.5 Influence of Dissolution Medium Osmotic Pressure

In order to investigate the effect of the osmotic pressure difference across the coating on the drug release, dissolution tests were conducted in solutions containing different concentrations of sodium chloride (NaCl). Figure 4.20 shows the dissolution profiles of paracetamol tablets coated with organic-based zein solution in distilled water and NaCl solutions of different strength: 0.25 M, 0.5 M and 1 M. The dissolution profiles showed a trend of decreasing in the drug release with increasing the NaCl concentration in the testing media, with the maximum drug release at 5 hours being 4.61%, 4.52%, 3.14% and 1.33% in distilled water, 0.25 M, 0.5 M and 1 M NaCl solutions, respectively.

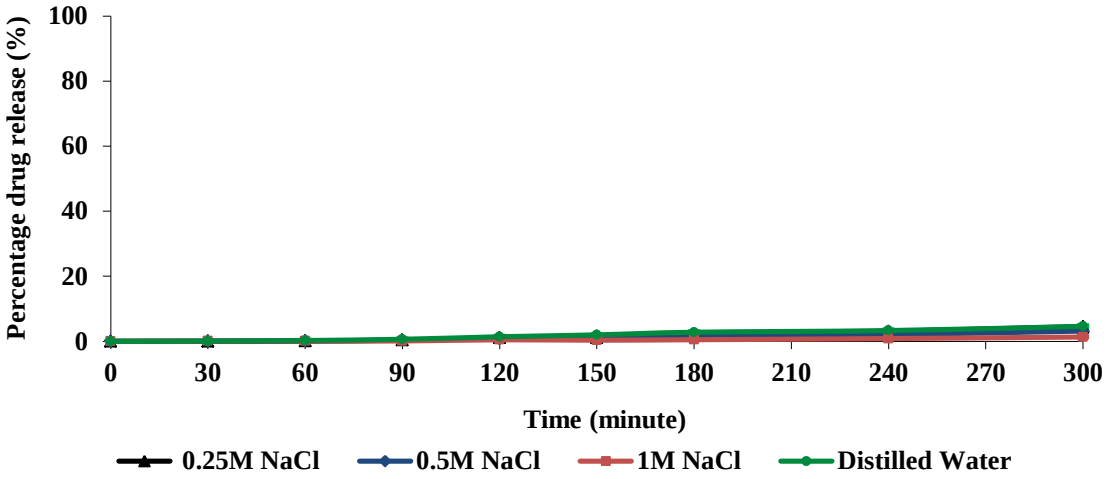


Figure 4.20 Dissolution profiles of paracetamol tablets coated with organic-based zein solution (TWG = 20%) in distilled water and NaCl solutions of different strength (0.25 M, 0.5 M and 1 M). Each point represents the mean ± SD (n = 3). (Error bars are very small).

The decrease in drug release rate might be attributed to the decrease in paracetamol solubility in NaCl solutions, which is dependent on NaCl concentration. Shaw et al. (2005) demonstrated that sodium chloride (NaCl) reduced the aqueous solubility of paracetamol by up to 20–30% compared to the intrinsic solubility and its effect was concentration-dependent. In addition, Oh et al. (2003) stated that the swelling behaviour of zein membranes is dependent on the ionic strength of the medium: NaCl concentrations < 0.1 M can change the electrostatic interactions between the amino acid side chain enhancing the membrane swelling, whereas higher NaCl concentrations reduce the water activity and hence suppress the swelling properties of zein. Consequently, the drug release from zein-coated tablets might be influenced by zein swelling behaviour which depends on the ionic strength of the medium, as well as the changes in the solubility of the drug in the dissolution media.

4.3.4.6 Influence of Presence of Digestive Enzymes

4.3.4.6.1 Presence of Pepsin

Zein is a protein and therefore it is essential to investigate the susceptibility of zein to the digestive action of pepsin, a protease, if tablets are to be administered orally and particularly if colon targetting is to be designed. Figure 4.21 shows the drug release in SGF

from tablets coated with organic-based zein solution up to 15%, 20% and 30% TWG in the presence of pepsin. It is clearly noted that the zein films were digested to a certain extent by pepsin. The integrity of the film coatings was thus impaired resulting in the rupture of the coating after a certain lag period. The lag phase duration was dependent on the coating level (expressed as TWG) i.e. longer lag periods were observed at higher coating thickness. The value was approximately 30 minutes, 3 and 5 hours for the three coating levels, respectively.

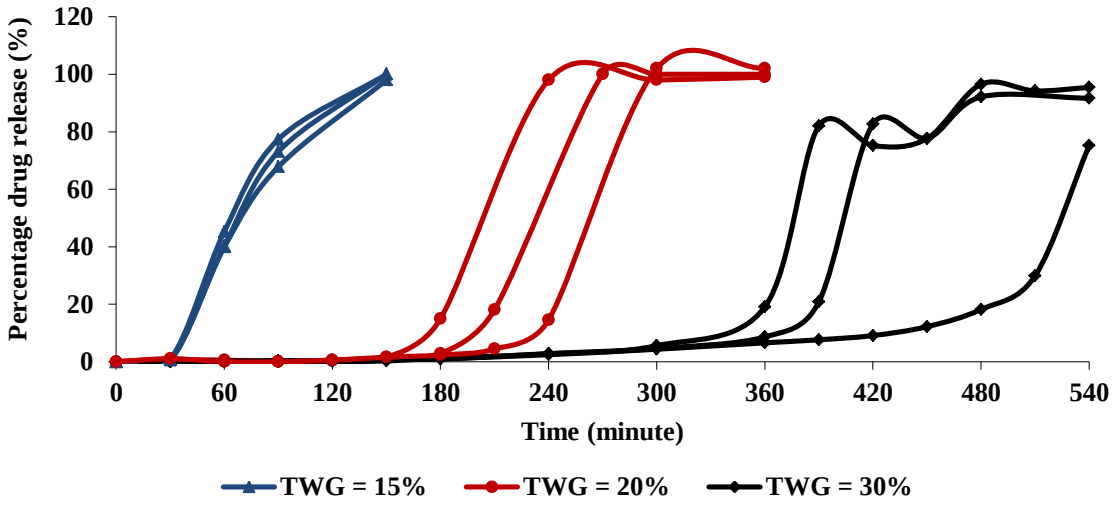


Figure 4.21 Dissolution profiles of paracetamol tablets coated with organic-based zein solution up to different coating levels (TWG = 15%, 20% and 30%) in SGF (with pepsin) pH 1.2. Each profile represents a single experiment.

A control experiment was carried out in SGF without pepsin to confirm that the rupture observed in the SGF with pepsin is due to the enzymatic influence rather than the coating efficiency; the results are shown in Figure 4.22. The dissolution profiles show that the drug release from coated paracetamol tablets (TWG = 20%) was retarded with no coating rupture over a 5 hour test duration in SGF without pepsin. Similarly, tablets coated up to 30% TWG did not exhibit rupture of the coating and the drug release was controlled even over an extended test duration of 9 hours. The lowest coating level (TWG = 15%) was associated with rupture of the film coating. This rupture is however more likely related to the mechanical stability of coating at this level suggesting that a coating level of 20% TWG is the lowest coating level that is required to control the drug release and prevent the abrupt rupturing of the coat.

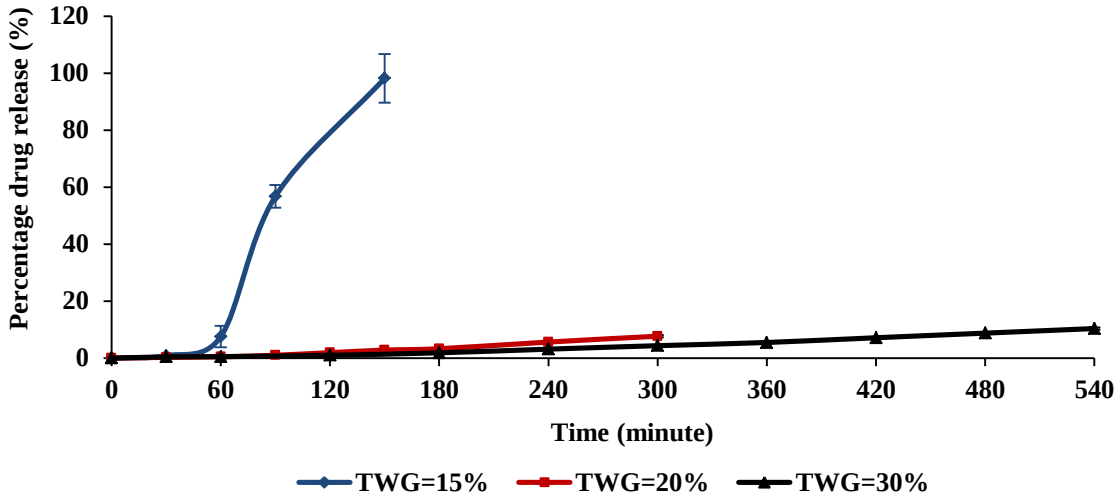


Figure 4.22 Dissolution profiles of paracetamol tablets coated with organic-based zein solution up to different coating levels (TWG = 15%, 20% and 30%) in SGF (without pepsin) pH 1.2. Each point represents the mean ± SD (n = 3).

The dissolution profile obtained when zein-coated tablets were tested in SGF, characterized by a lag period followed by an abrupt drug release, is quite different from that obtained by O'Donnell et al. (1997) where 100% of the drug was released from paracetamol tablets, coated with a 5% (of the tablet weight) zein pseudolatex containing 25% propylene glycol, 0.2% methyl paraben and 0.02% propyl paraben, within a 4 hour period in SGF with pepsin. This difference can be attributed to the different coating levels and composition used in this work.

The literature available on the susceptibility of zein to the digestive enzymes in the upper GIT is not conclusive. Katayama et al. (1992) reported that the drug release from zein tablets prepared by compressing spray-dried particles was facilitated by increasing the amount of pepsin in the release medium. On the other hand, organic-based free films of zein were found to be insoluble in gastric fluids but rapidly soluble in intestinal fluids by Kanig et al. (1962). Similarly, Liu et al. (2005) found out that only 50% of the tabletted microspheres of zein and ivermectin were degraded by pepsin after 8 days incubation and a complete degradation was obtained only after 13 days, suggesting that these microspheres and tabletted microspheres can endure the gastric degradation after oral uptake. Hurtado-Lopez et al. (2006) concluded that zein microspheres were extremely resistant to degradation in simple buffers in the absence of enzymes which explained the slow release of the entrapped solute in these media. However, significant and relatively fast degradation

of the microspheres occurred when they were incubated with pepsin and pancreatin enzymes, making the drug-loaded zein particles unsuitable for oral administration particularly if the drug is sensitive to extremes pH or to enzymatic degradation. Luo et al. (2010) found out that coating the selenite (hydrophilic nutrient)-loaded chitosan nanoparticles with zein greatly prolonged the drug release and offered sufficient protection for the nanoparticles against gastric conditions. Only 50% of the encapsulated selenite was released in SGF over 2 hours whereas 93% of the solute was released from the uncoated nanoparticles under the same conditions.

Takagi et al. (2003) and Fu et al. (2002) studied the digestibility of food allergens and non-allergenic proteins. Takagi et al. (2003) found that zein (prepared as a 0.5% solution in 10% dimethylsulfoxide) was rapidly digested in SGF with pepsin and SIF with pancreatin. Fu et al. (2002) measured the stability of proteins (prepared as 5 mg/mL in 0.03 M NaCl) in SGF and SIF (ratio of pepsin or pancreatin to test protein was 13:1) as the last time period in minutes that the protein could be seen in the SDS-PAGE gel; this was approximately 120 and 0.5 minutes when zein was incubated with pepsin and pancreatin, respectively. In summary, the studies quoted here used different sources of zein, different manufacturing methods and different testing protocols. Hence, a general conclusion to the susceptibility of zein to conditions resembling the upper gastrointestinal tract can not be easily drawn.

Figure 4.23 shows the influence of pepsin on the morphology of the tablet surface (TWG = 30 %), comparing the effects of exposure to SGF with pepsin (pH 1.2) and SGF without pepsin (pH 1.2) for 5 hours. The tablet surface was fairly smooth prior to any treatment (Figure 4.23, a) whereas some pores were formed onto a smooth and homogenous surface under acidic conditions without enzymes (Figure 4.23, b). Tablets tested in SGF with pepsin showed some signs of extensive digestion due to pepsin action (Figure 4.23, c).

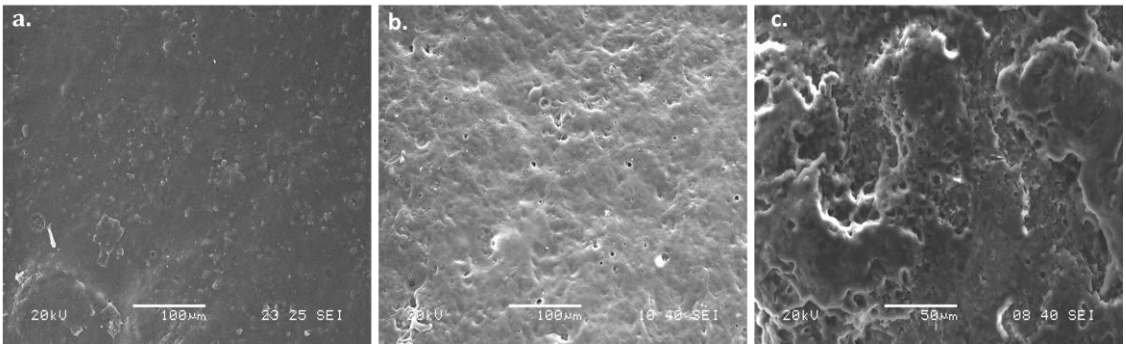


Figure 4.23 SEM images of paracetamol tablets coated with organic-based zein solution prior to any treatment (a, 250x) and after 5 hours test in SGF (without pepsin) (b, 250x) and SGF (with pepsin) (c, 500x).

4.3.4.6.2 Presence of Pancreatin

Pancreatin is a preparation of mammalian pancreas containing enzymes having protease, lipase and amylase activity. It may contain sodium chloride. Pancreatin contains in 1 mg not less than 1.4 units of free protease activity, not less than 20 units of lipase activity and not less than 24 units of amylase activity (British Pharmacopoeia, 2011). Figure 4.24 shows the influence of pancreatic enzymes on the drug release from paracetamol tablets coated with organic-based zein solution (TWG = 20%). The tablets remained intact and approximately 6% paracetamol was released in Simulated Intestinal Fluid (SIF) with pancreatin (pH 6.8) over 5 hours test duration. This result suggests that pancreatin, unlike pepsin, had no pronounced effect on zein films possibly due to the lower activity of the proteases present in the pancreatin. This result contradicts with the rapid digestion of zein in SIF (with pancreatin) seen in the aforementioned studies of Fu et al. (2002) and Takagi et al. (2003). This contradiction might be related to the difference in the way zein was presented to pancreatin.

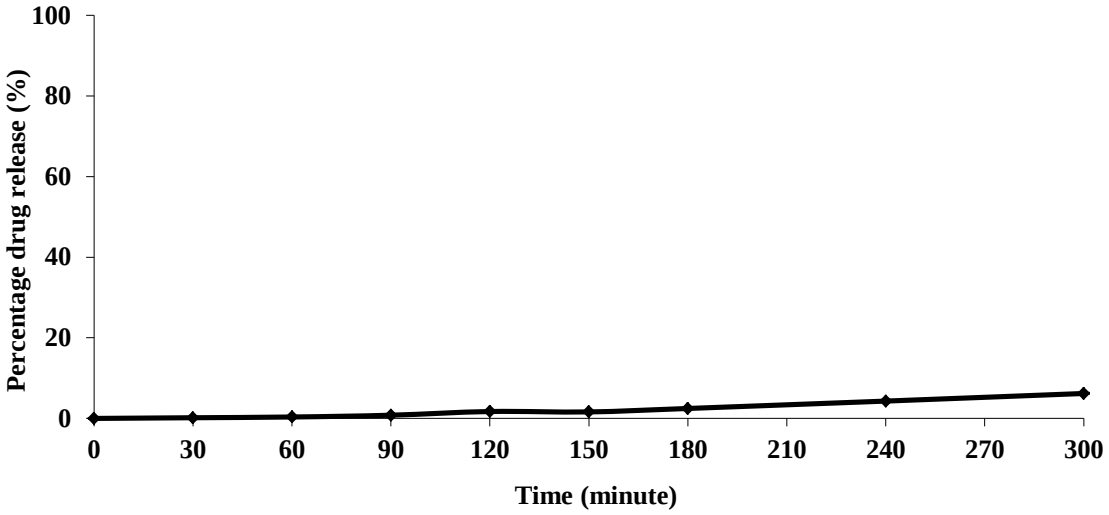


Figure 4.24 Dissolution profiles of paracetamol tablets coated with organic-based zein solution. (TWG = 20%) in SIF pH 6.8. Each point represents the mean ± SD (n = 3). (Error bars are very small).

Figure 4.25 shows the influence of pancreatin in SIF with pancreatin (pH 6.8) on the tablet surface as compared to the control condition i.e. phosphate buffer (pH 6.8). The tablet surface was relatively smooth prior to the test (Figure 4.25, a) whereas rimmed craters appeared on the surface of tablets tested in phosphate buffer pH 6.8 (Figure 4.25, b) and more significantly the tablet had a rough porous surface along with signs of digestion after a 5 hours test in SIF with pancreatin (pH 6.8) (Figure 4.25, c). However, the digestion was much less than that observed on tablets tested in SGF with pepsin over the same test duration (Figure 4.23, c), indicating that zein is more susceptible to the digestive action of pepsin in comparison with that of pancreatin. The susceptibility of zein to pancreatin as reported in literature is discussed in section 4.3.4.6.1.

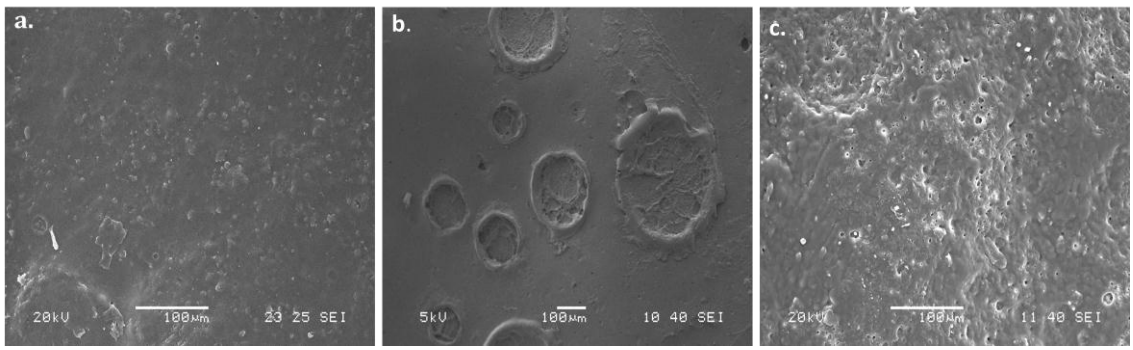


Figure 4.25 SEM images of paracetamol tablets coated with organic-based zein solution prior to any treatment (a, 250x) and after 5hours test in pH 6.8 (b, 100x) and SIF with pancreatin (pH 6.8) (c, 250x).

4.3.4.7 Dissolution in Fasting State Simulated Intestinal Fluid (FaSSIF)

A series of dissolution media which more closely reflect the biological situation than the simple SGF and SIF, has been developed in an attempt to better predict the in vivo performance of drug formulations, particularly where dissolution is the rate limiting step to drug absorption. Fasting state simulated intestinal fluid (FaSSIF) simulates the conditions in the proximal small intestine in the fasted state and was prepared according to Galia et al. (1998). Figure 4.26 presents the dissolution profile of paracetamol in FaSSIF medium with a pH value of 6.5, with no prior exposure to acid, from tablets coated with organic-based zein solution up to 20% TWG. The tablets remained intact with an average release of 9.5% after 8 hours test duration.

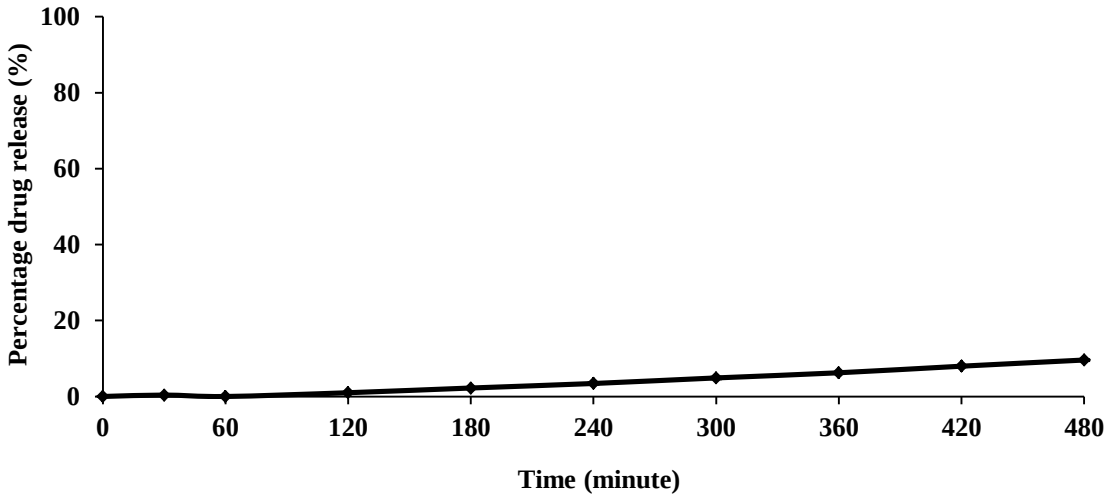


Figure 4.26 Dissolution profiles of paracetamol tablets coated with organic-based zein solution. (TWG = 20%) in FaSSIF pH 6.5. Each point represents the mean ± SD (n = 3). (Error bars are very small).

The drug release from coated tablets in FaSSIF (pH 6.5) was comparable to that obtained in the simple phosphate buffer (pH 6.8) indicating that the other components in this media, including the sodium taurochlorate (a bile salt) and lecithin (phosphatidylcholine), did not influence the integrity of zein coatings at least over the specified test duration (8 hours).

Figure 4.27 compares the morphology of the tablet surface when tested in FaSSIF (pH 6.5) as compared to simple phosphate buffer (pH 6.8). The surface of tablets tested in FaSSIF showed porous craters spread over a smooth surface (Figure 4.27, b); however, the craters were less in number and larger in diameter compared to those observed on the surface of tablets tested in phosphate buffer (Figure 4.27, c).

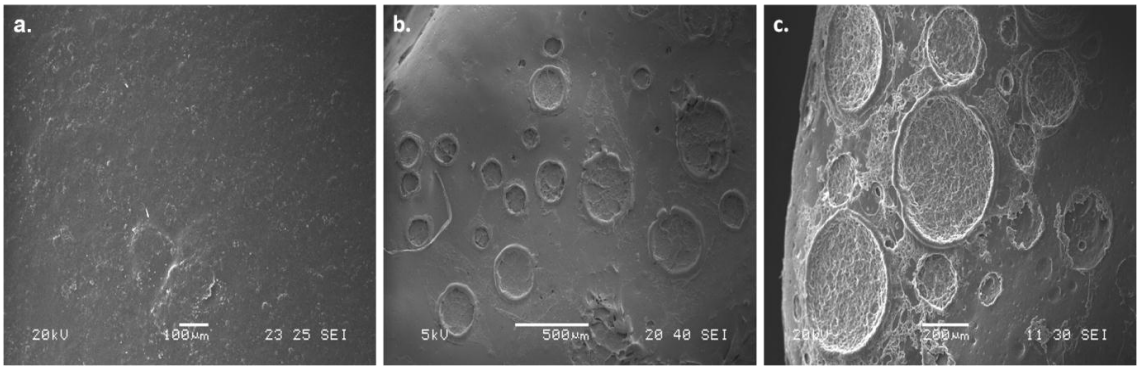


Figure 4.27 SEM images of paracetamol tablets coated with organic-based zein solution prior to any treatment (a, 100x) and after testing in phosphate buffer pH 6.8 (b, 50x) and FaSSIF pH 6.5 (c, 80x).

4.3.4.8 Dissolution in Simulated Colonic Fluid (SCoF)

Simulated colonic fluid (SCoF) was prepared according to Fotaki et al. (2005) (Table 4.4) and it was used to simulate the contents of the proximal colon. The pH is slightly acidic in the proximal colon, owing to the degradation of polysaccharides to short chain fatty acids with acetate, propionate and butyrate being the main organic anions (Leopold, 2001).

Table 4.4 The composition of Simulated Colonic Fluid (SCoF) according to Fotaki et al. (2005).

Buffer component	SCoF
NaOH	6.28 g
Acetic acid	10.20 g
Distilled water	To 1000 mL
pH	5.8

Figure 4.28 displays the dissolution profile of paracetamol tablets coated with organic-based zein solution up to 20% TWG in SCoF medium with a pH value of 5.8. The tablets remained intact with an average release of 8% after 8 hours test duration.

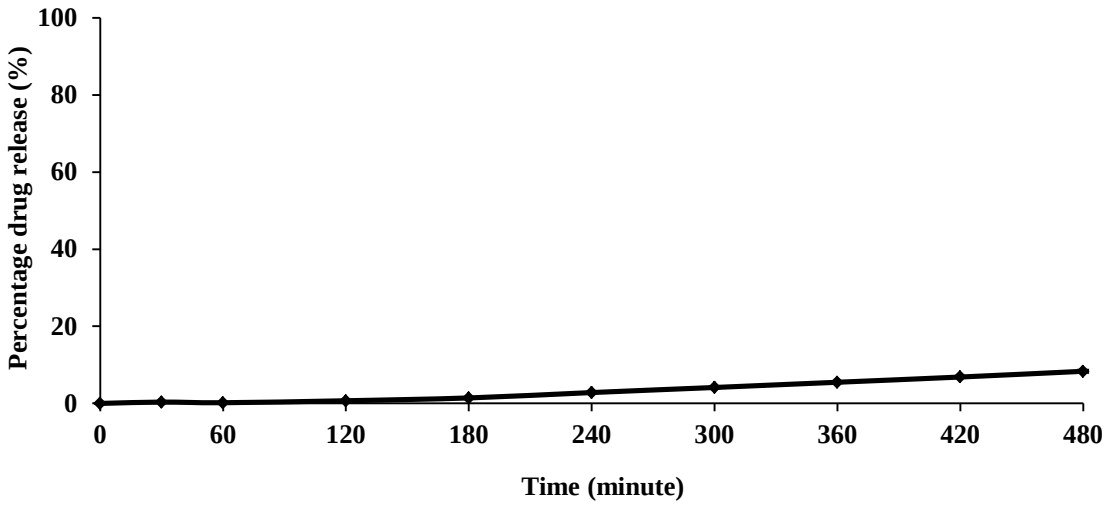


Figure 4.28 Dissolution profiles of paracetamol tablets coated with organic-based zein solution. (TWG = 20%) in SCoF pH 5.8. Each point represents the mean ± SD (n = 3). (Error bars are very small).

SEM images of the tablet prior to any treatment had a fairly smooth surface (Figure 4.29, a), whereas the surface developed some rimmed craters in phosphate buffer (pH 6.8) (Figure 4.29, b), but only some pores when tested in SCoF (pH 5.8) (Figure 4.29, c).

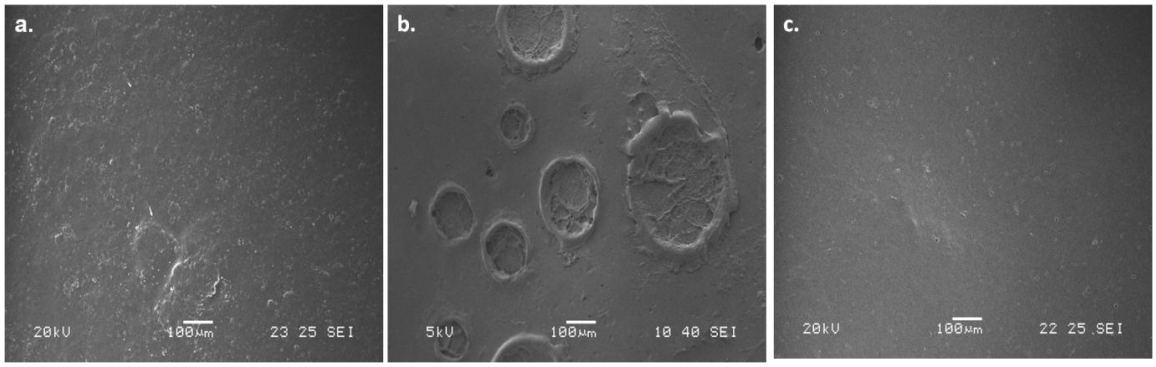


Figure 4.29 SEM images (100x) of paracetamol tablets coated with organic-based zein solution prior to any treatment (a) and after testing in pH 6.8 (b) and SCoF pH 5.8 (c).

4.4 Conclusions

Zein coatings were found to be quite resistant to degradation in simple buffers of acidic (pH 1) and basic (pH 6.8) pH values. The release pattern was found, as determined by a pH change method, to remain constant regardless of the pH of the medium with the maximum release being 14% after a total of 8 hours in SGF without pepsin (pH 1.2) and phosphate

buffer (pH 6.8). Zein was, however, susceptible to the digestive action of pepsin, a protease naturally secreted in the stomach, which resulted in breaking of the film coating. The period that preceded the coat rupture showed minimal drug release and was referred to as lag period. The lag period was dependent on the original coating thickness, i.e. thicker coats needed longer times before the rupture occurred. This can be explained by the fact that the different layers of the film coat were being digested gradually by the enzyme and therefore thicker coats needed more time before the remaining (undigested) film closer to the tablet core became too thin to counterbalance the internal pressure of the dissolved core material. On the other hand, the coatings seemed to be much less susceptible to pancreatin in SIF. The drug release rate and extent in the more complex media, i.e. FaSSIF (pH 6.5) was comparable to that obtained in the phosphate buffer (pH 6.8). Furthermore, neither the dissolution apparatus nor the rotation speed had an influence on the dissolution rate of the drug.

According to these results, zein coatings of the specified thickness (TWG = 20%) were not degraded under extreme pH values; and the digestive enzyme in SGF BP (pepsin 0.32% w/v) resulted in coating rupture only after 4 hours of the test. However, the transit time of a solid dosage form in the stomach is approximately 2 hours, as reviewed by Yang (2008). Moreover, the coating was not susceptible to the pancreatin action suggesting that the coated tablet might potentially enter the colon intact. Hence, these results highlight a potential use for zein coatings as a possible system for colonic-specific drug delivery.

Chapter 5 discusses the influence of incorporating polysaccharides into zein-based films on the drug release in the upper GIT. The potential use of zein alone and zein/polysaccharide mixed coatings for colon-specific drug delivery is investigated in Chapter 6.

5 Chapter 5 The Potential Use of Zein a Film Coating Material; b) applied as an aqueous dispersion (pseudolatex) and incorporating polysaccharide

5.1 Introduction

Film-coating polymers used in modified-release formulations are generally water-insoluble, a key attribute to achieving their intended functionality. Thus, solutions of these polymers in organic solvents have been used during film coating of tablets. Zein coatings applied as an organic solution were able to retard the drug release in conditions simulating the upper gastrointestinal tract (GIT), as discussed in Chapter 4, which can be explained by zein's water insolubility and its hydrophobic nature. On the other hand, polysaccharides such as pectin and high amylose maize starch (HAS) are hydrophilic in nature and are thus able to dissolve and/or swell in aqueous media. Zein could therefore be potentially used as a carrier of these polysaccharides in formulations designed for colon-specific drug delivery. However, the use of organic-based zein solutions (75% aqueous ethanol), described in Chapter 4, did not allow the addition of the aqueous-based systems of polysaccharides due to the decrease in the solubility of zein at higher water/ethanol ratio. In this part of the project, the development of aqueous coating formulations was therefore necessary to incorporate the aqueous-based solutions or dispersions of polysaccharides into zein-based coatings. Oral formulations intended for colonic targetting should ideally prevent extensive drug release in the upper GIT. Thus, the ability of zein to restrain the swelling properties of the polysaccharides and potentially retard the drug release in the upper GIT should be investigated if colonic drug delivery is to be achieved.

This chapter therefore investigates the use of aqueous dispersion (pseudolatex)-based zein film coatings and the drug release from tablets coated with such systems. The influence of some process parameters such as coating level, plasticization level and curing time is examined. More importantly, the influence of incorporating the aforementioned polysaccharides in pseudolatex-based zein coatings on the drug release, particularly in the upper GIT is also investigated.

5.2 Methods

This section describes the methods used in the preparation and film coating of paracetamol tablets and preparation of free films. The characterization techniques and their experimental parameters are also provided. A more detailed description of the principle of these techniques is given in Chapter 2.

5.2.1 Preparation of Aqueous Dispersions (Pseudolatexes)

Aqueous colloidal dispersions are classified into true latexes and pseudolatexes based on the technique of manufacture (Wheatley et al., 1997). Pseudolatexes are prepared by emulsification of an existing thermoplastic water-insoluble polymer, i.e. by preparing a polymer solution in an organic solvent, which is then emulsified in an aqueous phase containing surfactants and stabilisers. Homogenisation followed by solvent removal forms the aqueous colloidal dispersion. Aqueous zein dispersions or pseudolatex (10 % w/w) were prepared by a precipitation method (Li et al., 2010) and plasticized with 30% w/w (based on zein amount) polyethylene glycol 400 (PEG 400). To add the acetylated high amylose maize starch (HAS), three grams (3 g) of HAS was dispersed in 20 mL of distilled water and heated at 80 ± 5 ºC for 30 minutes, based on the work of Freire et al. (2009a). The required amount of the heat-processed HAS was transferred to another beaker and diluted with preheated water (80 ± 5 °C) to give a final concentration of approximately 1.8 % w/w. The attained dispersion was allowed to cool down to room temperature (circa 26 °C) and added to the plasticized aqueous zein dispersion. Different solid ratios of HAS and zein (1:7, 1:5 and 1:3) were mixed. The HAS/Zein dispersion was stirred overnight to achieve complete mixing of the two polymers.

Pectin was added to the aqueous dispersions of zein in 1:30 pectin/zein ratio. These dispersions were prepared by a precipitation method (Oshlack et al., 1994) and were plasticized with 30% w/w (based on zein amount) propylene glycol (PG); 0.2% w/v methyl parabens (MP) and 0.02% w/v of propyl parabens (PP) were added as preservatives (based on the final volume of the aqueous dispersion). The composition of aqueous dispersions (pseudolatexes) of zein is shown in Table 5.1.

Table 5.1 The composition of aqueous dispersions (pseudolatexes) of zein. The % w/w of each component in the film coating is shown in brackets.

Formulation	Composition of aqueous dispersion (100 mL)					
	Zein	Pectin	HAS	PEG400	PG/MP/PP	Tween 80
1 Pectin/Zein (1:30) aqueous dispersion	10 g	0.3 g			3 g/0.2 g/0.02 g	
2 Zein alone aqueous dispersion (control)	10 g				3 g/0.2 g/0.02 g	
3 HAS/Zein (1:5) aqueous dispersion	10 g (62.5%)		2 g (12.5%)	3 g (18.75%)		1 g (6.25%)
4 Zein alone aqueous dispersion (control)	10 g			2 or 3 g		1 g
5 HAS/Zein (1:3) aqueous dispersion	10 g (58%)		3.3 g (19%)	3 g (17%)		1 g (6%)

5.2.2 Preparation of Free Films

Free films were prepared by a casting/solvent evaporation technique. The aqueous dispersions (9 g each) of zein, HAS and HAS/Zein mixtures produced in Section 5.2.1 were poured onto 90 mm plastic petri dishes and dried in an oven at 45 ± 5 ºC for 24 hours. After drying, the films were stored in a desiccator over P_2O_5 (0% RH) at room temperature for subsequent studies. The film thickness was measured using a micrometer (Outside micrometer, 0-25 mm).

5.2.3 Film Coating of Paracetamol Tablets

Dibasic calcium phosphate-based paracetamol tablets, as prepared in Chapter 2, were initially subcoated with organic-based zein solution (10% w/w plasticized with 20% w/w glycerol) up to 2 to 3% total weight gain (TWG, based on tablet weight) using a top spray fluidized bed coater (Mini Coater/Drier 2, Caleva Process Solutions Ltd, UK). The tablets were subcoated to prevent the dissolution of the tablet surface on subsequent exposure to the aqueous dispersion. The tablets were then top coated with the zein aqueous dispersion. Dibasic calcium phosphate-based (n = 10) tablets were placed in the coating chamber. The atomization pressure was 0.7 bar and the pump rate was 1.09 rpm. The fan speed was 13 to

13.5 m/s (70 to 75%) with no agitation. The height for the spray head was 65 mm from the spray nozzle to the mesh in the base of the cone. After coating, the tablets were left in the coater (bed temperature = 45 ˚C) for 15 minutes to dry. The coating thickness is expressed in terms of the percentage total weight gain (TWG), and tablets with a TWG of 10 or 20% were obtained. These TWG values are, as determined in Chapter 4, equivalent to film thickness of circa 106 and 203 µm, respectively. Tablets coated with HAS/Zein pseudolatex were cured in an air oven at 45 ˚C for 10 hours, whereas those coated with pectin/zein aqueous dispersion were cured at the same temperature for 24 hours. Tablets were stored in sealed amber glass bottles until use.

5.2.4 Dissolution Studies

Drug release studies were performed using a BP Apparatus I dissolution bath in 900 mL of dissolution medium at 37 ± 0.5 ºC with a rotation speed of 50 rpm. To test the formulation stability in the upper GIT, freshly prepared simulated gastric fluid (SGF, 0.32% w/v pepsin) was used as the dissolution medium for the first 2 hours and was then replaced by freshly prepared simulated intestinal fluid (SIF, 1% w/v pancreatin) for an additional 6 hours. At predetermined sampling points, 10 mL of sample was collected from each vessel using a syringe, filtered through a 0.2 µm membrane filter and replaced with an equivalent volume of fresh medium. Samples that contained the pancreatic digestive enzymes were centrifuged at 13,000 rpm for 30 minutes prior to filtration and UV measurements. The paracetamol concentration was determined using a UV-VIS spectrophotometer set at as wavelength of 243 nm and reference to an appropriate standard curve. The dissolution studies were carried out in triplicate under sink conditions and the average drug release ± SD was calculated. The drug release profiles were compared on the basis of the area under the curve (AUC) calculated by the trapezoidal method using a 2-tailed T-test (2 samples).

5.2.5 Characterization Techniques

5.2.5.1 Scanning Electron Microscopy (SEM)

The surface morphology of coated tablets before and after dissolution tests and of zein alone and HAS/Zein free films was observed using SEM. Samples were mounted onto stubs using double-sided tape and were gold coated by a Polaron SC7640 sputter gold

coater manufactured by Quorum Technologies. The thickness of the gold coating was about 15 nm. The imaging process was performed in a high vacuum environment. Imaging process was performed with a JEOL JSM5900 LV SEM (Japan), mounted with a tungsten filament with an acceleration voltage of 5-20 kV.

5.2.5.2 Laser Diffraction (LD)

The particle size distribution of zein alone, HAS alone and HAS/Zein aqueous dispersions was measured using a Sympatec HELOS/BF (HI146, TYPE: cuv-50 mL/US, Germany) laser diffraction particle size analyser. A droplet of the dispersion was added to the quartz cuvette using water as the dispersion medium and each sample was scanned 4 times using a small lens of size detection range of 0.25/0.45 to 87.5 µm. Measurements were performed in triplicate and the average density distribution (y-axis) was then plotted against the particle size on a log scale (x-axis).

5.2.5.3 X-Ray Powder Diffraction (XRPD)

X-Ray Powder Diffraction analysis of HAS samples (prior and after heat-treatment) was performed with a XRPD, Thermo ARL Xtra model (Switzerland) equipped with a copper X-ray tube (1.540562 Å). Samples were exposed to an X-ray beam with a voltage of 45 kV and a current of 40 mA. Other test conditions were: scanning range at 2θ of 5 to 60°, step size 0.01°, and time per step: 0.5 seconds.

5.2.5.4 Attenuated Total Reflectance-Fourier Transform Infra-Red (ATR-FTIR)

Infrared spectra of zein powder, HAS samples (prior and after heat-treatment), zein alone free films and HAS/Zein mixed films were recorded in the wave number range of 4000 to 550 cm^{-1} using an IFS-60/S Fourier transform infrared (Bruker Optics, Coventry, UK) with an attenuated total reflectance (ATR) accessory (SPECAC, Orpington, UK). For each sample a total of 16 scans were performed at a resolution of 4 cm^{-1}. Spectra were analysed using OPUS software (version 6.0).

5.2.5.5 Thermogravimetric Analysis (TGA)

Thermogravimetric analysis was performed using Hi-Res TGA 2950 (TA Instruments, Newcastle, USA) in order to measure the water content of the free films. To identify water content as measured by weight loss due to sample dehydration, samples were heated from room temperature to 100 ºC at a heating rate of 20 ºC/minute, and then were held (isothermally) at 100 ºC for 10 minutes.

5.2.5.6 Modulated Temperature Differential Scanning Calorimetry (MTDSC)

Thermal behaviour of various samples including zein alone free films and HAS/Zein mixed films was studied by means of MTDSC using a Differential Scanning Calorimeter (Q2000, TA Instruments, Newcastle, USA). Samples (n = 3) were accurately weighed (1 to 3 mg) into pinhole aluminium pans (Perkin Elmer) in order to minimize the influence of water on the sample's behaviour. Samples were scanned between -20 ºC and 250 ºC with optimized modulation temperature amplitude of ± 1.0 ºC, modulation time of 60 s and a ramp rate of 2 ºC/minute. Temperature and cell constant calibration of the DSC instrument was performed with indium, n-octadecane and tin by using their melting point, whereas the heat capacity was calibrated with aluminium oxide in modulated mode.

5.2.5.7 Nano-Thermal Analysis (n-TA)

Nano-thermal analysis (n-TA) was performed using an Anasys Instruments Nano TA2 Controller with Veeco Calibre AFM head. Thermal nanoprobes (AN-2 probes, Anasys Instruments, Santa Barbara, CA) were used. The scan area for all AFM images was 50 µm x 50 µm with a resolution of 512 pixels and a scan rate of 0.5 Hz. The nanoprobes were heated from room temperature to 200 °C at a heating rate of 10 °C/s. Tapping mode was operated by oscillating the probe at its resonant frequency and scanning with the same parameters as mentioned previously; however, the probes were held at constant temperature during the run using the nano TA2 controller.

5.3 Results and Discussion

5.3.1 Dissolution Studies on Tablets Coated with Zein Alone Pseudolatex

In this section, the influence of some formulation parameters and testing conditions on the drug release from paracetamol tablets coated with zein alone pseudolatex (formulation 4, Table 5.1) is studied. These include the plasticization level, coating level, curing time, pH value of the dissolution medium and the presence of digestive enzymes.

5.3.1.1 Influence of Plasticizer Level

Film-coating polymers are known to be brittle in nature, and therefore plasticizers are generally added to the formulation to modify the physical properties of the polymer (Porter, 2007). Polyethylene glycol 400 (PEG 400) is a water soluble plasticizer and was incorporated in zein aqueous dispersions at two levels: 20 and 30% w/w (based on the amount of zein). Figure 5.1 shows the influence of the plasticizer level (20 and 30%) on the drug release from tablets coated with zein alone pseudolatex up to 10% TWG in 0.1 M HCl (pH 1). Both release profiles showed a lag time of circa 1 hour prior to approaching a linear release profile over the remaining test duration (5 hours total). The drug release was, however, slightly ($p > 0.05$) faster at the higher plasticizer level (30 % PEG 400). The smaller error bars associated with the dissolution profile of tablets coated with a 10% (TWG) zein pseudolatex coating containing 30% w/w PEG 400 (based on zein amount), compared to those coated with zein dispersion plasticized with 20% w/w PEG 400, can be attributed to the improved integrity of the coating achieved by the plasticization effect of the higher quantity of PEG 400.

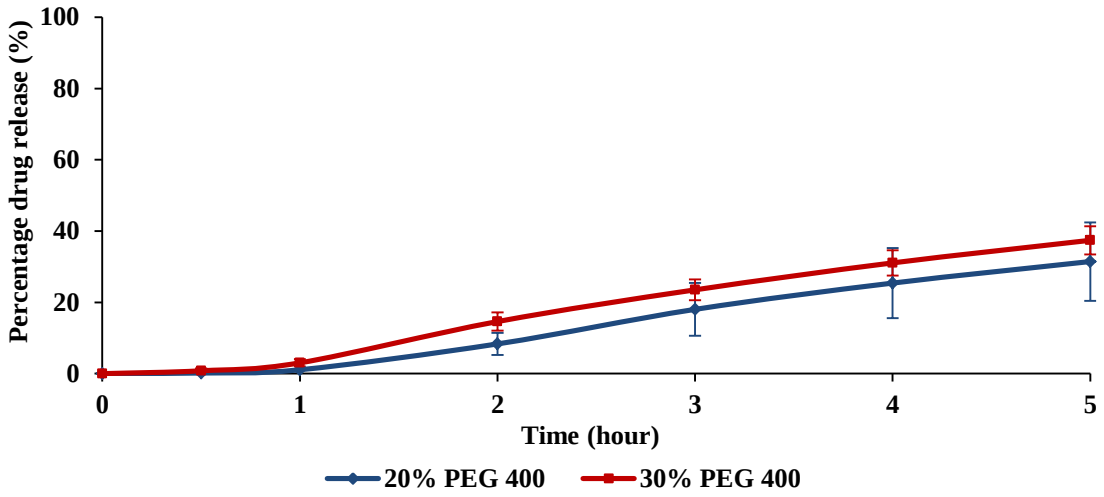


Figure 5.1 Dissolution profiles in 0.1 M HCl of paracetamol tablets coated (TWG = 10%) with zein alone pseudolatex (plasticization level = 20% and 30% PEG 400). Each point represents the mean value ± SD (n = 3).

Figure 5.2 shows the influence of the PEG 400 level (20 and 30 %) on the drug release from tablets coated with zein alone pseudolatex up to 20 % TWG in 0.1 M HCl (pH 1). The dissolution profiles showed a lag time of circa 2 hours, which is longer than that observed at lower coating levels (TWG = 10%). The drug release in 0.1 M HCl from tablets with the film coating plasticized with 30 % PEG 400 was significantly ($p < 0.05$) higher than that obtained with the lower level of plasticizer (20 %), with the maximum release at 5 hours being 9% and 6%, respectively.

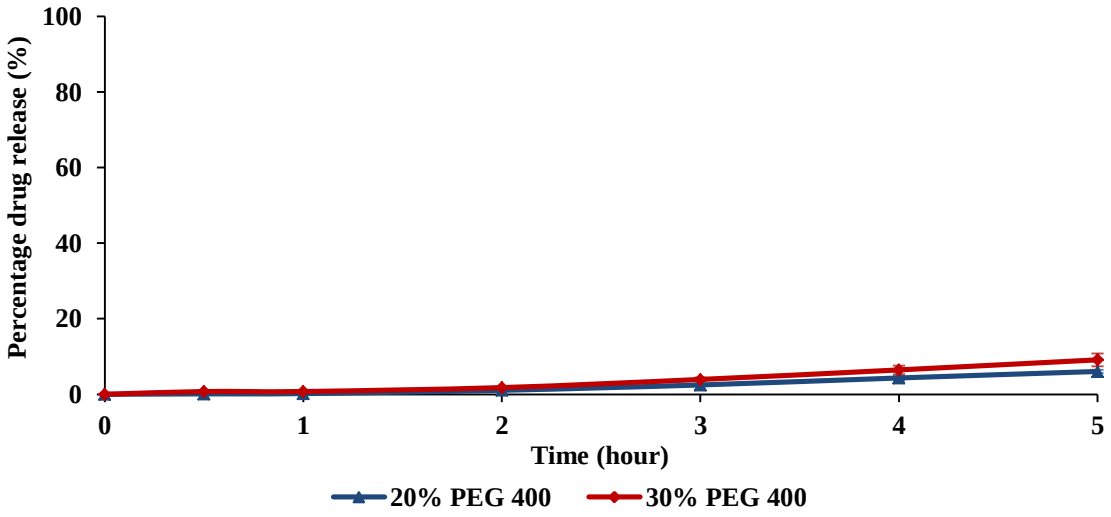


Figure 5.2 Dissolution profiles in 0.1 M HCl of paracetamol tablets coated (TWG = 20%) with zein alone pseudolatex (plasticization level = 20% and 30% PEG 400). Each point represents the mean value ± SD (n = 3). (Error bars are very small).

This increase in dissolution with the higher plasticization level at both coating thicknesses can be explained by the fact that PEG 400 is water soluble and is expected to dissolve once in contact with aqueous media forming pores within the film coat. Higher porosity and subsequently higher permeation and release rates are therefore expected at higher plasticization levels. In addition, plasticizers tend to increase the free volume and facilitate polymer chain motion within the coating structure; therefore, higher plasticizer levels will also facilitate the drug diffusion through the spaces (aqueous channels) formed between the polymeric chains by enhancing the polymeric chain motion. It is worth noting that regardless of the plasticization level, the drug release is still very low at the higher coating level (TWG = 20%) and the difference although statistically significant might possibly not be biologically significant.

5.3.1.2 Influence of Coating Level

The influence of coating thickness (TWG = 10 or 20%) on paracetamol release from tablets coated with zein alone pseudolatex with a plasticization level of 20% w/w (based on zein amount) in 0.1 M HCl is shown in Figure 5.3. Figure 5.4 shows the influence of the coating thickness on the drug release from pseudolatex-based zein coatings plasticized with 30 % PEG 400. Figure 5.3 and Figure 5.4 use the data previously shown in Figure 5.1 and Figure 5.2. The dissolution profiles show that the drug release rate is inversely proportional to the thickness of the coat; increasing the coating level from 10 to 20% slowed down the drug release, regardless of the plasticization level. The duration of the lag time also decreased when coatings of lower thicknesses were used; lag periods of approximately 1 and 2 hours were obtained at coating levels (TWG) of 10 and 20 %, respectively, regardless of the % of PEG 400.

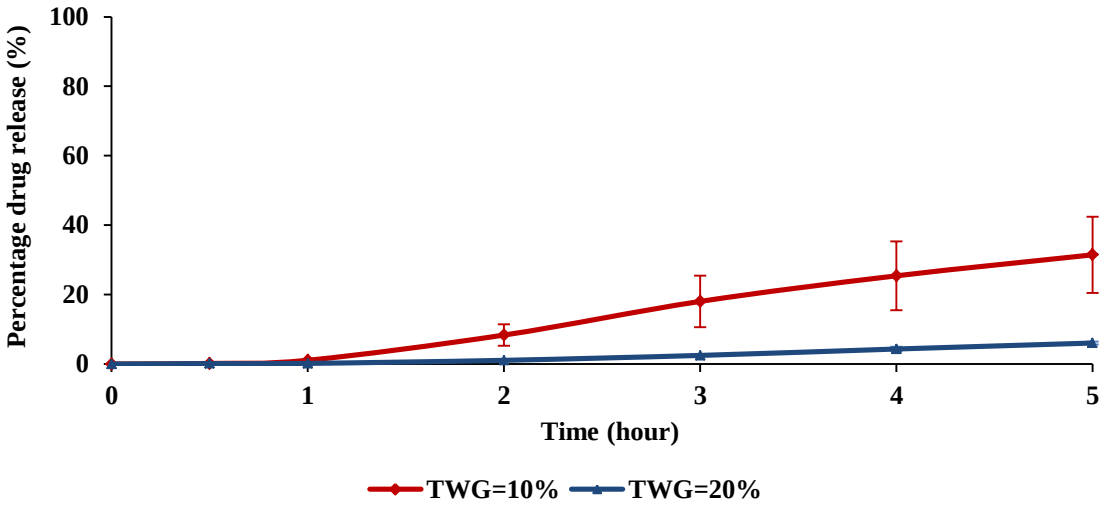


Figure 5.3 Dissolution profiles in 0.1 M HCl of paracetamol tablets coated (TWG = 10 % and 20 %) with zein alone pseudolatex (plasticization level = 20% PEG 400). Each point represents the mean value ± SD (n = 3).

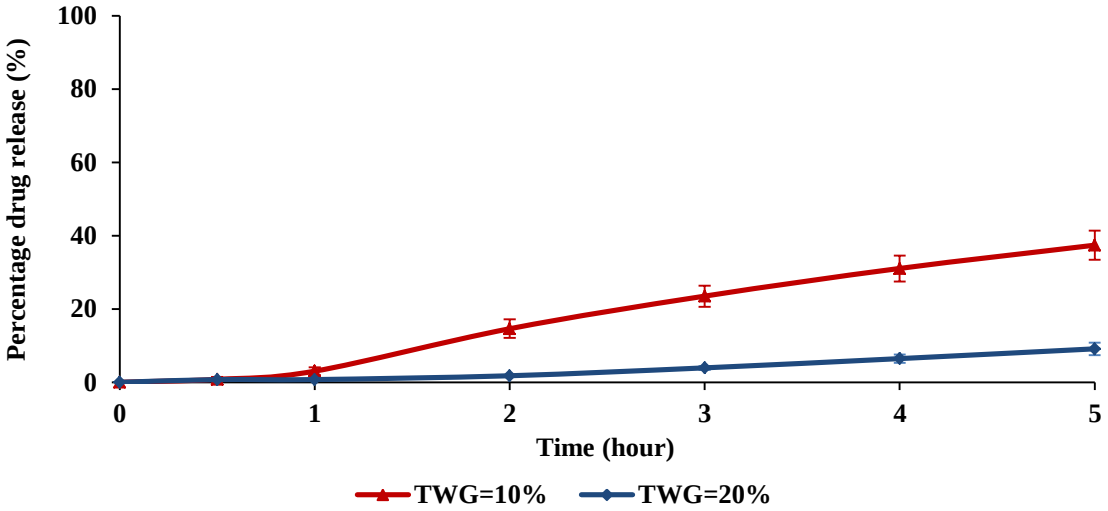


Figure 5.4 Dissolution profiles in 0.1 M HCl of paracetamol tablets coated (TWG = 10 % and 20 %) with zein alone pseudolatex (plasticization level = 30% PEG 400). Each point represents the mean value ± SD (n = 3).

The increase in drug release upon decreasing the coating thickness from 20 % to 10% TWG was significant ($p < 0.05$) at both plasticization levels 20 % and 30 %, suggesting that the film coat was controlling the drug release process. This result can be partly explained by the longer diffusion path the aqueous medium needs to cross into the tablet core and the dissolved drug needs to cross for the release to occur from tablets with higher coating levels. In addition, if one wishes to consider the influence of the osmotic gradient formed across the membrane (discussed in further detail in section 5.3.1.4) and the subsequent film stretching and cracking or pore widening, thicker coatings are known to

offer higher mechanical strength and hence higher ability to restrain the swelling of tablets upon contact with the aqueous media. In short, a stronger expansion is required to stretch and perforate coatings of high thickness (Zhang et al., 2003). Moreover, coatings of higher thickness are composed of a higher number of polymeric layers which will minimize the influence of coating process defects such as the probability of forming continuous water-filled channels through which the drug release can occur, and enhance the reproducibility of dissolution (Siepmann et al., 2007). The larger error bars obtained at lower coating levels (10%) compared to the higher levels (20%), as noticed in Figure 5.3 and Figure 5.4 reflect a poorer reproducibility of the performance of the coatings at lower coating levels.

5.3.1.3 Influence of Curing Time

Curing of tablets directly after coating with aqueous dispersions involves thermal treatment at elevated temperatures and aims to accelerate the film formation process, to achieve a better coalescence of the colloidal polymer particles and hence to avoid stability problems upon storage such as changes in drug release profiles (Wesseling et al., 1999). Figure 5.5 represents the influence of curing time on paracetamol release from tablets coated with zein alone aqueous dispersions and tested in 0.1 M HCl directly after the coating and curing steps. The dissolution profiles of coated tablets without a curing step and of those cured for 10 and 24 hours showed similar release kinetics with the maximum release being 9% after 5 hours dissolution test in 0.1 M HCl. This result suggests that the film coatings were completely formed right after the coating and the subsequent drying steps in the coater. The high plasticization levels might have eliminated the need for the curing step. Bodmeier et al. (1994) reported that curing had little effect at plasticizer levels above 25 % due to the good coalescence of the polymer particles achieved during the coating step. No obvious difference in the drug release profiles of the coated tablets cured for 10 or 24 hours as compared to those of the non-cured coated tablets. However, the importance of this step in minimizing changes in drug release profile over the age of the substrate on storage (i.e. aging effects) has been reported in literature. The tablets were, therefore, cured at 45 ºC, over an intermediate curing time of 10 hours. The influence of curing on the stability of the pseudolatex-based zein coated tablets was, however, not investigated in this part of the study.

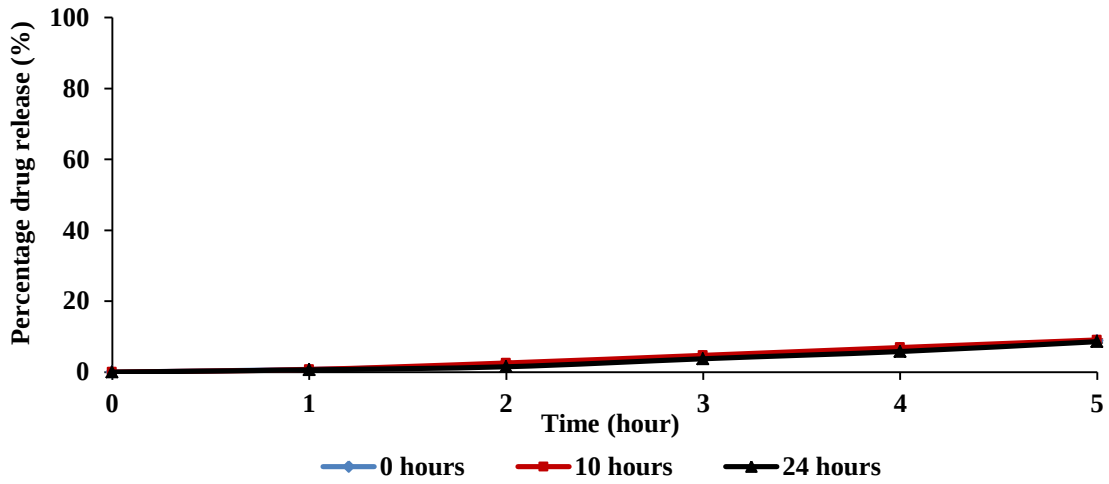


Figure 5.5 Dissolution profiles in 0.1 M HCl of paracetamol tablets coated (TWG = 20%) with zein alone pseudolatex (plasticization level = 30% PEG 400) and cured for 0, 10 and 24 hours. Each point represents the mean value ± SD (n = 3). (Error bars are very small).

5.3.1.4 Influence of Dissolution Media pH

Figure 5.6 presents the average percentage release of paracetamol from tablets coated (TWG = 20%) with zein alone aqueous dispersion (plasticization level = 30% of PEG 400) in 0.1 M HCl (pH 1) and phosphate buffer (pH 6.8). The drug release was higher ($p < 0.05$) in 0.1 M HCl compared to the more basic conditions with the maximum release being 66% and 49%, respectively, after 24 hours. Zero-order release kinetics were obtained in both 0.1 M HCl (pH 1) and phosphate buffer (pH 6.8) with r^2 values of 0.9970 and 0.9906, respectively. Both release profiles showed a lag period of circa 2 and 3 hours, respectively. The lag time is thought to be the time needed to form aqueous channels within the film coating. Aqueous channels can potentially be formed by the dissolution of the water-soluble components of the coating membrane such as the plasticizer (i.e. PEG 400). It has been also suggested by Oh et al. (2010) that zein membranes hydrate and swell, forming aqueous channels through which drug diffusion may occur.

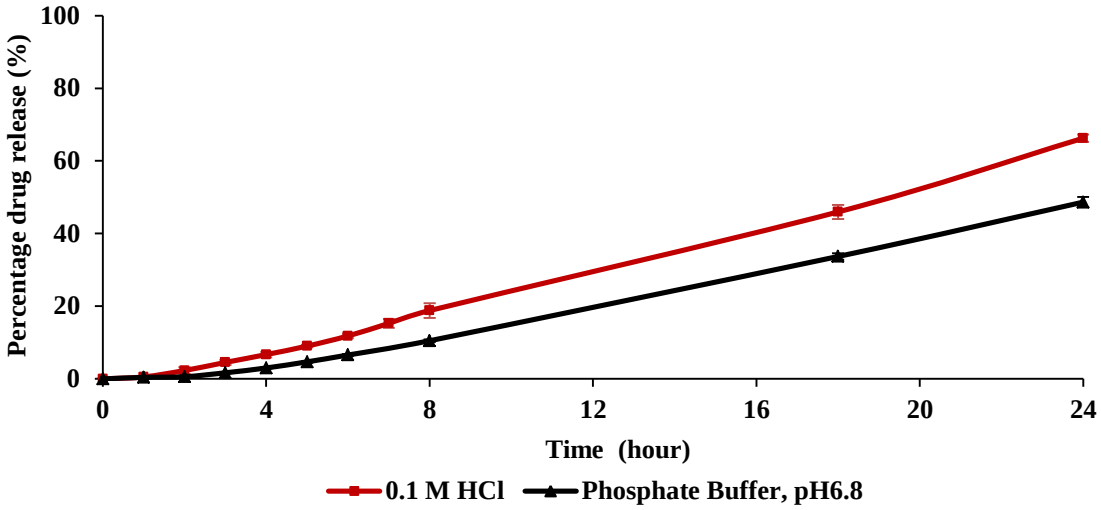


Figure 5.6 Dissolution profiles in 0.1 M HCl (pH 1) and phosphate buffer (pH 6.8) of paracetamol tablets coated (TWG = 20%) with zein pseudolatex alone (plasticization level = 30% of PEG 400). Each point represents the mean value ± SD (n = 3).

The higher drug release at low pH can be partially explained by the preferential solubility of the tablet core excipient in 0.1 M HCl as compared to its solubility in phosphate buffer (pH 6.8). Dibasic calcium phosphate (DCP), which counts for 57% of the tablet core, is a salt that dissolves in dilute acids. Therefore, the tablet core is expected to dissolve in 0.1 M HCl once the aqueous channels through which the medium can penetrate form. Thus, high osmotic pressures would be generated within the coated tablet which will work as a driving force for the water influx. The osmotic pressure may cause swelling of the tablet core and can ultimately affect the drug and solvent permeability possibly by decreasing the film thickness and widening of the inherent pores in the coating upon swelling of the tablet core and stretching of the polymeric coating.

O'Donnell et al. (1997) found that the drug release from paracetamol tablets coated with zein pseudolatex occurred at a faster rate in 0.1 M HCl and the rate decreased with increasing the pH, suggesting a greater solubility of the zein in an acidic environment. On the other hand, the pH of the dissolution media had a negligible effect on the release of paracetamol from tablets coated with a 4.39% (of the tablet weight) zein pseudolatex as stated by Oshlack et al. (1994). In Chapter 4, the influence of the pH value of dissolution medium on the drug release from tablets coated with an organic-based zein solution was assessed by subsequent changing of the pH of the medium. The release pattern remained constant regardless of the pH of the medium with the maximum release being 14% after a

total of 8 hours in SGF (without pepsin) and phosphate buffer (pH 6.8). Thus, the osmotic effect of the DCP is more likely responsible for the higher drug release in acidic conditions.

SEM images of paracetamol tablets coated with zein alone pseudolatex prior to any treatment and after testing in 0.1 HCl (pH 1) and phosphate buffer (pH 6.8) are shown in Figure 5.7. The images of the tablets surface showed similar surface features in both media; however, a greater porosity level was observed at low pH compared to the relatively smooth tablet surface prior to any treatment.

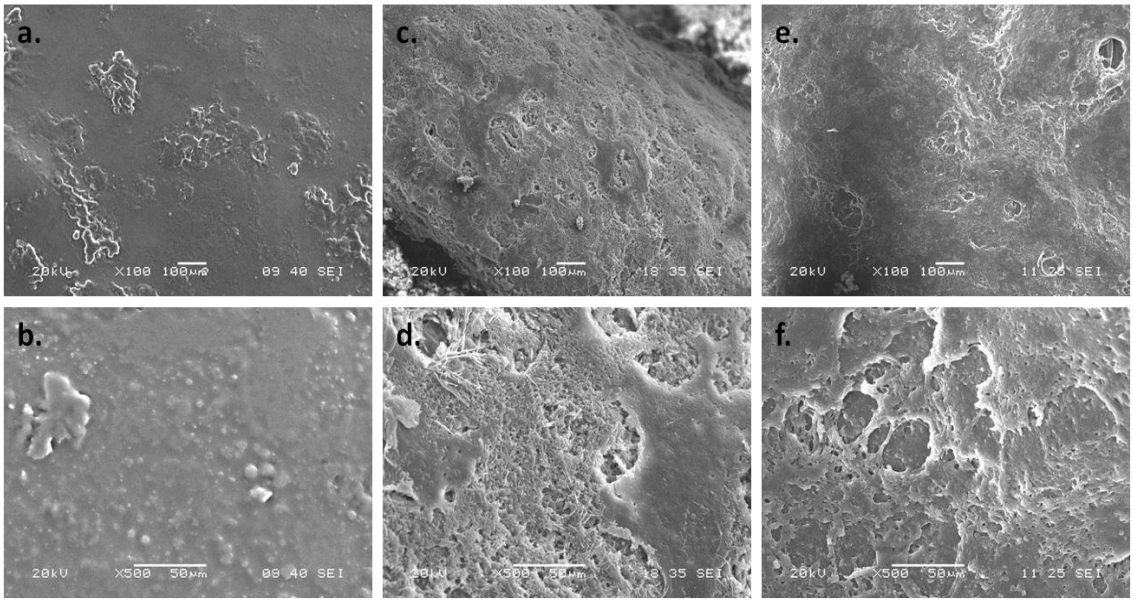


Figure 5.7 SEM images (100x and 500x) of paracetamol tablets coated with zein alone pseudolatex prior to any treatment (a and b) and after 24 hours test in 0.1 M HCl (c and d) and phosphate buffer pH 6.8 (e and f).

5.3.1.5 Influence of Digestive Enzymes

The drug release was also assessed in conditions simulating the stomach and the small intestine, where the transit times of a solid dosage form are approximately 2 hours and 4hours, respectively, as reviewed by Yang (2008). Figure 5.8 shows the paracetamol release from tablets coated (TWG = 20%) with zein alone pseudolatex (plasticized with 30% PEG 400) tested in simulated gastric fluid with pepsin (SGF with pepsin) for 2 hours and subsequently in simulated intestinal fluid with pancreatin (SIF with pancreatin) for a further 6 hours. Only 1.65% of the drug content was released at the end of the 2 hours test

in SGF with pepsin and the drug release was almost zero during the first hour. The dissolution profile indicates that zein films confer release protection under conditions simulating the upper GIT, with only 19% of the drug being released after a total of 8 hours in SGF with pepsin and SIF with pancreatin.

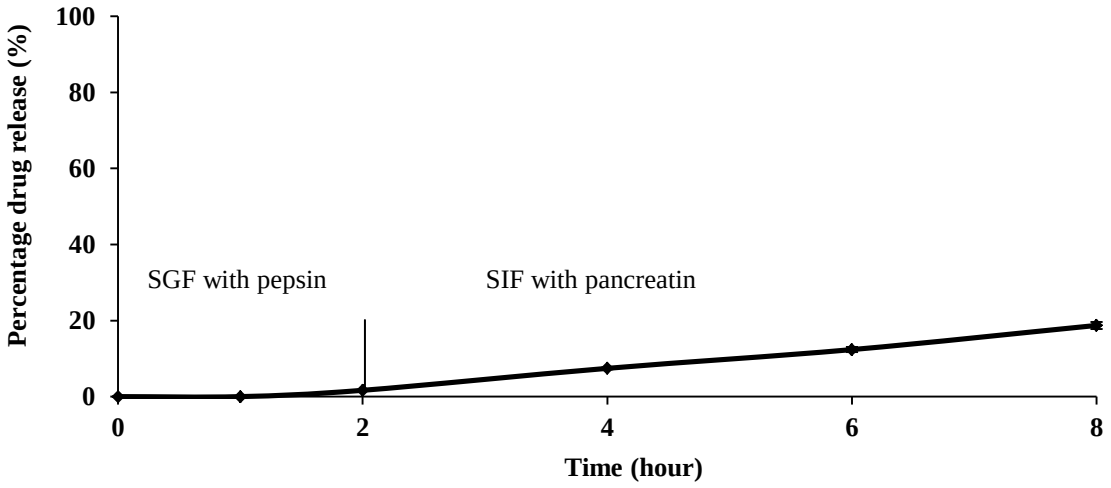


Figure 5.8 Dissolution profiles of paracetamol tablets coated (TWG = 20%) with zein alone pseudolatex (plasticization level = 30% PEG 400) in SGF (with pepsin) pH 1.2 (2 hours) then SIF (with pancreatin) pH 6.8 (6 hours). Each point represents the mean value ± SD (n = 3). (Error bars are very small).

Comparing the drug release in SGF with pepsin (1.65 %) to that obtained (2.3%) in 0.1 M HCl at the end of 2 hours (Section 5.3.1.4), it can be concluded that the film integrity was not compromised by the presence of pepsin, at least over the test duration. This result is quite different from that obtained by O'Donnell et al. (1997) where 100% of the drug was released from paracetamol tablets, coated with a 5% (of the tablet weight) zein pseudolatex containing 25% propylene glycol, 0.2% methyl parabens and 0.02% propyl parabens, within a 4 hours period in SGF with pepsin. This difference can be attributed to the different coating levels and composition and the test duration in SGF with pepsin. In this study, tablets coated up to 20% (TWG) with zein alone pseudolatex plasticized with 30% PEG 400, were used and tested for 2 hours in SGF with pepsin before a complete substitution of the medium with SIF with pancreatin.

SEM images of the coating surface before and after the release study are shown in Figure 5.9. The coated tablet had a fairly smooth and tight surface before the release but became porous and showed some evidence of digestion after the release.

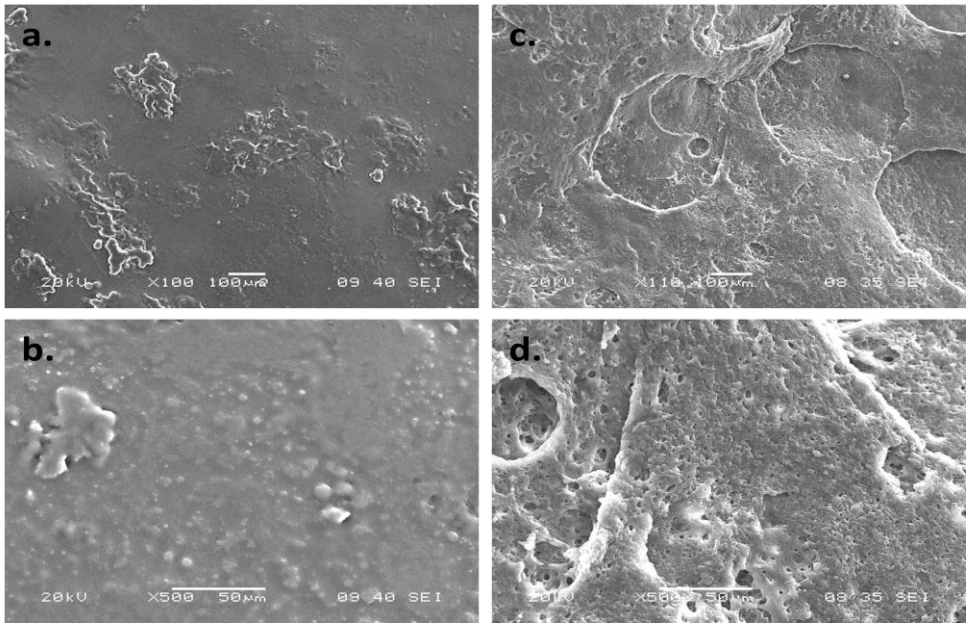


Figure 5.9 SEM images (100x and 500x) of paracetamol tablets coated with zein alone pseudolatex prior to any treatment (a and b) and after a total of an 8 hours test in SGF (with pepsin) and SIF (with pancreatin) (c and d).

5.3.2 Dissolution Studies of Pectin/Zein Pseudolatex Coated Systems

Oral drug delivery systems designed to release the drug in the colon should ideally prevent drug release in the stomach and small intestine, but trigger the release once it reaches the colon. Bacterially degraded polysaccharides such as pectin have been used for such purposes. The aqueous solubility of pectin has prevented its use as a single component film coat, and therefore, it has been incorporated with water-insoluble polymers to overcome its dissolution in the upper GIT. Promising results were obtained upon mixing pectin with ethylcellulose polymers for coating tablets and pellets by Wakerly et al. (1997) and He et al. (2008).

In this section of the project, pectin from apple (USP grade), a high methoxylated pectin, was incorporated into pseudolatex-based zein coatings. The drug release from paracetamol tablets coated with this system was investigated to evaluate the potential use of zein as a carrier of these polysaccharides in oral formulations intended for colonic targetting.

5.3.2.1 Influence of Adding Pectin in 1:30 Ratio

Pectin was added to the aqueous dispersions of zein in a 1:30 pectin/zein ratio plasticized with 30% w/w (based on zein amount) propylene glycol (PG); 0.2% w/v methyl parabens (MP) and 0.02% w/v of propyl parabens (PP) being added as preservatives (based on the final volume of the aqueous dispersion). Tablets coated with zein alone pseudolatex were also tested for comparison purposes (formulations 1 and 2 in Table 5.1). Figure 5.10 shows the release of paracetamol into distilled water from tablets coated (TWG = 12%) with zein alone and pectin/zein (1:30) aqueous dispersions.

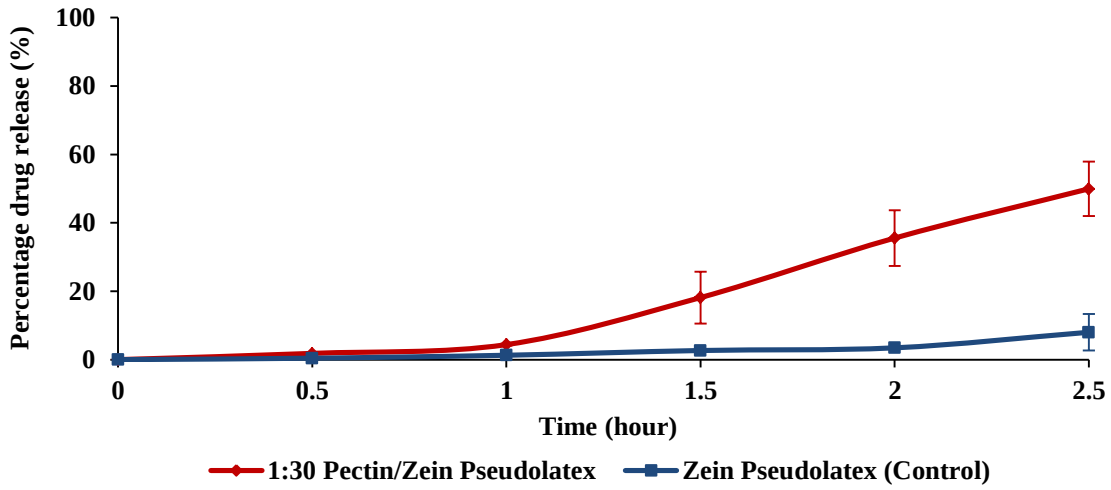


Figure 5.10 Dissolution profiles in distilled water of paracetamol tablets coated (TWG = 12%) with pectin/zein pseudolatex (1:30) and zein alone pseudolatex. Each point represents the mean value ± SD (n = 3).

The dissolution profile shows that the addition of pectin increased the extent and rate of drug release as compared to the release from tablets coated with zein alone pseudolatex, particularly after one hour of the dissolution test. This lag period can be explained by the time the coating materials need to hydrate and swell. The drug release is also expected to be influenced by the addition of water-soluble polymers such as pectin to the insoluble film coatings of zein. Pectin will dissolve and leach out once in contact with an aqueous medium leading to a disruption in the film structure and the formation of aqueous filled pores through which drug release can occur. Thus, the permeability of the film coating and hence the rate of drug release are expected to increase as pectin dissolves. A similar explanation of the drug release mechanism from paracetamol tablets coated with ethylcellulose/pectin mixed coating systems in phosphate buffer pH 6 was reported by Wakerly et al. (1997).

5.3.2.2 Influence of Coating Level

Semde et al. (1998) investigated the kinetics of pectin leaching (diffusing out) from mixed pectin/cellulosic or acrylic polymer films and reported that the diffusion rate highly depends on the level of pectin in the mixed films (higher levels being associated with more progressive leaching) as well as the size of pectin molecule and the type of film-forming polymer. Figure 5.11 shows the average percentage drug released into distilled water from tablets coated with 1:30 pectin/zein pseudolatex up to 12% and 20% TWG. The dissolution profile shows that an average of 43% of the drug was released within 6 hours from tablets coated with 1:30 pectin/zein pseudolatex up to a coating level of 20% TWG, and a complete drug release was obtained from those coated up to 12% TWG at the end of the 6 hours test.

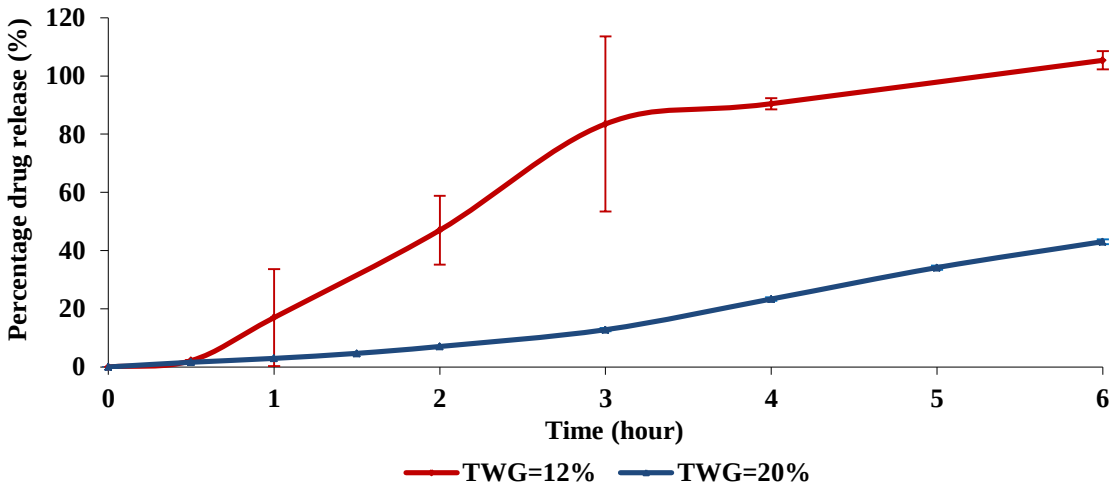


Figure 5.11 Dissolution profiles in distilled water of paracetamol tablets coated with pectin/zein (1:30) pseudolatex up to 12% and 20% TWG. Each point represents the mean value ± SD (n = 3).

These results, therefore, indicate that zein polymers could not prevent the fast swelling and dissolution of pectin at concentrations as low as 1:30 pectin/zein ratio and high coating levels as high as 20% TWG during the transit time in the upper GIT. Therefore, the leaching out of pectin from tablets coated with 10 and 20% TWG pectin/zein coatings induced a premature drug release in the upper GIT, rendering these systems of low potential value for colonic targetted delivery system.

5.3.3 Dissolution Studies of HAS/Zein Pseudolatex Coated Systems

Amylose is another polysaccharide that has been investigated for use in mixed coating systems intended for colonic targetting purposes. Research has been performed on amylose-butan-1-ol complex (or glassy amylose) (Milojevic et al., 1996a; Milojevic et al., 1996b; Siew et al., 2000; Wilson et al., 2005) and on high amylose starches (Freire et al., 2009a; Freire et al., 2009b; Freire et al., 2010a; Freire et al., 2010b). In this study, the acetylated form of high amylose maize starch (HAS) has been used and incorporated into aqueous zein pseudolatex coatings in an attempt to study the influence of this polysaccharide on the release of paracetamol from tablets coated with such a polymeric mix under conditions simulating the upper GIT.

In this section, the influence of the pH value of the dissolution medium, presence of digestive enzymes, the HAS/Zein ratio and the coating level, on the drug release from paracetamol tablets coated with HAS/Zein pseudolatex (formulation 3, Table 5.1) is studied. It also includes the characterization of zein alone, HAS alone and HAS/Zein aqueous dispersions, in terms of particle size distribution (PSD) and the influence of the heat treatment step, involved in the incorporation of HAS into HAS/Zein mixed films (Section 5.2.1), on the physico-chemical properties of the HAS fraction. Lastly, this section deals with the characterization of HAS alone, zein alone and HAS/Zein pseudolatex-based free films.

5.3.3.1 Influence of Dissolution Media pH

HAS was added to the aqueous dispersions of zein in 1:5 HAS/Zein ratio plasticized with 30% w/w (based on zein amount) polyethylene glycol 400 (PEG 400). Tablets coated with zein alone pseudolatex were also tested for comparison purposes (formulations 3 and 4 in Table 5.1). Figure 5.12 shows the drug release from tablets coated (TWG = 20%) with 1:5 HAS/Zein pseudolatex plasticized with 30% w/w PEG 400, in 0.1 M HCl (pH 1) and phosphate buffer (pH 6.8). The dissolution profiles show a significantly higher ($p < 0.05$) drug release under the more basic conditions compared to the acidic ones, but the drug release in both media followed zero-order kinetics with r^2 values of 0.9982 and 0.9976, respectively.

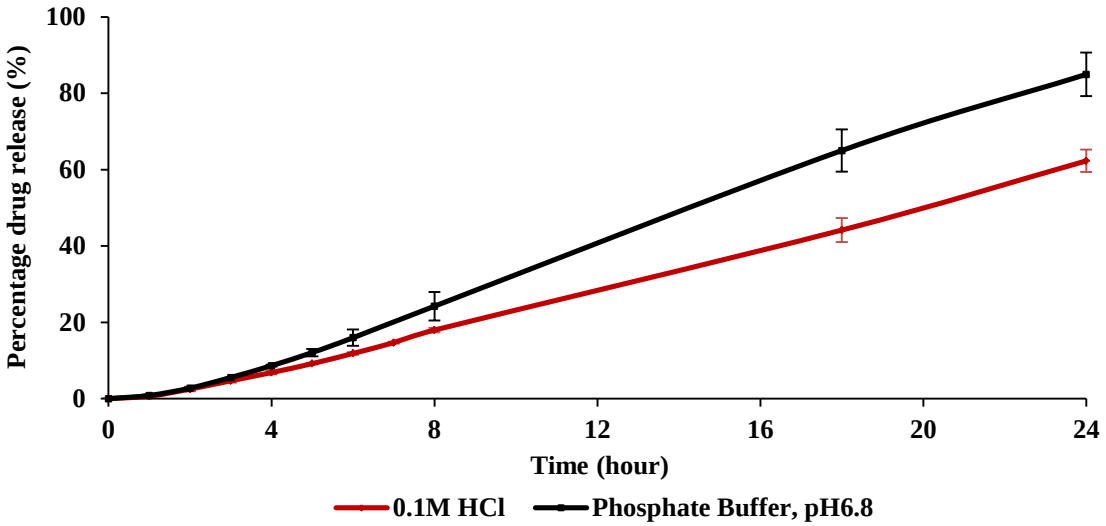


Figure 5.12 Dissolution profiles in 0.1 M HCl and phosphate buffer (pH 6.8) of paracetamol tablets coated (TWG = 20%) with 1:5 HAS/Zein pseudolatex (plasticization level = 30% PEG 400). Each point represents the mean value ± SD (n = 3).

The drug release from tablets coated with 1:5 HAS/Zein aqueous dispersions is expected to be influenced by the presence of amylose in the zein film. In the presence of an aqueous medium, the amylose tends to swell and therefore disrupt the film structure forming aqueous filled pores through which drug diffusion can occur (Siew et al., 2000). The higher release in the phosphate buffer, however, suggests that the swelling of HAS is pH-dependent, resulting in a more porous film structure and ultimately higher permeability to the drug molecules under more basic conditions. SEM images of paracetamol tablets coated (TWG = 20%) with 1:5 HAS/Zein pseudolatex prior to any treatment (a and b) and after 24 hours testing in 0.1 M HCl (c and d) and phosphate buffer pH 6.8 (e and f) are shown in Figure 5.13. The images showed a higher porosity under the more basic conditions (e and f) in comparison with the fairly smooth tablet surface prior to the study (a and b) and the less porous surface of the tablets treated in acidic conditions (c and d). SEM images of HAS/Zein coatings prior to and after the release study correlate well with the suggested pH-dependent swelling properties of this particular starch type.

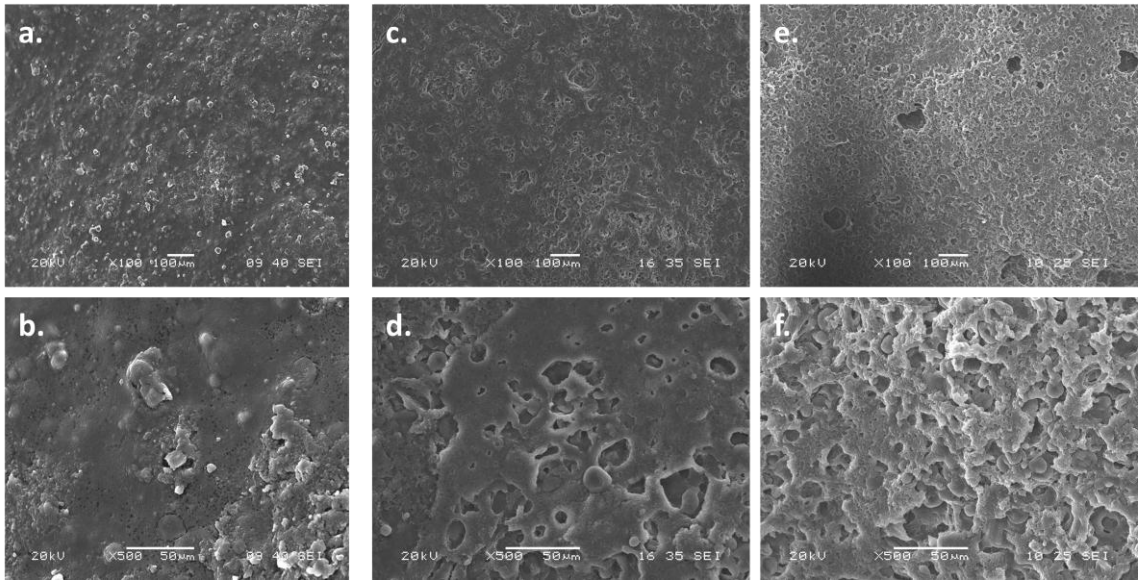


Figure 5.13 SEM images (100x and 500x) of paracetamol tablets coated with 1:5 HAS/Zein pseudolatex prior to any treatment (a and b) and after 24 hours testing in 0.1 M HCl (c and d) and phosphate buffer pH 6.8 (e and f).

Figure 5.14 compares the drug release from tablets coated with zein alone pseudolatex and 1:5 HAS/Zein pseudolatex under acidic (0.1 M HCl, pH 1) conditions. Figure 5.15 presents the dissolution profiles of the same formulations under more basic conditions (phosphate buffer pH 6.8). The incorporation of HAS did not influence the drug release at low pH values with 66% and 62% being released from zein alone and 1:5 HAS/Zein pseudolatex coated tablets, respectively, after 24 hours testing in 0.1 M HCl. Under the more basic conditions, the drug release from 1:5 HAS/Zein coated tablets was, however, significantly higher ($p < 0.05$) than that released from tablets coated with zein alone with 85% and 49% being released from both coatings, respectively, after 24 hours. These results support the suggested pH-dependent swelling behaviour of the HAS incorporated into zein films; HAS tends to swell to a greater extent at higher pH values and thus induces more pore formation and faster drug release under these conditions. As mentioned earlier, the transit times of a solid dosage form are approximately 2 hours and 4hours in the stomach and the small intestine, respectively. The dissolution tests either in 0.1 M HCl or phosphate buffer pH 6.8 were, however, carried out for 24 hours in order to stress the formulation and more fully understand its behaviour.

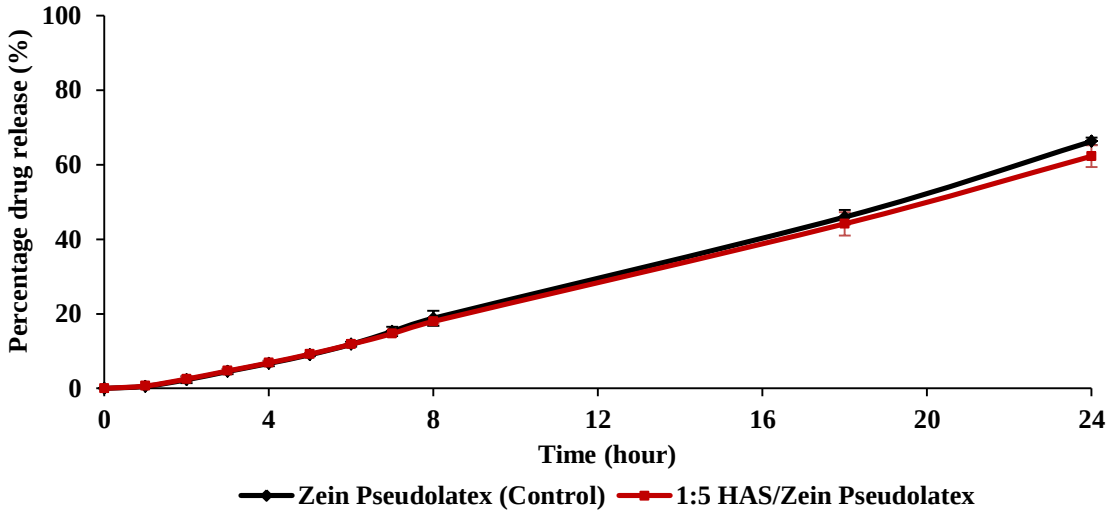


Figure 5.14 Dissolution profiles in 0.1 M HCl of paracetamol tablets coated (TWG = 20%) with zein alone pseudolatex (control) and 1:5 HAS/Zein pseudolatex (plasticization level = 30% PEG 400). Each point represents the mean value ± SD (n = 3).

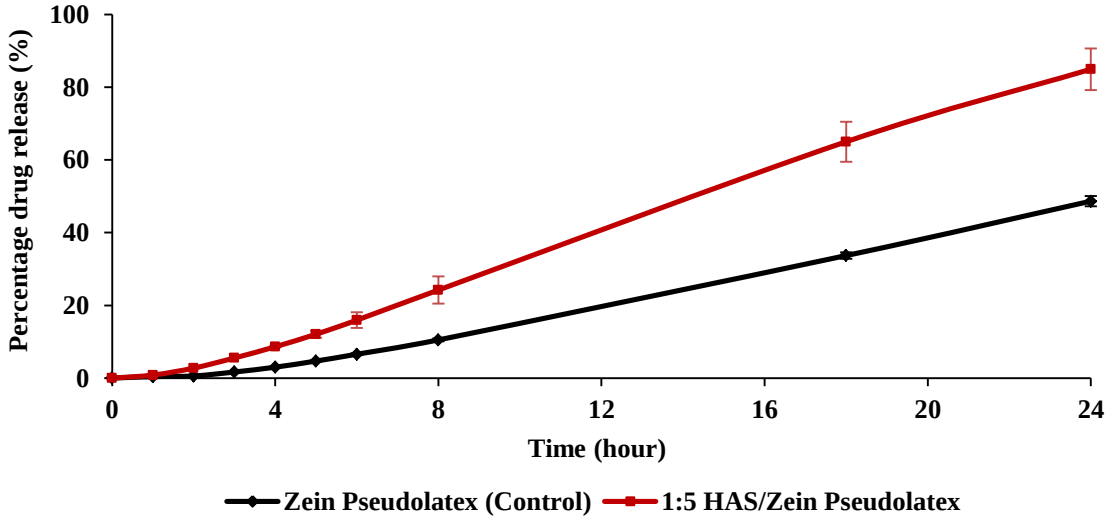


Figure 5.15 Dissolution profiles in phosphate buffer pH 6.8 of paracetamol tablets coated (TWG = 20%) with zein alone pseudolatex (control) and 1:5 HAS/Zein pseudolatex (plasticization level = 30% PEG 400). Each point represents the mean value ± SD (n = 3).

5.3.3.2 Influence of Digestive Enzymes

As mentioned earlier, a colonic-targetted drug delivery system is expected to prevent premature drug release in the upper GIT. Figure 5.16 shows the drug release from paracetamol tablets coated (TWG = 20%) with zein alone pseudolatex and 1:5 HAS/Zein pseudolatex (30% PEG 400) in simulated gastric fluid with pepsin (SGF with pepsin) for 2

hours and subsequently in simulated intestinal fluid with pancreatin (SIF with pancreatin) for a further 6 hours. The dissolution profile indicates that both films conferred release protection through the upper GIT with only 19% and 21%, respectively, of the drug being released after a total of 8 hours in SGF with pepsin and SIF with pancreatin. The drug release from zein alone and 1:5 HAS/Zein coatings was linear (zero-order kinetics) with r^2 values of 0.9957 and 0.9937, respectively. This figure also suggests that HAS containing coatings showed no difference in drug release rate and extent compared to zein alone coatings i.e. the digestive enzymes are not being particularly selective to either zein or HAS polymers, at least over this test duration.

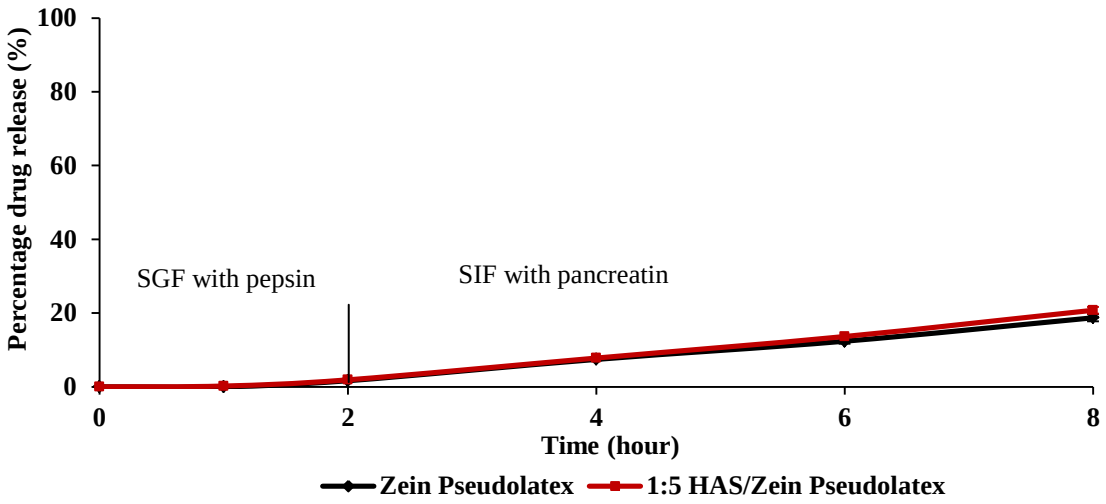


Figure 5.16 Dissolution profiles of paracetamol tablets coated (TWG = 20%) with zein pseudolatex alone and 1:5 HAS/Zein pseudolatex (plasticization level = 30% PEG 400) in SGF (with pepsin) pH 1.2 (2 hours) then SIF (with pancreatin) pH 6.8 (6 hours). Each point represents the mean value ± SD (n = 3). (Error bars are very small).

SEM images of 1:5 HAS/Zein coated tablets before and after the release study are shown in Figure 5.17. The tablets had a relatively smooth surface before the release but became porous after an 8 hour test in SGF and SIF. Conversely, SEM images of tablets coated with zein alone pseudolatex (Figure 5.9) showed a less porous surface indicating that the enzymatic media influenced the tablet surface to a lesser extent compared to the HAS containing one, possibly due to the swelling properties of the latter in basic conditions.

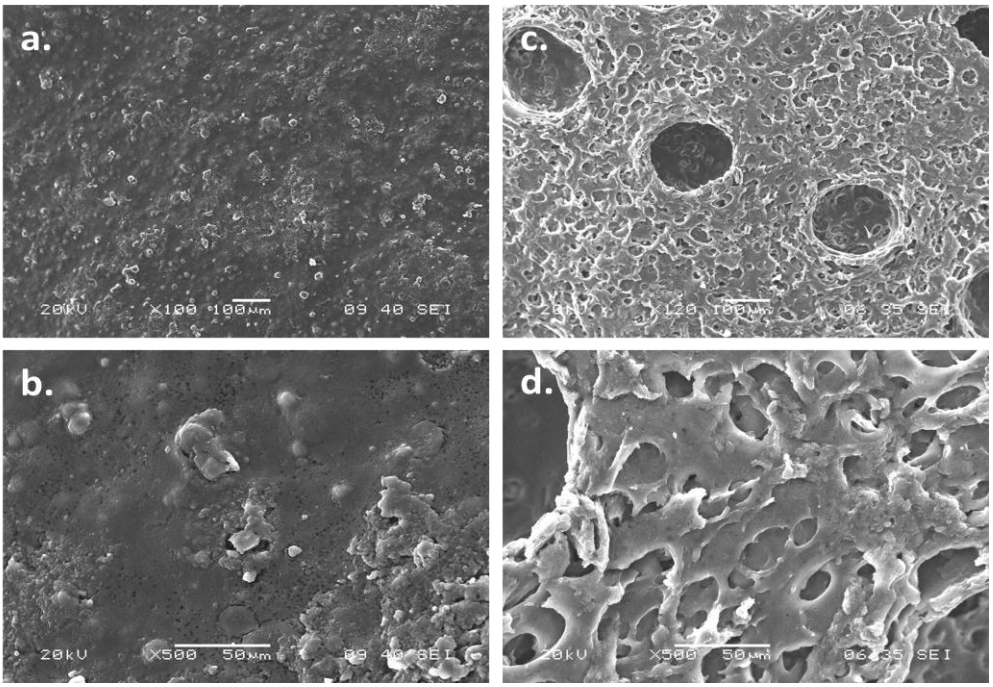


Figure 5.17 SEM images (100x and 500x) of paracetamol tablets coated (TWG = 20%) with 1:5 HAS/Zein pseudolatex prior to any treatment (a and b) and after a total of an 8 hours test in SGF (with pepsin) and SIF (with pancreatin) (c and d).

5.3.3.3 Influence of HAS/Zein Ratio

In HAS/Zein mixed coating systems designed for colon-specific drug delivery systems with particular reference to those activated by the colonic microbiota, the HAS would potentially serve as a substrate for the colonic enzymes. The drug release is expected to occur following the digestion of the starch domains within the film and the subsequent pore formation. The enzymatic digestion of these pore formers, as explained by Freire et al. (2010b), follows the Michaelis–Menten mechanism; higher digestion rates are thus expected at higher concentration of the substrate (i.e. HAS). Accordingly, higher levels of HAS were incorporated into pseudolatex-based zein coatings. Figure 5.18 shows the release into 0.1 M HCl of paracetamol from tablets coated (TWG = 20%) with pseudolatex-based coatings of different HAS/Zein ratios (1:3 and 1:5) and from tablets coated with zein alone pseudolatex (control).

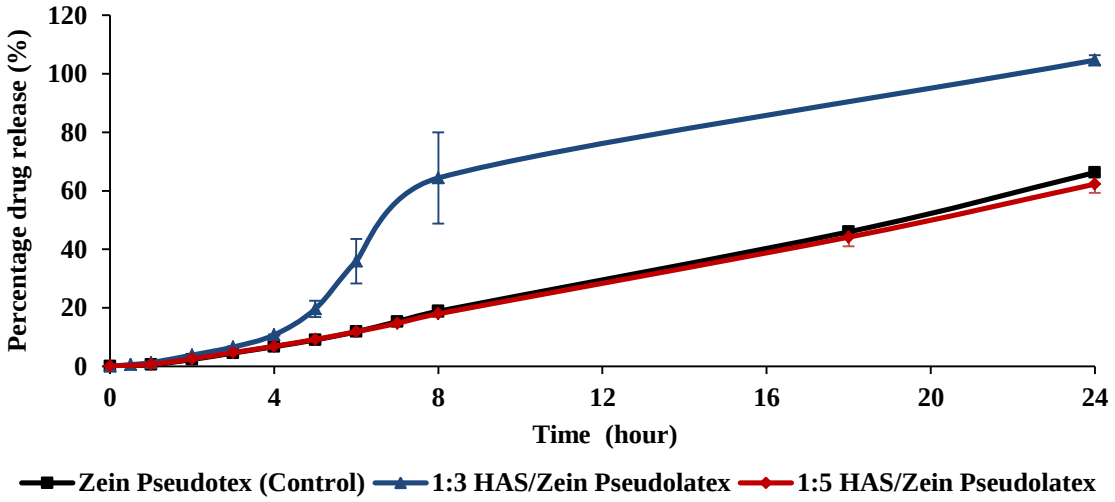


Figure 5.18 Dissolution profiles in 0.1 M HCl of paracetamol tablets coated (TWG = 20%) with zein alone (control), 1:3 HAS/Zein and 1:5 HAS/Zein pseudolatexes (plasticization level = 30% PEG 400). Each point represents the mean value ± SD (n = 3).

The dissolution profiles show a comparable drug release of both zein alone and 1:5 HAS/Zein coatings; however, a faster release was obtained at the higher HAS/Zein ratio (i.e. 1:3). Increasing the HAS/Zein ratio up to 1:3 resulted in a fast release of the drug accompanied by film cracking. This result can be attributed to the zein's ability to suppress the swelling of amylose in HAS/Zein films at the higher HAS content; zein could not control the swelling of HAS at the higher level (1:3). Thus, a premature drug release in the upper GIT is expected to occur from tablets coated with 1:3 HAS/Zein pseudolatex at a coating level of 20% TWG. Similarly, reducing the coating thickness of the lower HAS/Zein ratio, i.e. 1:5, pseudolatex-based coatings led to a premature film rupture and drug release in 0.1 M HCl (data not shown). These results thus limit the potential use of the 1:3 HAS/Zein coatings (TWG = 20%) and the 1:5 HAS/Zein coatings (TWG = 10%) for colonic targetting purposes.

5.3.3.4 Characterization of Zein Alone, HAS Alone and HAS/Zein Aqueous Dispersions

Aqueous zein dispersions (10% w/w) were prepared by a precipitation method (Li et al., 2010) and plasticized with 30% w/w (based on zein amount) polyethylene glycol 400 (formulation 4 in Table 5.1). Acetylated high amylose maize starch (HAS) was dispersed in distilled water in 14:86 ratio and heated at 80 ± 5 °C for 30 minutes, based on the work

of Freire et al. (2009a). The resulting HAS dispersion (13.04 % w/w), called thereafter as heat-processed HAS stock, was very viscous and formed an opaque gel upon cooling down to room temperature. Therefore, the required amount of the heat-processed HAS stock was diluted with preheated water (80 ± 5°C) to approximately 1.8 % w/w and then allowed to cool down to room temperature (circa 26 °C). The diluted aqueous dispersion of HAS, named thereafter as heat-processed diluted HAS, was unstable at room temperature where it precipitated gradually with time; however, aggregation did not occur and the dispersion could be retrieved upon stirring. The dilution to concentrations < 2% w/w was determined by experimentation. Ellis et al. (1985) determined the concentration (C* = 1.5 %) that separates gelation and precipitation of amylose on cooling the solution; precipitation occurs at polymer concentrations < C* whereas opaque gels are observed at concentrations > C*. Figure 5.19 shows a photograph of the heat-processed HAS stock (a) and the heat-processed diluted HAS (b) dispersions. The diluted HAS dispersion was then added to the pre-plasticized (30 % PEG 400 based on zein amount) aqueous dispersion of zein drop wise upon continuous stirring. The resulting aqueous dispersions of zein alone and HAS/Zein (formulations 3 and 4 in Table 5.1) were then used for coating tablets using a top spray fluidized bed coater or to prepare free films by a casting/solvent evaporation technique.

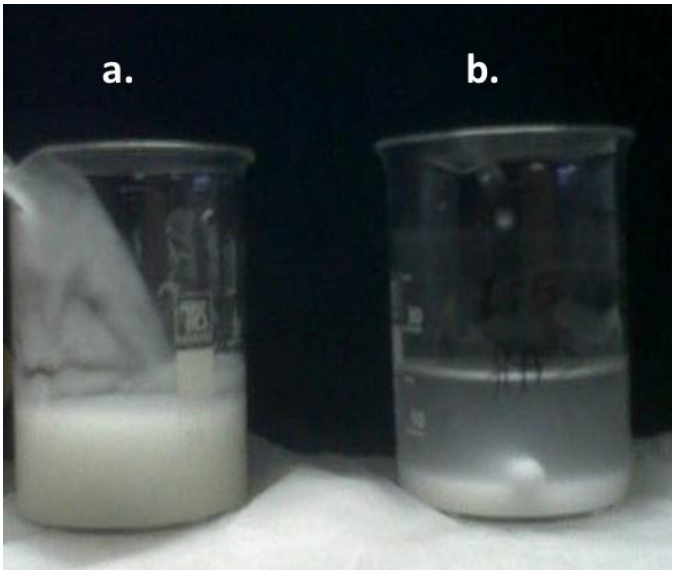


Figure 5.19 A photograph of the heat-processed HAS stock (a) and the heat-processed diluted HAS (b) dispersions.

5.3.3.4.1 Particle Size Distribution (PSD) of Aqueous Dispersions

Particle size is a key element to stability, or resistance to settling and sedimentation, and subsequent film forming mechanisms of these pseudolatexes. Oshlack et al. (1994) described that the particle size of zein in the aqueous dispersion is in the range of 0.01 to 10 µm and preferably less than 10 µm. Laser diffraction particle analysis (LD) was used to determine the particle size distribution (PSD) of the aqueous dispersions. Figure 5.20 represents the PSD of zein alone pseudolatex, heat-processed diluted HAS dispersion and 1:5 HAS/Zein pseudolatex plotted on a logarithmic scale. The distributions are roughly normally distributed and therefore the mean size X_{50} can be used to represent these data. Zein pseudolatex had X_{50} values of 10.17 ± 1.59 µm whereas the HAS dispersion had higher values of 20.23 ± 0.11 µm. The 1:5 HAS/Zein pseudolatex had an X_{50} value that is very close to that obtained with the zein alone aqueous dispersion and was 10.82 ± 1.77 µm. However the density distribution curve of 1:5 HAS/Zein pseudolatex is wider owing to the contribution of the larger particles of HAS dispersion.

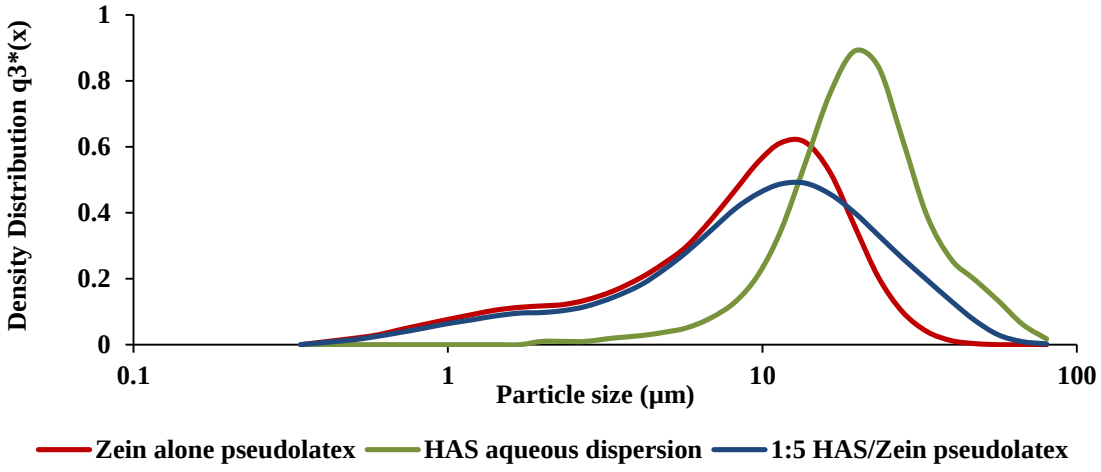


Figure 5.20 Particle size distribution of zein alone pseudolatex, HAS aqueous dispersion and 1:5 HAS/Zein pseudolatex using the laser diffraction (LD) technique.

5.3.3.4.2 Effect of Heat Treatment on the Physico-chemical Properties of High Amylose Maize Starch (HAS)

Film coatings made from heat-treated acetylated high amylose maize starch (HAS) and the water-insoluble zein protein, were designed for potential uses in colon-specific drug delivery. The treatment of starch granules at high temperatures (80 ± 5 °C) induces

gelatinization which involves swelling and disruption of the granules and the leaching out of amylose. This treatment is thought to increase starch digestibility, but it was found that upon cooling amylose is given enough time to rearrange itself in a more crystalline structure, and this phenomenon is called retrogradation (Miles et al., 1985). Retrograded starch is known to be more resistant to enzymatic attacks by pancreatic α-amylases, yet susceptible to those present in the colon (Freire et al., 2009a). This observation supports the potential use of heat-treated starch samples as a coating material to deliver drugs to the colon. Chemically modified starch, such acetylated amylose starch, is also classified as resistant starch. In this work, acetylated high amylose maize starch (DS = 0.06) was used. The investigation of the influence of moisture-heat treatment and dilution steps during the preparation of HAS samples on the physicochemical properties of the high amylose maize starch was thus necessary and evaluated using X-ray and FT-IR techniques.

Figure 5.21 shows the FT-IR spectra of HAS free film, heat-processed diluted HAS dispersion, heat-processed HAS stock dispersion, unprocessed HAS aqueous dispersion (prepared by dispersing the raw powder in distilled water without heat treatment) and unprocessed HAS raw powder in the region of 1500 to 800 cm^{-1}. HAS raw powder showed distinct peaks at 1005 cm^{-1}, 1080 cm^{-1} and 1153 cm^{-1} in addition to a small peak at 1240 cm^{-1} which is typical of acetylated starches. These results are in agreement with those reported by Diop et al. (2011). These bands were maintained in the spectrum of the unprocessed HAS aqueous dispersion; however, a small shoulder appeared at approximately 1050 cm^{-1}. Heat-processed HAS samples (including HAS free film, heat-processed diluted HAS dispersion and heat-processed HAS stock dispersion) showed the same three main peaks of the raw powder, albeit at lower intensities possibly due to the different HAS concentration in these samples. These results suggest that treating the HAS at high temperature and diluting the heat-treated samples did not induce significant changes in their FT-IR spectra. Freire et al. (2009a) reported similar results where no significant changes in the FT-IR spectra of the acetylated starch (DS = 1.5) were detected after heat treatment.

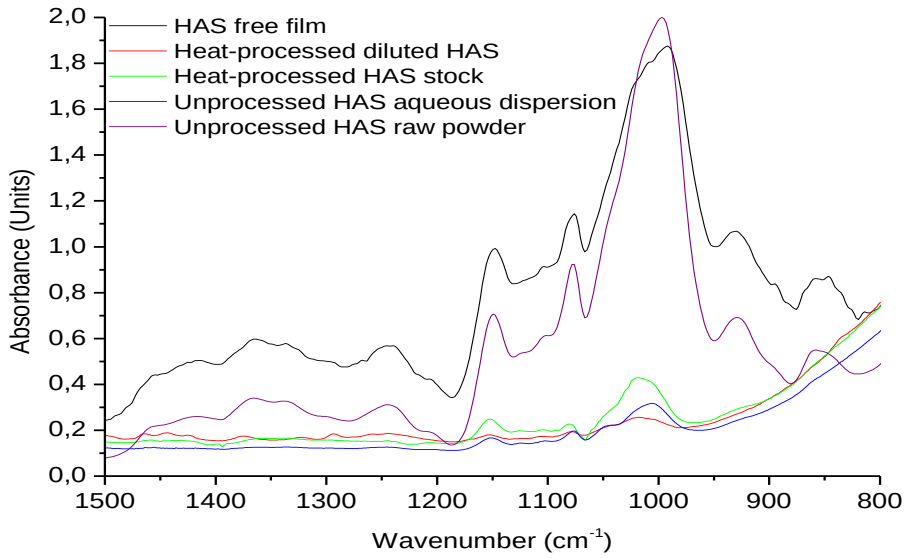


Figure 5.21 FT-IR spectra of HAS free film, heat-processed diluted HAS dispersion, heat-processed HAS stock dispersion, unprocessed HAS aqueous dispersion and unprocessed HAS raw powder in the region of 1500 to 800 cm^{-1}.

The X-ray diffractograms of the heat processed HAS samples (HAS free film, heat-processed diluted HAS dispersion and heat-processed HAS stock dispersion) and the unprocessed HAS aqueous dispersion and raw powder of HAS are compared in Figure 5.22. The raw powder of HAS showed diffraction peaks of considerably low intensity at 2θ of approximately 17°, 19° and 23°. These peaks are however different from the wide peaks at 2θ of approximately 9° and 20° determined by Diop et al. (2011) for acetylated starch. This could be attributed to the low degree of substitution (DS = 0.06) of the acetylated starch used in this study as compared to the modified starch of high DS levels (DS ≥ 1.83) studied by these authors. As compared to the raw powder, wider peaks at 2θ of approximately 30° and 40° were observed in the diffractogram of the unprocessed HAS aqueous dispersion. The right shift and widening of the peaks observed upon hydrating the starch sample is possibly related to the swelling of the starch granules. After heating the samples at 80 °C the X-ray diffractograms showed significant changes; the samples were converted essentially into an amorphous form as seen by the lack of diffraction peaks in the diffractograms of the heat treated samples. Freire et al. (2009a) reported a similar result with the acetylated starch which was fully amorphous after the heat treatment possibly due to its lower gelatinisation temperature.

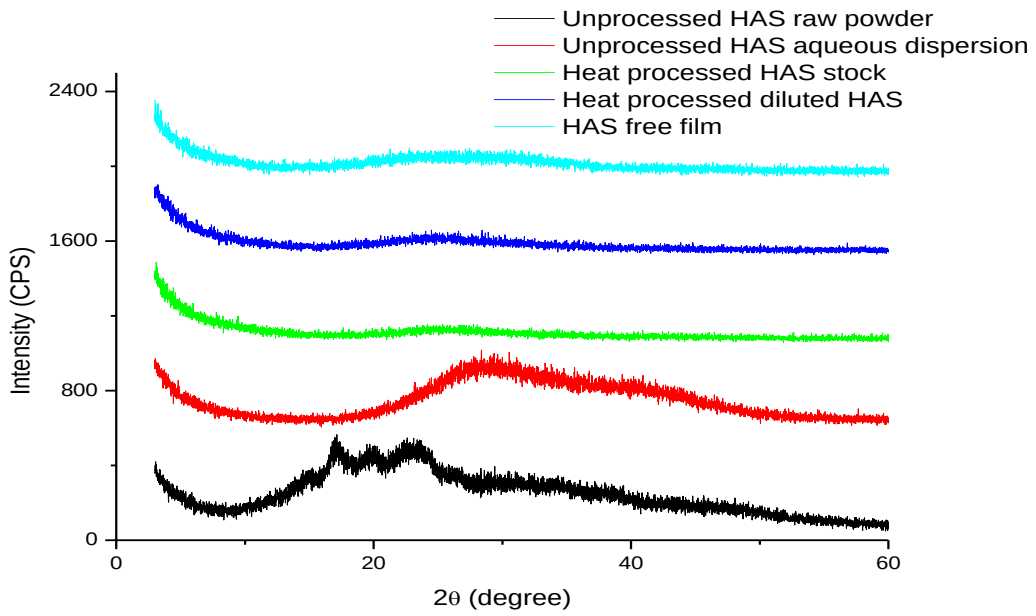


Figure 5.22 X-ray diffractograms of HAS free film, heat-processed diluted HAS dispersion, heat-processed HAS stock dispersion, unprocessed HAS aqueous dispersion and unprocessed HAS raw powder.

In conclusion, heat-treated HAS free film exhibited a comparable molecular arrangement to that of the unprocessed HAS powder, as was seen in the FT-IR spectrum. However, the X-ray diffraction peaks were completely lost upon heat-processing of the starch sample. These data suggest that retrogradation has not occurred in the samples in this study, as the heat-treated samples would have been expected to show some well-ordered arrangements owing to the retrograded form.

5.3.3.5 Characterization of Pseudolatex-based Zein Alone and HAS/Zein Films

Pseudolatex-based zein alone and HAS/Zein free films (formulations 4 and 5, Table 5.1), as prepared in Section 5.2.2, and HAS alone films, as prepared in Section 5.3.3.4.2, were stored in a desiccator over P_2O_5 (0% RH) at room temperature for subsequent studies. The film thickness was measured using a micrometer (Outside micrometer, 0-25 mm) (Table 5.2). A number of characterization techniques were used to study the free films and these include scanning electron microscopy (SEM), modulated temperature differential scanning calorimetry (MTDSC), thermogravimetric analysis (TGA), nano-thermal analysis (n-TA) and attenuated total reflectance-Fourier transform infra-red (ATR-FTIR) spectroscopy.

Table 5.2 Thickness values (mm, mean ± SD) of zein alone, HAS alone, and mixed HAS/Zein films measured by a micrometer.

Film type	HAS alone	Zein alone	1:7 HAS/Zein	1:5 HAS/Zein	1:3 HAS/Zein
Thickness (mm), n = 10	0.05 ± 0.001	0.075 ± 0.039	0.148 ± 0.015	0.112 ± 0.024	0.122 ± 0.013

5.3.3.5.1 Scanning Electron Microscopy (SEM)

SEM images of the surfaces of free films offered information regarding the miscibility of the two polymers and the overall quality of the films. SEM images of the surfaces of the free films obtained using different HAS and zein ratios (w/w) are presented in Figure 5.23. The unplasticized HAS alone films (Figure 5.23, a) showed some globular features of non-uniform size, whereas plasticized zein alone films (Figure 5.23, b) had a compact surface made up of small spherical particles. Mixed films of 1:7 and 1:5 HAS/Zein ratio (Figure 5.23, c and d) showed a smooth surface along with some spherical features that resemble those observed with zein alone films. Increasing the HAS/Zein ratio to 1:3 resulted in a fully smooth and homogenous surface. These images might suggest a poor adhesion between the two components at lower HAS/Zein ratios whereas a complete fusion occurs at higher HAS/Zein ratios.

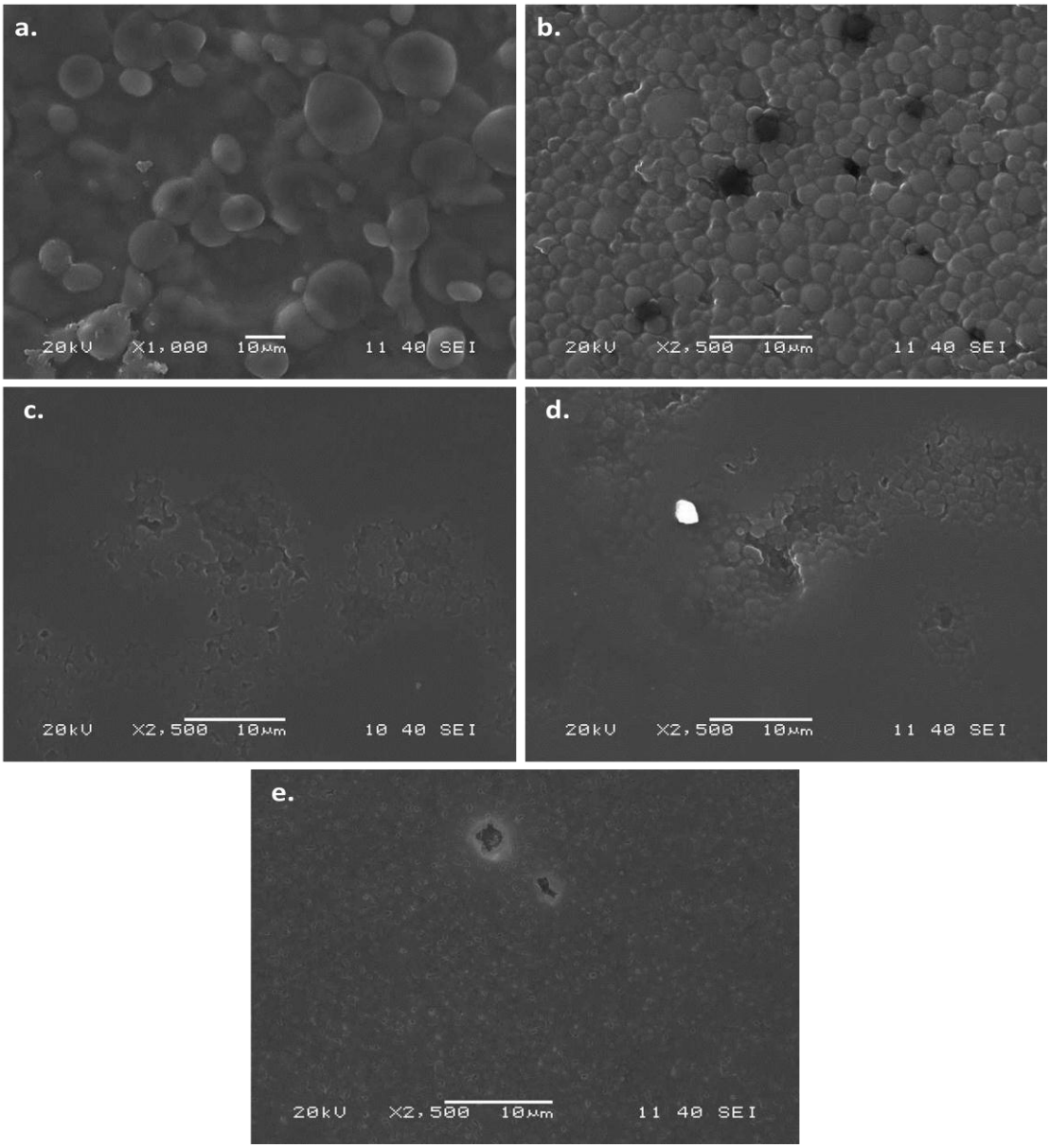


Figure 5.23 SEM images of HAS alone (a, 1000x), zein alone (b, 2500x), 1:7 HAS/Zein (c, 2500x), 1:5 HAS/Zein (d, 2500x) and 1:3 HAS/Zein (e, 2500x) films.

5.3.3.5.2 Modulated Temperature Differential Scanning Calorimetry (MTDSC) and Thermogravimetric Analysis (TGA)

Standard thermal analytical techniques such as differential scanning calorimetry (DSC), modulated temperature DSC (MTDSC) and thermogravimetric analysis (TGA) have been used widely to study pharmaceutical systems, e.g. (McPhillips et al., 1999). The aim of this part of the study is to investigate the thermal behaviour of the mixed polymeric films with limited moisture content and to assess the miscibility of the two polymers (HAS and zein). As a prerequisite for the use of these mixed films in colon-specific delivery, the two

polymers (in this case HAS and zein) should be immiscible i.e. retaining their individual thermal transitions, so that the starch domains in the film are readily accessible to the colonic microbiota (Freire et al., 2009b).

Unplasticized free films of HAS alone and plasticized zein alone and mixed HAS/Zein films were stored in a desiccator over P_2O_5 (0% RH) at room temperature for a minimum of 24 hours before being tested. Table 5.3 presents the water content (%) of HAS alone, zein alone and mixed HAS/Zein films. The HAS free films had the highest moisture content, possibly due to the hydrophilic nature of HAS. Zein alone films were plasticized with 30% w/w (based on the amount of zein) PEG 400, which might explain the relatively high water content of these films (3.6%). The mixed HAS/Zein films showed a trend of increasing the moisture content with increasing the HAS fraction in the mixed films.

Table 5.3 A summary of the water content (%) of HAS alone, zein alone and mixed HAS/Zein films (mean ± SD) as measured by TGA.

Film type	HAS alone	Zein alone	1:7 HAS/Zein	1:5 HAS/Zein	1:3 HAS/Zein
Water content (%) (n = 3)	4.4 ± 0.7	3.6 ± 1.6	1.9 ± 0.6	2.3 ± 0.2	2.9 ± 0.3

All films showed an endothermic peak at around 40 to 60 ºC in the non-reversing MTDSC signal. The glass transition (Tg) of dry zein has been stated to be approximately 165 ºC (Shukla et al., 2001); however, the addition of plasticizers can significantly decrease the glass transition temperature. The water had a confounding effect on the detection of zein's Tg; a plasticized Tg, rather than the Tg of zein reported in the literature, was seen in the reversing MTDSC signal. In addition, the incorporation of HAS into zein films made it more difficult to detect the glass transition temperature of zein. The investigation of the thermal behaviour of pure HAS raw powder and HAS alone free films was difficult under the study conditions, i.e. no transitions were detected in the reversing MTDSC signal, along with a water loss signal on both the total and non-reversing signals. Zeleznak et al. (1987) revealed that the glass transition (T_g) of wheat starch samples could be only identified within a narrow moisture range (13 to 22%). Under limited moisture contents (< 13%), the small change in heat capacity made it difficult to determine the T_g. The water content of the free films under study (Table 5.3) was between 1.9 and 4.4% which might

explain the inability to determine the glass transition of the HAS component of the free films. The MTDSC results were not conclusive and of limited use in this case, i.e. the determination of the miscibility of HAS and zein, and gave no evidence for phase separation.

5.3.3.5.3 Nano-Thermal Analysis (n-TA)

Nano-thermal analysis (n-TA) is a recently introduced technique that combines thermal analysis with scanning probe microscopy (SPM) and allow site-specific thermal characterization compared to bulk techniques such as DSC and MTDSC (Dai et al., 2009). The instrumentation is based on atomic force microscopy (AFM); however, the conventional SPM ultra-sharp tip is replaced by a thermal probe. The temperature at the tip of these probes can be adjusted by supplying a controlled voltage (Craig et al., 2002). This technique can be used to attain topographic images and moreover local thermomechanical analysis (LTA) can be carried out once a topographic image is obtained. The latter involves placing the probe on the area of interest in a topographic image and a temperature ramping is applied linearly inducing changes in the mechanical properties of the material on heating. These changes involve thermal expansion of the sample forcing the cantilever upwards or softening at glass-rubber transitions or melts causing the probe tip to indent the sample. LTA allows the study of the miscibility of polymeric samples on specific regions of a sample and at interfaces between its components.

Contact mode topography images of HAS alone, zein alone and HAS/Zein mixed films are displayed in Figure 5.24. The AFM images correlate well with the SEM images (Figure 5.23). HAS alone free films exhibited huge circular features and examination of the height difference (4000 nm) suggests that the surface is quite rough (Figure 5.24, a). The surface of zein alone films (Figure 5.24, b) appeared to be made up of spherical particles and was fairly rough, but smoother than that of the HAS alone films. HAS/Zein mixed films showed different structural patterns which were intermediate between the features displayed by the single components and were dependent on the HAS content of the film. Films having the lowest starch content (1:7 HAS/Zein) showed mainly spherical particles but some of these structures started to smooth (Figure 5.24, c). Films with the highest starch content (1:3 HAS/Zein) (Figure 5.24, e) had the smoothest surface (height difference = 400 nm). HAS/Zein films of 1:5 ratio (Figure 5.24, d) were made up of spherical

particles along with flatter smoother areas and the surface was as rough as that of the zein alone films. It can be noted that the films became smoother at higher HAS/Zein ratios which might suggest a poor adhesion between the two polymers at lower ratios (1:7 and 1:5) compared to the higher ratios (1:3) as was also implied by the SEM images (Figure 5.23).

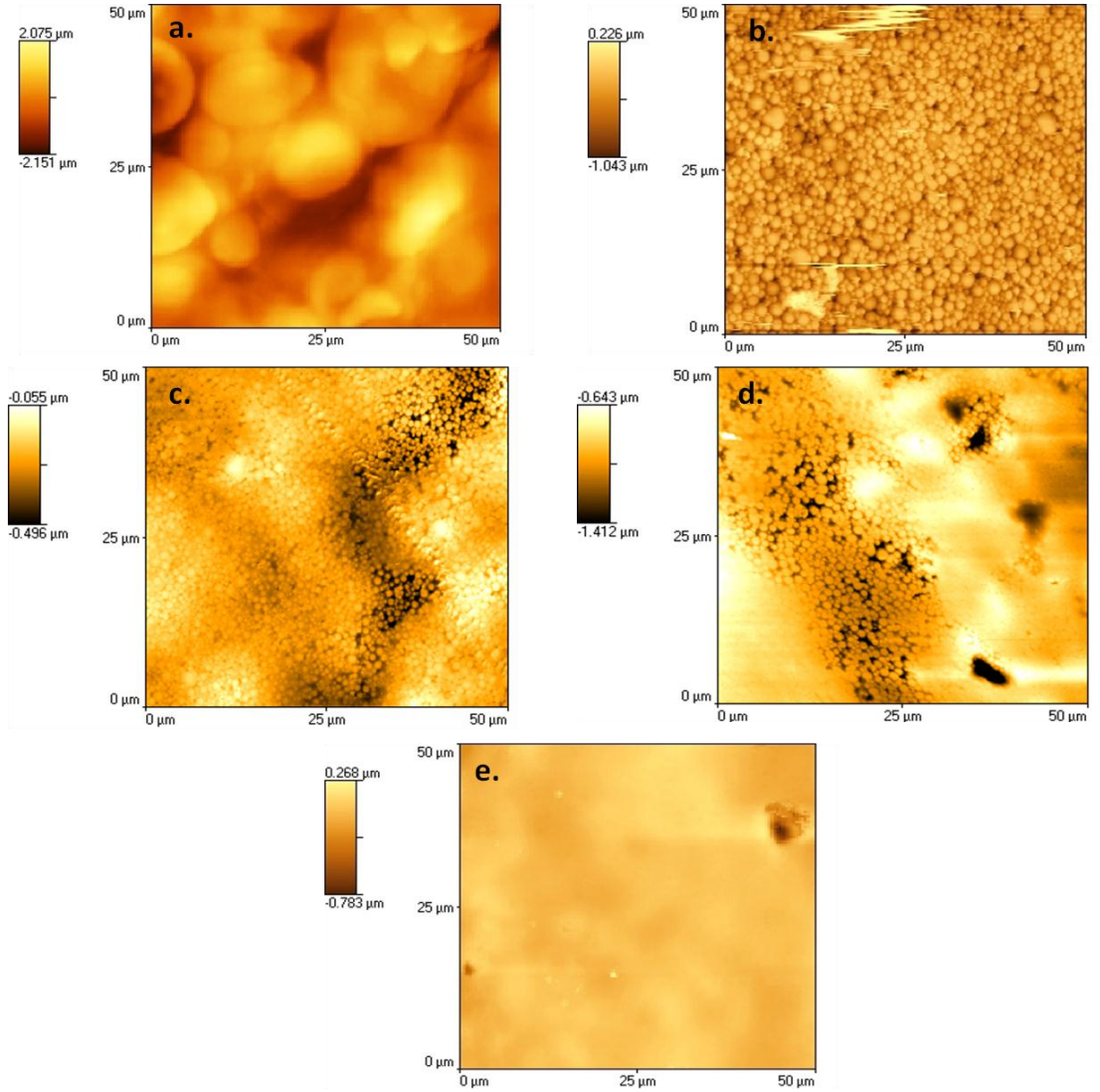


Figure 5.24 AFM images of HAS alone (a.), zein alone (b.), 1:7 HAS/Zein (c.), 1:5 HAS/Zein (d.) and 1:3 HAS/Zein (e.) free films.

Localized thermomechanical analysis (LTA) studies were performed on the surface of HAS alone, zein alone and HAS/Zein free films (Figure 5.25 to Figure 5.28). No transitions were observed when HAS alone films were tested. Zein alone films were relatively consistent with softening occurring at around 90 °C. This softening temperature is related to the glass transition of zein. HAS/Zein mixed films of 1:7, 1:5 and 1:3 ratios

showed a trend whereby one softening point, in most cases, was detected at circa 110, 130 and 140 °C, respectively. It can be noted that the softening temperature increased with increasing the HAS/Zein ratio. It is worth noting that although the topographical images revealed some structural differences based on HAS/Zein ratio, the LTA data suggested homogenous surfaces as the thermal transitions were fairly close to each other.

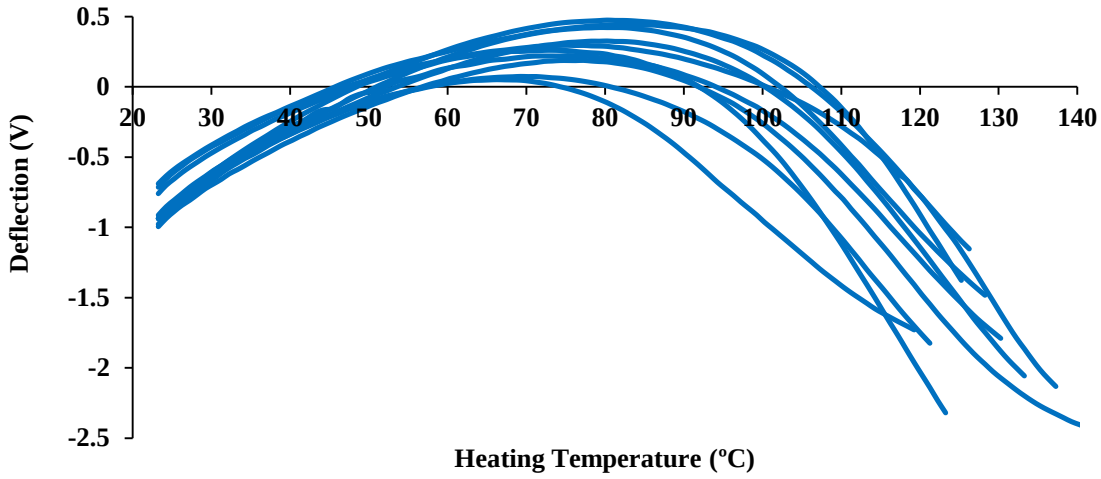


Figure 5.25 Localized thermomechanical analysis of zein alone film surface, showing initial expansion followed by probe penetration due to polymer softening. Each line represents a separate experiment.

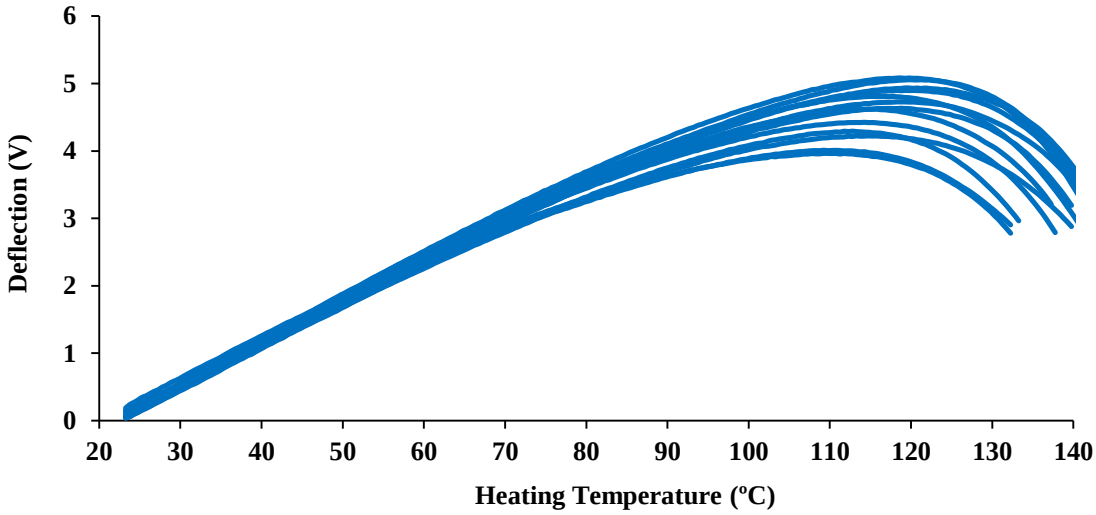


Figure 5.26 Localized thermomechanical analysis of 1:7 HAS/Zein film surface, showing initial expansion followed by probe penetration due to polymer softening. Each line represents a separate experiment.

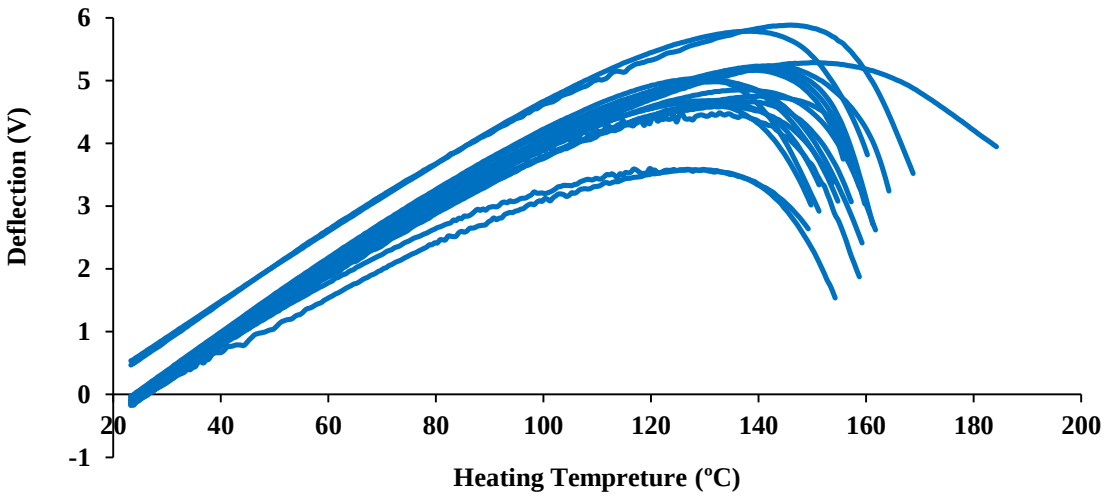


Figure 5.27 Localized thermomechanical analysis of 1:5 HAS/Zein film surface, showing initial expansion followed by probe penetration due to polymer softening. Each line represents a separate experiment.

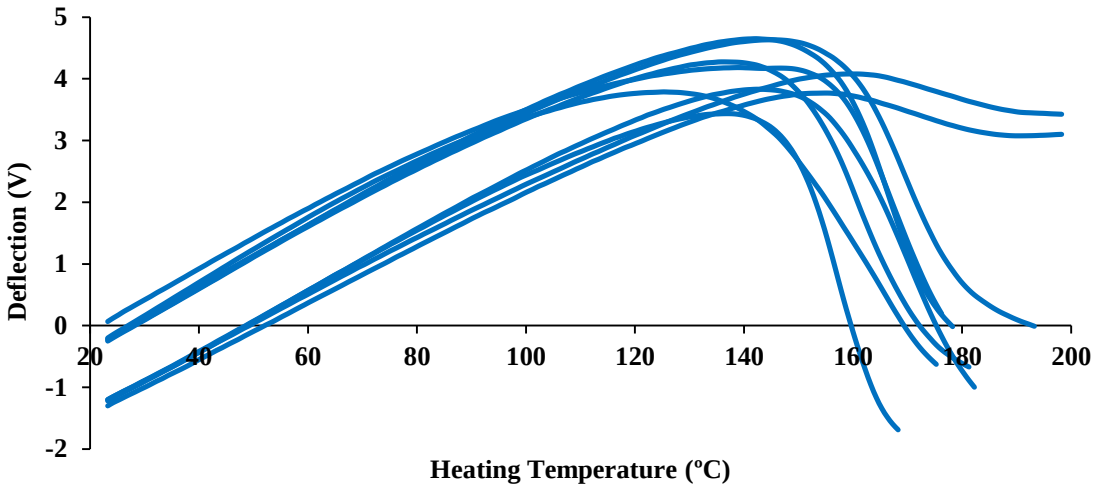


Figure 5.28 Localized thermomechanical analysis of 1:3 HAS/Zein film surface, showing initial expansion followed by probe penetration due to polymer softening. Each line represents a separate experiment.

Another application of the n-TA includes tapping mode where the probe is oscillated at a constant frequency, providing intermittent contact with the sample, while maintaining a constant temperature of the probe tip. By heating the probe to the softening temperature of a particular material in a multi-component sample, it is possible to deliberately alter the mechanical properties of a particular material and provide greater differences in the phase of the probe. To further elucidate the results from the above studies, some free film samples were therefore tested using the 'tapping' mode in AFM imaging (TM-AFM). The

use of phase imaging along with topographical imaging is useful for studying phase-separated systems as it can allow mapping of spatial distribution of phases and components with different mechanical and physicochemical properties (Turner et al., 2007).

Figure 5.29 presents TM-AFM images of 1:3 HAS/Zein film surface, showing the topography (top row) and the phase image (bottom row) at room temperature, 40 °C and 120 °C. The phase images did not exhibit different phases or components and were essentially the same at the temperature values used. These data are in line with the LTA data and suggest that the surfaces are homogeneous. The images also suggest that the structural differences elucidated in Figure 5.24 are not compositional differences or phase-separated systems.

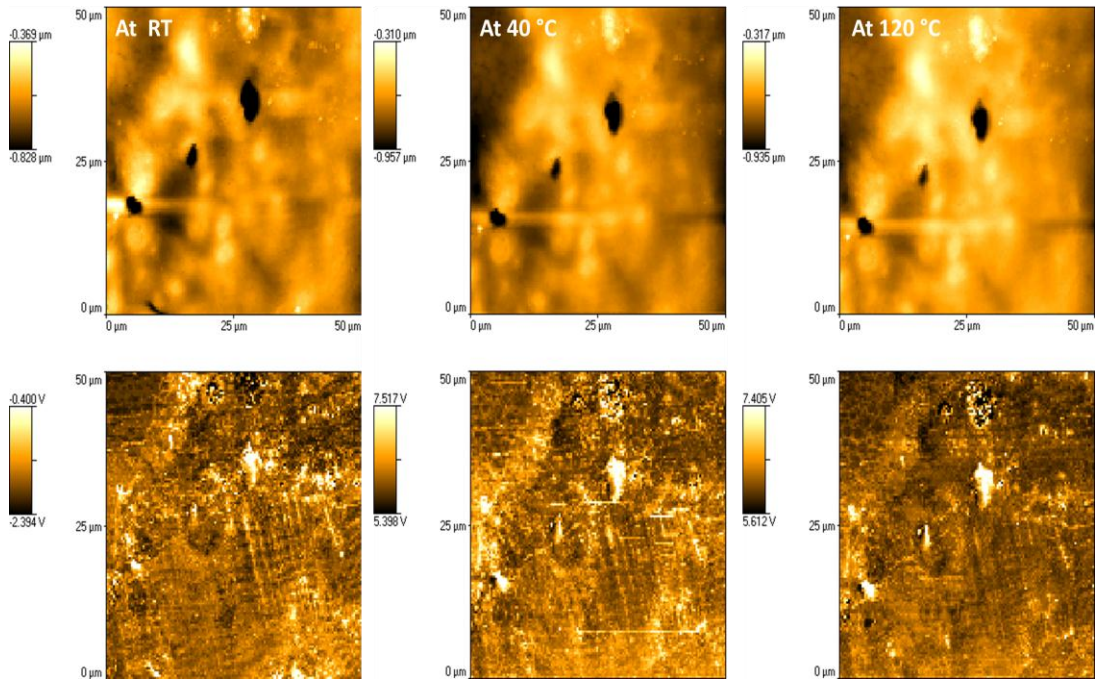


Figure 5.29 TM-AFM images of 1:3 HAS/Zein film surface, showing the topography (top row) and the phase image (bottom row) at room temperature, 40 °C and 120 °C.

5.3.3.5.4 Fourier-Transform Infrared Analysis (FT-IR)

FT-IR spectra of mixed films with various HAS/Zein ratios, shown in Figure 5.30 to Figure 5.32, were obtained in the spectral region of 4000 to 500 cm^{-1} to investigate any possible interactions between the two polymers. HAS alone and zein alone films were also scanned for comparison. The FTIR spectrum of zein protein consists of the typical protein bands including amide I from 1750 to 1600 cm^{-1} and amide II from 1500 to 1400 cm^{-1}

(Forato et al., 2003). HAS alone film showed distinct peaks at 1005 cm^{-1}, 1080 cm^{-1} and 1153 cm^{-1} in addition to a small peak at 1240 cm^{-1} which is typical of acetylated starches. No spectra of the mixed HAS/Zein films, regardless of the ratio, exhibited any significant shifts in the amide I and II peaks and no new peaks could be detected even at the highest HAS content, suggesting a lack of chemical interaction between the two polymers.

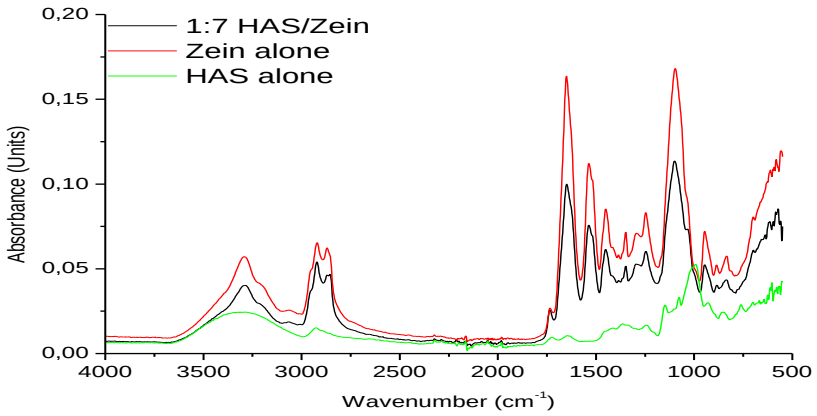


Figure 5.30 FT-IR spectra of zein alone film, HAS alone film, and 1:7 HAS/Zein film in the spectral region of 500 to 4000 cm^{-1}.

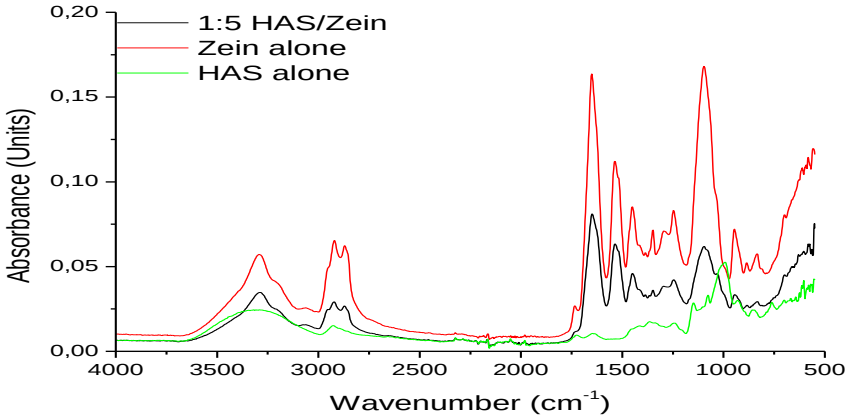


Figure 5.31 FT-IR spectra of zein alone film, HAS alone film, and 1:5 HAS/Zein film in the spectral region of 500 to 4000 cm^{-1}.

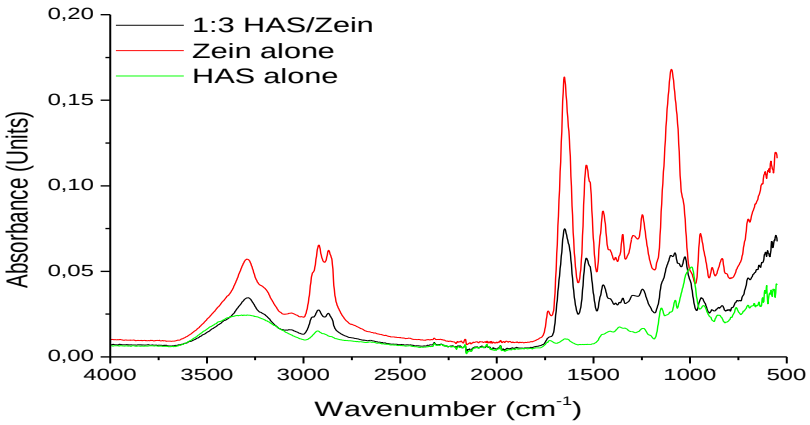


Figure 5.32 FT-IR spectra of zein alone film, HAS alone film, and 1:3 HAS/Zein film in the spectral region of 500 to 4000 cm^{-1}.

5.4 Conclusions

Pseudolatex-based zein coatings were successfully produced and used for coating paracetamol tablets. The drug release was sensitive to the plasticization level (20 and 30% w/w, based on zein amount) with more drug being released at higher plasticization levels. The release of paracetamol from tablets coated with zein alone pseudolatex was controlled by the coat thickness (TWG = 10 and 20%), with higher drug release rate and extent obtained at the lower coating levels in 0.1 M HCl. The curing time at 45 ºC had no influence on the drug release in 0.1 M HCl from tablets coated with zein alone pseudolatex, plasticized with the higher plasticization level (30% w/w) and tested directly after the coating and the subsequent drying steps in the coater. The drug release from these systems was sufficiently controlled in conditions resembling the upper gastrointestinal tract (GIT) with only 19% of the drug being released after a total of 8 hours in SGF with pepsin and SIF with pancreatin. The incorporation of polysaccharides into the film coat, however, altered the performance of zein alone coatings. Pectin/zein polymeric coatings were not able to suppress the paracetamol release even at a very low pectin/zein ratio (1:30), with circa 43% of the drug being released into distilled water from tablets coated with pectin/zein pseudolatex (20% TWG) in 6 hours. These systems are expected to be associated with premature drug release in the upper GIT and are thus inappropriate candidates for colon-specific delivery.

On the other hand, 1:5 HAS/Zein coatings (TWG = 20%) were able to prevent extensive drug release in the upper GIT. The extent and rate of drug release from tablets coated with zein alone and HAS/Zein films were essentially the same in enzyme containing simulated gastric and simulated intestinal fluids, with only 19% and 21%, respectively, of the drug being released after a total of 8 hours in SGF with pepsin and SIF with pancreatin. This implies that the digestive enzymes of the upper GIT are not being particularly selective to either zein or HAS polymers, at least over the test duration (chosen to reflect the average transit time of solid dosage forms in the stomach and small intestine). This study thus shows that the heat-treated acetylated high amylose maize starch was resistant to digestion by the pepsin and pancreatic α-amylases and confirms the enzyme resistant form of this heat-treated starch-type. Thus, HAS/Zein (1:5) film coatings (TWG = 20%) have the potential to be used in film coating for colon-specific drug delivery. However, increasing the HAS/Zein ratio up to 1:3 at a coating level of 20% TWG or reducing the coating thickness of 1:5 HAS/Zein coatings from 20% to 10% TWG, resulted in faster drug release accompanied by film cracking and premature drug release in the upper GIT, indicating that zein could not control the swelling of HAS under these conditions.

As mentioned earlier, the two polymers of mixed coatings (zein and HAS in this case) designed to deliver the drug to the colon should be immiscible so that the starch domains in the film are readily accessible to the colonic microbiota. The individual thermal transition of the starch (HAS) fraction could not be determined using either the MTDSC or the n-TA. Thus, the MTDSC results were not conclusive and gave no evidence for phase separation. The FT-IR spectra of the HAS/Zein films suggested a lack of chemical interaction between the two polymers. Although both SEM and the topographical images of n-TA showed some structural differences based on HAS/Zein ratio, the LTA data and the tapping mode AFM images suggested that the surfaces are homogenous and that these structural differences are not compositional differences or phase-separated systems.

The susceptibility of pseudolatex-based zein alone, pectin/zein and HAS/Zein coatings to digestion by the colonic microbiota is investigated using batch culture fermentation batches in Chapter 6.

6 Chapter 6 Colonic Targetting: An Investigation of Zein Susceptibility to Colon Microbiota and the Influence of Film Coat-Incorporated Polysaccharides

6.1 Introduction

Colonic drug targetting is of considerable importance for the selective treatment of colonic disorders and the targetted delivery of peptides and proteins (Leopold, 2001). To achieve this, dosage forms may be coated with polymers that selectively release the drug in the colon, either by being degraded themselves or by incorporating colonic-sensitive agents in an inert film. An ideal colonic delivery system should prevent the drug release in the stomach and small intestine, but initiate the release once it reaches the colon. Zein alone and mixed HAS/Zein coatings have conferred release protection in the upper gastrointestinal tract (GIT) as discussed in Chapter 5, potentially making these films good candidates for colonic targetting purposes.

In this chapter, the susceptibility of organic-based and aqueous dispersion zein coatings to the colonic microbiota is investigated. In particular, the influence of incorporation of polysaccharides such as pectin or acetylated high amylose maize starch (HAS) in aqueous zein dispersion coatings on paracetamol release in conditions simulating colonic microbiota is examined.

6.2 Materials and Methods

This section briefly describes the methods used in the preparation of the aqueous dispersions of zein and in the coating of paracetamol tablets. It also describes the dissolution and fermentation studies used to evaluate the drug release kinetics from the coated tablets. A detailed comparison between the two protocols used in the fermentation studies along with the materials used is provided in this section. The characterization techniques are also described.

6.2.1 Preparation of Film Coating

Organic-based zein coatings (10% w/v solution of zein in 75% aqueous ethanol) were prepared and plasticized with glycerol (20 % w/w, based on the amount of zein). Aqueous zein dispersions or pseudolatex (10% w/w) were prepared by a precipitation method (Li et al., 2010) and plasticized with 30% w/w (based on zein amount) polyethylene glycol 400 (PEG 400). To add the acetylated high amylose maize starch (HAS), three grams (3 g) of HAS was dispersed in 20 mL of distilled water and heated at 80 ± 5 ºC for 30 minutes based on the work of Freire et al. (2009a). The required amount of the heat-processed HAS was transferred to another beaker and diluted with preheated water (80 ± 5 °C) to give a final concentration of approximately 1.8 % w/w. The attained dispersion was allowed to cool down to room temperature (circa 26 °C) and added to the plasticized aqueous zein dispersion as required. Different solid ratios of HAS and zein (1:5 and 1:3) were mixed. The HAS/Zein dispersion was stirred overnight to achieve complete mixing of the two polymers.

Pectin was added to the aqueous dispersions of zein in 1:30 pectin/zein ratio. These dispersions were prepared by a precipitation method (Oshlack et al., 1994) and were plasticized with 30% w/w (based on zein amount) propylene glycol (PG), with 0.2% w/v methyl parabens (MP) and 0.02% w/v of propyl parabens (PP) being added as preservatives (based on the final volume of the aqueous dispersion). The composition of aqueous dispersions (pseudolatexes) of zein is shown in Table 6.1.

Table 6.1 The composition of aqueous dispersions (pseudolatexes) of zein.

Formulation	Composition of aqueous dispersion (100 mL)					
	Zein	Pectin	HAS	PEG 400	PG/MP/PP	Tween 80
1 Pectin/Zein (1:30) aqueous dispersion	10 g	0.3 g			3 g/0.2 g/0.02 g	
2 Zein alone aqueous dispersion (control)	10 g				3 g/0.2 g/0.02 g	
3 HAS/Zein (1:5) aqueous dispersion	10 g		2 g	3 g		1 g
4 Zein alone aqueous dispersion (control)	10 g			2 or 3 g		1 g

6.2.2 Film Coating of Paracetamol Tablets

Dibasic calcium phosphate-based paracetamol tablets, as prepared in Chapter 2, were initially subcoated with an organic-based zein solution (10% w/w plasticized with 20% w/w glycerol) up to 2 to 3% total weight gain (TWG, based on tablet weight) using a top spray fluidized-bed coater (Mini Coater/Drier 2, Caleva Process Solutions Ltd, UK). The tablets were subcoated to prevent the dissolution of the tablet surface on subsequent exposure to the aqueous dispersion. The tablets were then top coated with zein aqueous dispersion. Dibasic calcium phosphate-based (n = 10) tablets were placed in the coating chamber. The atomization pressure was 0.7 bar and the pump rate was 1.09 rpm. The fan speed was 13 to 13.5 m/s (70 to 75%) with no agitation. The height for the spray head was 65 mm from the spray nozzle to the mesh in the base of the cone. After coating, the tablets were left in the coater (bed temperature = 45 ˚C) for 15 minutes to dry. The coating thickness is expressed in terms of the percentage total weight gain (TWG), and tablets with a TWG of 10 or 20% were obtained. These TWG values are, as determined in Chapter 4, equivalent to film thickness of circa 106 and 203 µm, respectively. Tablets coated with HAS/Zein pseudolatex were cured in an air oven at 45 ˚C for 10 hours, whereas those coated with pectin/zein aqueous dispersion were cured at the same temperature for 24 hours. Tablets were stored in sealed amber glass bottles until use.

6.2.3 Dissolution Studies

Drug release studies were performed using a BP Apparatus I dissolution bath in 900 mL of dissolution medium at 37 ± 0.5 ºC with a rotation of 50 rpm. To test the formulation stability in the upper GIT, freshly prepared simulated gastric fluid (SGF, 0.32% w/v pepsin) was used as the dissolution medium for the first 2 hours and was then replaced by freshly prepared simulated intestinal fluid (SIF, 1% w/v pancreatin) for an additional 6 hours. At predetermined sampling points, 10 mL of sample was collected from each vessel using a syringe, filtered through a 0.2 µm membrane filter and replaced with an equivalent volume of fresh medium. Samples that contain the pancreatic digestive enzymes were centrifuged at 13,000 rpm for 30 minutes prior to filtration and UV measurements. The paracetamol concentration was determined using a UV-VIS spectrophotometer set at as wavelength of 243 nm with reference to an appropriate standard curve. The dissolution studies were carried out in triplicate under sink conditions and the average drug release ± SD was calculated. The drug release profiles were compared on the basis of the area under

the curve (AUC) calculated by the trapezoidal method using a 2-tailed T-test (2 samples).

6.2.4 Fermentation Studies

Conventional dissolution tests have been used to evaluate the drug release from microbiota sensitive colon-specific drug delivery systems in simulated colonic fluid containing commercially available enzymes (Yang, 2008; Freire et al., 2010b). However, *in vitro* human faecal fermentation systems may be a more suitable model for human colonic bacterial activity. Colonic studies can be thus performed in a continuous colonic model, a triple-stage chemostat gut model (Macfarlane et al., 1998; Yang, 2008) (Figure 6.1, a), or using batch culture fermentation system. In this project, the drug release from coated tablets was assessed under simulated colonic conditions using batch culture fermentation system for simplicity and shorter preparation time purposes.

Batch culture fermenters simply consist of human faeces homogenized in a physiological buffer, of total volume of 100 mL, of near neutral pH and maintained under reduced (almost anaerobic) conditions. The fermenters used in this work consisted of a glass vessel filled with prereduced basal culture medium of pH 6.8 (described fully in sections 6.2.4.1.1 and 6.2.4.2.1) and maintained at 37 ºC in an anaerobic cabinet (Figure 6.1, b). The medium was then inoculated with faecal slurry which was prepared by homogenizing fresh human faeces in phosphate-buffered saline (PBS, 8 g L^{-1} NaCl, 0.2 g L^{-1} KCl, 1.15 g L^{-1} Na_2HPO_4 and 0.2 g L^{-1} KH_2PO_4) pH 7.3.

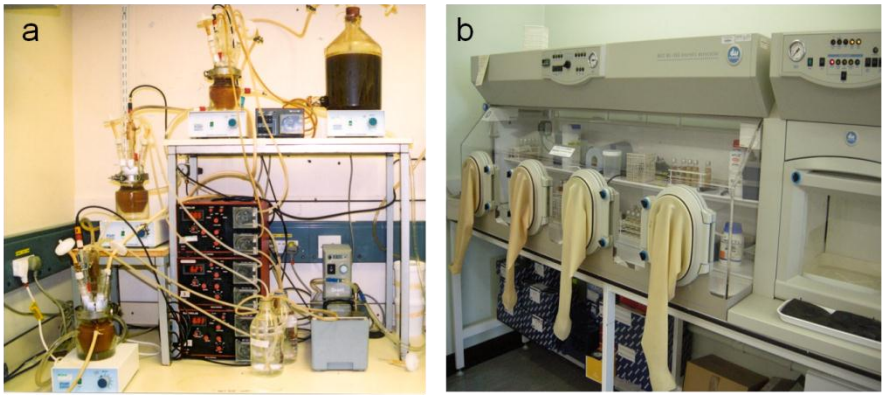


Figure 6.1 Pictures showing: a) Continuous colonic model and b) An anaerobic cabinet where batch culture fermentation takes place.

Throughout these colonic studies, two different protocols have been used. Protocol I was proposed by Institute of Food Research, IFR (Norwich, UK), and based mainly on the work of Baines et al. (2005) with slight modifications. Protocol II was slightly modified from a previously described system (Hughes et al., 2008) with the assistance of Prof. Abdul Basit's group (private communication). Differences between Protocol I and II in terms of preparation of basal media, faecal inoculation and post sample treatment and analysis will be highlighted in sections 6.2.4.1 and 6.2.4.2. It is worth mentioning that these two models exhibit some significant differences, but both attempt to mimic the *in vivo* colonic conditions. These *in vitro* models offer a reasonable simulation of the low fluid, viscous, bacteria-rich and anaerobic environment of the colon (Siew et al., 2004), however, these models are only qualitatively similar to conditions present in the colon (Yang, 2008) and are therefore not entirely predictive of *in vivo* behaviour (McConnell et al., 2008c).

6.2.4.1 Protocol I

6.2.4.1.1 Preparation of Basal Media

The basal medium is a complex medium which supports the bacterial growth and consists of thirteen components, as displayed in Table 6.2, based on the work of Baines et al. (2005). The powders were weighed accurately into a clean beaker and dissolved in distilled water. The mixture was stirred for 30 minutes to ensure all ingredients were dissolved. Haemin was dissolved in a few drops of NaOH before being added to the solution. Vitamin K1 and Tween 80 were added at the end. The medium was autoclaved in 250 mL glass bottles. The bottles of media were allowed to cool down prior to being kept in an anaerobic cabinet with loosened lids overnight to reduce the dissolved oxygen level. The fermenter vessels along with the magnetic stirrers and dissolution baskets used in the batch fermentation were also autoclaved and kept under anaerobic conditions overnight before the experiment day (i.e. faecal inoculation).

Table 6.2 Basal medium composition- Protocol I and Protocol II

Ingredient	Amount (g/L)	
	Protocol I	**Protocol II**
Peptone water	2.00	2.00
Yeast extract	2.00	2.00
NaCl	0.10	0.10
K_2HPO_4	0.04	0.04
KH_2PO_4	0.04	-
$MgSO_4 \cdot 7H_2O$	0.01	0.01
$CaCl_2 \cdot 2H_2O$	0.01	0.01
$NaHCO_3$	2.00	2.00
Tween 80	2.00 mL	2.00 mL
Vitamin K1	10.0 µL	10.0 µL
L-Cysteine•HCl	0.50	0.50
Bile salts	0.50	0.50
Haemin	0.005	0.005
Resazurin solution 0.02%	-	5.00 mL

6.2.4.1.2 Faecal Inoculation

Freshly voided faecal sample was diluted in pre-reduced (kept in an anaerobic cabinet overnight) phosphate-buffered saline (PBS, pH 7.3) in a 1:10 ratio, stomached (homogenized) using Stomacher 400 circulator (Seward) at 230 rpm for 45 seconds and filtered through filter bags (Stomacher® lab system, BA6141/STR bag filters made for Seward LTd.). The filtrate was collected into an autoclaved glass bottles. The media were inoculated with the faecal material to 10% v/v. The final pH was adjusted to 6.8. For the control experiments, the basal medium was instead diluted with PBS to 10% v/v.

The batch fermentation model used in this work (Figure 6.2) consists of 250 mL sterile glass bottles (fermentation vessels) filled with the inoculated basal medium. Dissolution baskets were attached to the bottles lids and used to keep the coated tablets suspended in

the medium during the test. A magnetic stirrer was added into each vessel and vessels were placed on stirring plates running at 100 rpm. Stirring is essential in order to prevent the precipitation of the suspended faecal material and to provide a sufficient drug distribution. The working volume of each culture was 100 mL. Coated tablets were added to the baskets and then into the test media. Experiments representing the colonic (with faecal content) conditions were performed in triplicate, whereas a single control (without faecal content) experiment was carried out.

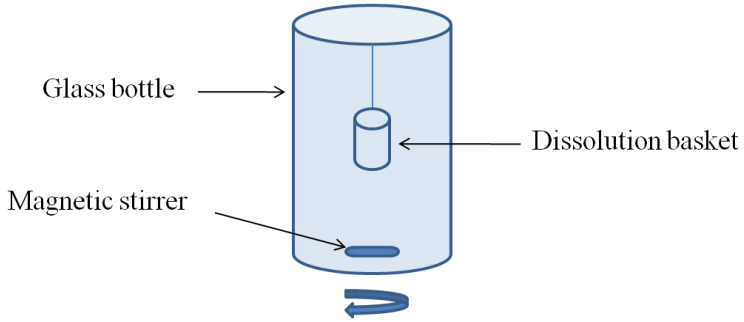


Figure 6.2 A schematic representation of the colonic batch fermentation model which consists of a fermenter (glass bottle), a magnetic stirrer and a dissolution basket.

6.2.4.1.3 Post-Sample Treatment and Analysis

Samples were withdrawn at predetermined time points (either in 0, 3, 6, 9, 12 and 24 hours sets or 0, 4, 8, 10 and 24 hours sets) and centrifuged at 14,000 rpm using an Eppendorf Centrifuge 5418 (Eppendorf AG, 22331 Hamburg, Germany) for 10 minutes. The supernatant was then filtered through 0.2 µm filters (Minisart High-Flow single use syringe filter, Sartorius Stedim Biotech GmbH, Germany) and kept at - 20 ºC until analysis.

Samples were analysed using two different methods. A simple chemical extraction method of the model drug (paracetamol) was used in the preliminary stages of the colonic studies. The withdrawn sample was thawed, and 3-times extracted with ethyl acetate in a similar manner to that used by Dalton et al. (2007) to extract the same drug from blood samples. The ethyl acetate was evaporated to dryness under reduced pressure using a rotary evaporator (Buchi rotary evaporator, Switzerland) and the solid residue was dissolved in distilled water using a 10 mL volumetric flask. The drug amount was then determined spectrophotometrically (Perkin-Elmer Lambda XLS spectrophotometer) at 243 nm. This preliminary technique was used to analyse the samples of the experiments performed in

section 6.3.1 and 6.3.2. Once the colonic targetting concept was established, a more accurate method using HPLC was developed to quantify the drug amount in the withdrawn samples.

6.2.4.2 Protocol II

6.2.4.2.1 Preparation of Basal Media

The basal media of protocol II was based on work by Hughes et al. (2008). Table 6.2 (Section 6.2.4.1.1) presents the components of the basal medium used in protocol II. Peptone water, yeast extract, and resazurin were dissolved in 4/5 the volume of medium intended to be prepared. The solution was fully reduced using the Hungate column technique (i.e. the medium was boiled on a hot plate for 15 minutes while being purged with oxygen-free nitrogen and was subsequently cooled in ice while being gassed with oxygen-free $N_2/H_2/CO_2$ (85:10:5) gas), autoclaved, and stored in an anaerobic cabinet until use. The remaining ingredients were kept in an anaerobic cabinet for a minimum of 6 hours in order to minimize their exposure to oxygen, and were then dissolved in the remaining one-fifth of the volume (water) that had been previously reduced using the Hungate column. This latter solution was then filtered-sterilized (Cellulose Nitrate Membrane filters 0.2 µm, Whatman, Germany) and added to the larger volume inside an anaerobic cabinet. The resulting basal media was then kept in an anaerobic cabinet overnight. The fermenter vessels along with the dissolution baskets used in the batch fermentation were also autoclaved and kept under anaerobic conditions overnight before the experiment day (i.e. faecal inoculation).

6.2.4.2.2 Faecal Inoculation

Freshly voided faeces were diluted down to 40% w/w with PBS, which had been previously reduced using the Hungate column, stomached using Stomacher 400 circulator (Seward) at 230 rpm for 45 seconds and filtered through filter bags (Stomacher® lab system, BA6141/STR bag filters made for Seward LTd.). The filtrate was collected into an autoclaved glass bottle. Subsequently, it was further diluted down to 20% w/w using the pre-prepared reduced and sterilized basal medium. Each fermenter vessel was inoculated to 10% w/w with faecal sample (samples were taken from the 20% w/w dilution). This two-

dilution step of the faecal sample was to reduce the amount of PBS incorporated into the medium and to maintain the basal medium buffering capacity. The whole medium was inoculated all at once; the final pH was adjusted to 6.8 inside the anaerobic cabinet, and then poured into separate autoclaved and reduced 250 mL glass bottles. The working volume of each culture was 100 mL. For the control experiments, the 40% w/w dilution of the faecal material was autoclaved to kill the bacteria and inactivate the enzymes, and then allowed to cool down to room temperature prior to being stored refrigerated in a tightly closed glass bottle. Control medium was prepared exactly the same way as the colonic media; however, the autoclaved faecal sample was used.

The batch fermentation model consists of 250 mL sterile glass bottles filled with the inoculated basal medium and dissolution baskets, which kept the coated tablets suspended in the medium. Coated tablets were added to the baskets and then into the test media. The bottles were placed on a shaker running at a speed of 100 rpm in order to prevent the precipitation of the suspended faecal material. Experiments representing the colonic (with faecal content) conditions and control (with autoclaved faecal content) experiments were performed in triplicate on two consecutive days. The use of autoclaved faecal material as a control medium allowed a closer simulation of the density and viscosity of the colonic medium (inoculated with fresh faecal material), yet with no bacterial activity.

6.2.4.2.3 Post-Sample Treatment and Analysis

One millilitre (1 mL) samples were withdrawn at 0, 3, 6, 9, 12 and 24 hours and centrifuged at room temperature at 14,000 rpm using an Eppendorf Centrifuge 5418 (Eppendorf AG, 22331 Hamburg, Germany) for 10 minutes. The supernatant was then filtered through 0.2 µm (Minisart High-Flow single use syringe filter, Sartorius Stedim Biotech GmbH, Germany) and kept at -20 ºC until analysis for drug concentration by HPLC. The HPLC method used in this work is based on the work published by Isariebel et al. (2009).

The samples were allowed to thaw and analysed using a Dionex (Leeds, UK) HPLC, equipped with a Dionex ASI-100 automated sample injector and a Dionex PDA-100 photodiode array detector. The sample (10 µL) was injected into a C18, 5 µm, 250 x 4.6 mm column (Phenomenex, Macclesfield, Cheshire, UK). The mobile phase consisted of

acetonitrile (MeCN)-water (25:75 v/v) adjusted to pH 2.5 with phosphoric acid (flow rate = 1.7 mL min^{-1}). UV detection was carried out at 245 nm. The retention time of paracetamol was 2.38 minutes with this system.

6.2.5 Characterization Techniques

6.2.5.1 Scanning Electron Microscope (SEM)

Changes in the surface morphology of tablets coated with zein alone and HAS/Zein after dissolution and fermentation studies were observed using SEM. Samples were mounted onto stubs using double-sided tape and were gold coated by a Polaron SC7640 sputter gold coater manufactured by Quorum Technologies. The thickness of the gold coating was about 15 nm. The imaging process was performed in a high vacuum environment. Imaging process was performed with a JEOL JSM5900 LV SEM (Japan), mounted with a tungsten filament with an acceleration voltage of 5-20 kV.

6.2.5.2 Light Microscopy

Images of the tablets under study were captured using a light microscope (Leica Mz75, Switzerland) connected to a digital camera (IC D model).

6.3 Results and Discussion

This section presents the results of the dissolution and fermentation studies of zein alone, pectin/zein and HAS/Zein coatings. The influence of incorporated polysaccharides such as pectin and high amylose maize starch (HAS) on the susceptibility of zein coatings to digestion by enzymes produced by colonic microbiota is investigated. Moreover, the influence of using different protocols on the results of the fermentation studies is verified.

6.3.1 Faecal Fermentation of Organic-based Zein Coated Tablets, Protocol I

As discussed in Chapter 4, organic-based zein coatings were quite resistant to digestion by the upper GIT enzymes and they offered drug release protection from tablets coated with these polymeric coatings making them good candidates for colonic-specific delivery. Paracetamol tablets coated with organic-based zein were therefore tested in colonic (with

faecal content) and control (without faecal content) conditions using the batch culture fermenters. Figure 6.3 presents the average percentage release of paracetamol from tablets coated (TWG = 20%) with organic-based zein solution plasticized with 20% w/w glycerol in colonic and control conditions and phosphate buffer pH 6.8 plotted against time. The dissolution profiles show higher drug release under colonic conditions compared to control conditions except at the second sampling point (8 hours) with the maximum drug release at the end of the 24-hour study being 64% and 50%, respectively. The drug release was the slowest in phosphate buffer pH 6.8 with the maximum release being 35%.

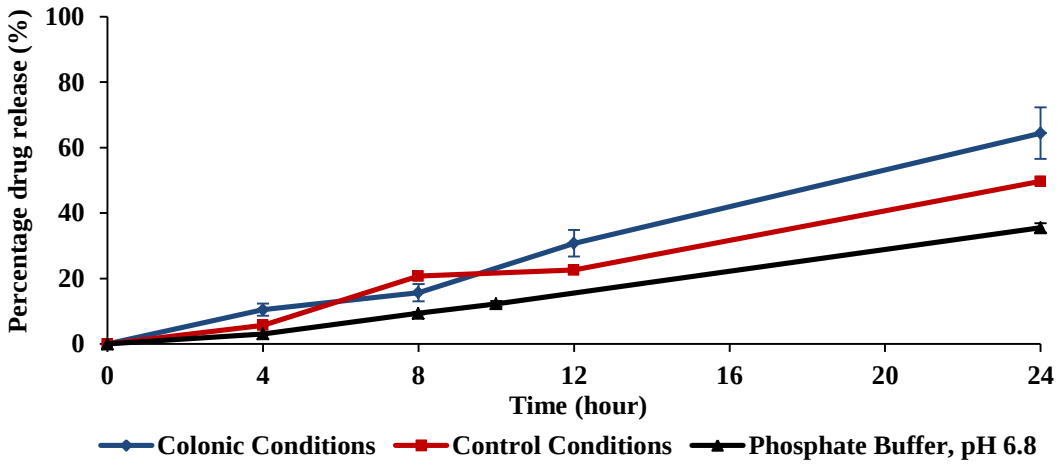


Figure 6.3 Dissolution profiles of paracetamol tablets coated (TWG = 20%) with organic-based zein solution (20% w/w glycerol) under Protocol I colonic conditions (n = 3), Protocol I control conditions (n = 1) and phosphate buffer (pH 6.8) (n = 3). Bars represent standard deviation (SD).

These results suggest that zein itself is degraded to some extent by the microbiota. The difference in drug release between colonic and control conditions can be related to various factors including the susceptibility of zein to the colonic enzymes, the bacterial count in the colonic medium and differences in composition, viscosity and density of the control and colonic media. The susceptibility of zein to digestion by the colonic enzymes is expected to influence the rate of the degradation process of the film coating. Other film ingredients such as polysaccharides might therefore be added to improve the susceptibility of these coatings to the enzymatic activity in the colon. In control conditions, the test medium consisted of the basal growth medium diluted with PBS whereas the medium used to mimic the colonic conditions was inoculated with faecal material. The control medium is therefore less dense and less viscous than the colonic medium. The low density and viscosity of the control medium might have improved water diffusion into the tablet core

and hence enhancing the dissolution of the drug and other tablet core additives. As a result, higher pressure builds up within the tablet pushing the dissolved drug out to the external medium.

Furthermore, the basal medium (Table 6.2) is quite complex with different components, that although present at the same concentration in the colonic medium, could compromise the protein coatings integrity to a higher extent in control conditions. This assumption can be explained either by a solubilising effect induced by certain components such as Tween 80 and bile salts, or by changes in the swelling behaviour of zein. The swelling properties of zein films control their permeability and were reported to be altered by the ionic strength of the aqueous environment by Oh et al. (2003). The impact of the non-ionic surfactant (Tween 20) on the release of encapsulated compounds from spray-dried microcapsules of zein and Tween 20 was reported by Xiao et al. (2011). The action of anionic (Ruso et al., 2004; Mehta et al., 2010) and cationic (Mehta et al., 2009) surfactants as denaturants and as solubilising agents for zein has been also reported. These assumptions might therefore suggest that the control experiment might not have offered relevant control conditions and a new control needs to be used. Lastly, the colonic microbiota is mostly anaerobic in nature and hence the bacterial count might have been reduced during the first steps of preparation (faecal dilution and homogenization) that took place on lab benches.

The release of paracetamol was the slowest in phosphate buffer pH 6.8 which indicates that the release in control conditions (with no faecal content) is not simply dependant on the pH and it is further influenced by the composition of the basal medium as explained earlier.

6.3.2 Faecal Fermentation of Pectin/Zein Pseudolatex Coated Tablets, Protocol I

A selection of polysaccharides are known to avoid digestion in the upper GIT but are digested by the enzymes produced by the colonic microbiota. These naturally occurring, nontoxic and biodegradable compounds are therefore good candidates for bacterially-triggered colon-specific drug delivery. The main drawback of these polysaccharides is their propensity to swell or dissolve in aqueous conditions and they are therefore often mixed with water insoluble polymers to overcome this. Pectin from apple (USP grade), a high methoxylated pectin, is an example of such polysaccharides. Pectin is digested by the

colonic microbiota; however, its solubility in water is a major disadvantage. Therefore, it is usually mixed with water-insoluble polymers such as ethyl cellulose in order to control the polysaccharide swelling properties and prevent premature drug release in the upper GIT. In this work, pectin was mixed with the water-insoluble zein proteins (formulations 1 and 2, Table 6.1) and used for coating paracetamol tablets.

Figure 6.4 shows the average percentage of drug released from tablets coated with 1:30 pectin/zein pseudolatex up to 12% and 20% TWG in distilled water. This figure was presented in Chapter 5 and it is shown here again for clarity. The dissolution profile shows that tablets coated with 1:30 pectin/zein pseudolatex up to a coating level of 20% TWG released an average of 43% of the drug within the first 6 hours, whereas a complete drug release was obtained from those coated up to 12% TWG in distilled water. These results, as discussed in Chapter 5, show that there was a poor retardation of the drug release and indicate that zein polymers could not control the swelling and dissolution of pectin efficiently even at low pectin/zein ratio (1:30) and high coating levels (20% TWG). The dissolution of pectin therefore resulted in the disruption of the film integrity creating pores through which the drug release could occur. Thinner coatings induced faster drug release as the formation of continuous channels within the film thickness via the dissolution of pectin is expected to be faster as compared to thicker films. Moreover, the drug needs to overcome a narrower barrier before it reaches the external medium. With a transit time through the stomach and small intestine of approximately 2 and 4 hours (Yang, 2008), respectively, this would suggest that circa 43% of the drug would be released into these areas from tablets coated with pectin/zein pseudolatex (20% TWG). This result suggests that the 1:30 pectin/zein coating system is not ideal for colonic targetting applications as premature drug release in the upper GIT is expected to occur.

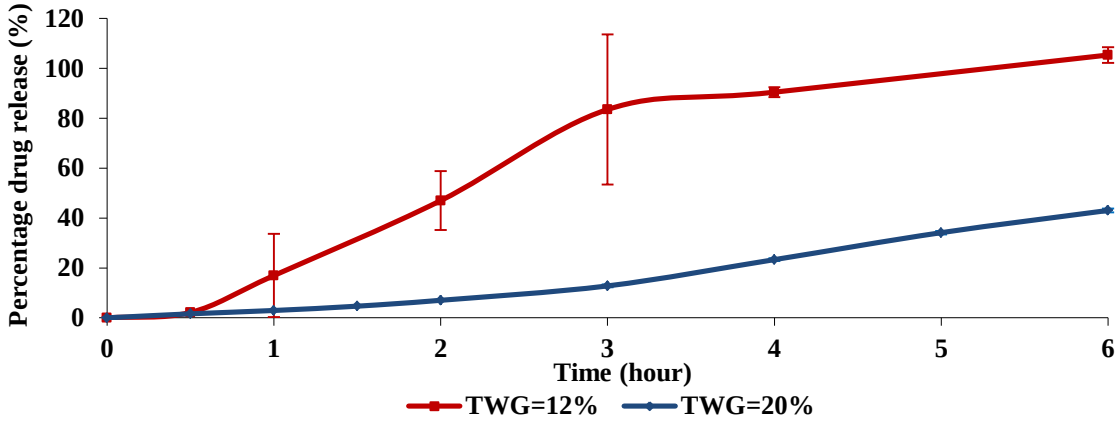


Figure 6.4 Dissolution profiles of paracetamol tablets coated with (1:30) pectin/zein pseudolatex up to 12% and 20% TWG in distilled water. Each point represents the mean ± SD (n = 3).

Although pectin/zein coatings did not prove ideally suitable for colonic delivery, the drug release from such systems with the higher coating level was studied under colonic and control conditions and is shown in Figure 6.5. The dissolution profiles did not reveal a large difference in the drug release rate and extent between the two conditions. This can be due to the very low level of pectin in the film coatings which could not be recognized by the colonic microbiota. Pectin is therefore assumed to have functioned as a pore former that dissolved upon contact with the aqueous environment of both colonic (with faecal content) and control (without faecal content) media. As a result, aqueous channels were formed within the coating structure of tablets tested in both control and colonic conditions. The rate and extent of drug release from these tablets in both conditions were therefore comparable.

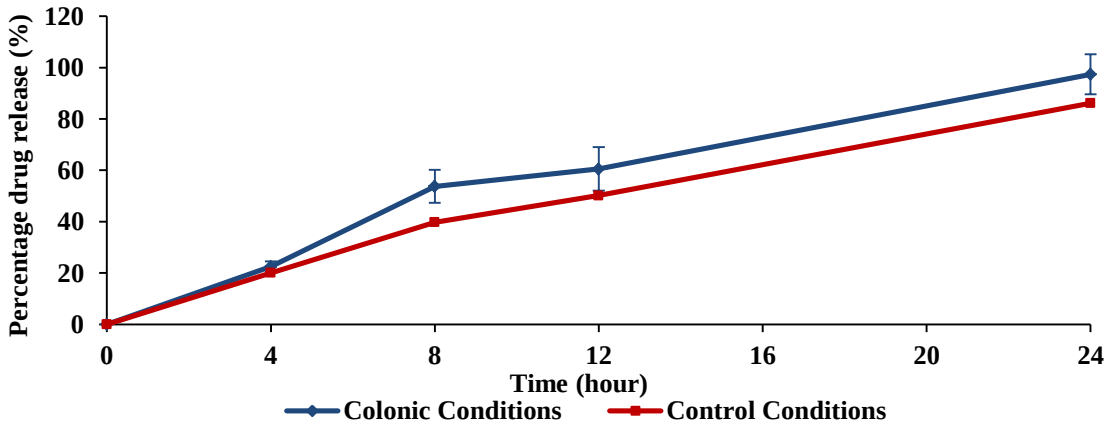


Figure 6.5 Dissolution profiles of paracetamol tablets coated (TWG = 20%) with (1:30) pectin/zein pseudolatex under Protocol I colonic (n = 3) and Protocol I control conditions (n = 1). Bars represent standard deviation (SD).

6.3.3 Faecal Fermentation of Zein Alone and HAS/Zein Pseudolatex Coated Tablets, Protocol I

Resistant starch is the fraction of starch that is neither digested nor absorbed in the small intestine, but is digested in the large bowel by the colonic microbiota. Resistant starch is classified into four types. Type 3, known as retrograded starch, forms after certain heat-moisture treatments of the starch, whereas type 4 is chemically modified starch such acetylated amylose starch. In this work, the dispersion of acetylated high amylose maize starch (HAS) was prepared via heat/moisture treatment (Section 6.2.1). Amylose swells upon contact with aqueous media but, unlike pectin, is water-insoluble. The aqueous dispersion of HAS was mixed with zein aqueous dispersion. HAS/Zein aqueous dispersion coatings were then tested for their susceptibility to digestion by enzymes produced by the colonic microbiota.

A colonic-targetted drug delivery system is expected to prevent premature drug release in the upper GIT. The ability of zein alone and HAS/Zein films to confer release protection through the upper GIT was examined in Chapter 5; however, the data is shown here again for clarity. Figure 6.6 represents the influence of upper GIT enzymes and conditions on the integrity of zein pseudolatex and 1:5 HAS/Zein coatings (formulations 3 and 4, Table 6.1) and hence the drug release from tablets coated (TWG = 20%) with such polymeric systems. It indicates that these films conferred release protection through the upper GIT with only 19% and 21%, respectively, of the drug being released after a total of 8 hours in SGF with pepsin and SIF with pancreatin. This figure also suggests that HAS containing coatings showed no difference in drug release rate and extent compared to zein alone coatings and confirms that HAS containing films were resistant to digestion by the enzymes of the upper GIT at least over this test duration. The fact that amylose is water-insoluble might account for the obtained similar drug release pattern in the upper GIT.

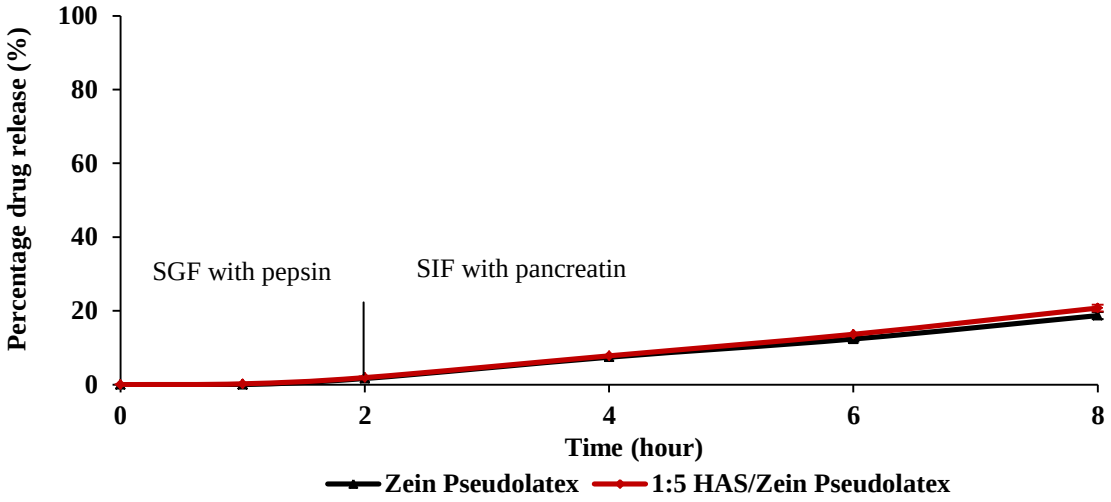


Figure 6.6 Dissolution profiles of paracetamol tablets coated (TWG = 20%) with zein alone pseudolatex and 1:5 HAS/Zein pseudolatex, in SGF with pepsin pH 1.2 (2 hours) then SIF with pancreatin pH 6.8 (6 hours). Each point represent the mean ± SD (n = 3). (Error bars are very small).

The release of paracetamol from tablets coated with zein alone and 1:5 HAS/Zein pseudolatexes was then investigated in colonic and control conditions following protocol I, as shown in Figure 6.7 and Figure 6.8. Both zein alone and 1:5 HAS/Zein pseudolatex coated tablets showed higher drug release under control conditions in comparison with the colonic conditions. This result can be partially attributed to the density and composition of the basal growth medium (control medium), the susceptibility of zein to the colonic enzymes, and the bacterial count in the colonic medium, as explained earlier in section 6.3.1.

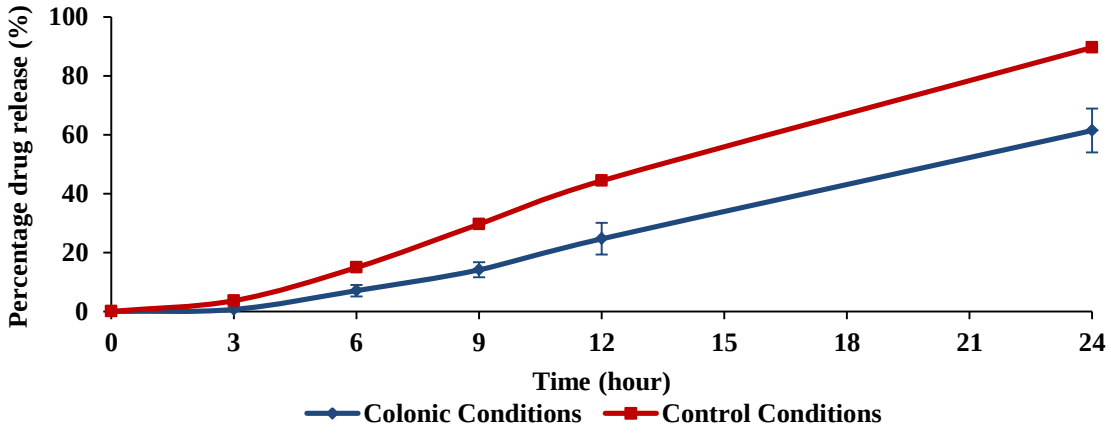


Figure 6.7 Dissolution profiles of paracetamol tablets coated (TWG = 20%) with zein alone pseudolatex under Protocol I colonic (n = 3) and Protocol I control conditions (n = 1). Bars represent standard deviation (SD).

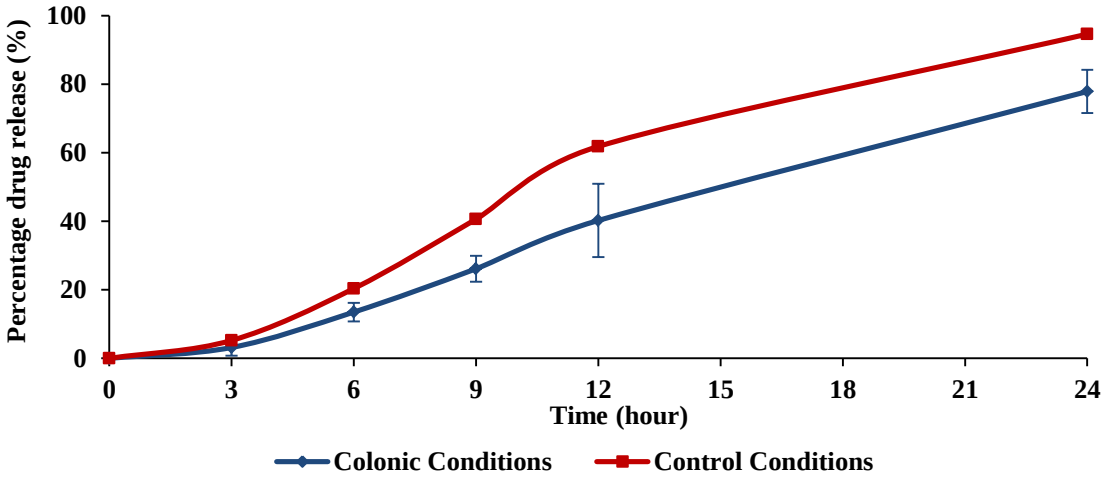


Figure 6.8 Dissolution profiles of paracetamol tablets coated (TWG = 20%) with 1:5 HAS/Zein pseudolatex under Protocol I colonic (n = 3) and Protocol I control conditions (n = 1). Bars represent standard deviation (SD).

As previously suggested (Section 6.3.1), the control experiment might not have offered the relevant control conditions and therefore one can compare the release profiles of both zein alone and 1:5 HAS/Zein pseudolatex coated tablets only under colonic conditions. Figure 6.9 displays the drug release from zein alone and 1:5 HAS/Zein coated tablets under colonic conditions. The release of paracetamol from 1:5 HAS/Zein coated tablets in the presence of the colonic microbiota is significantly ($p < 0.05$) higher than from those coated with zein alone pseudolatex. This result confirms that the HAS is being selectively digested by the microbiota.

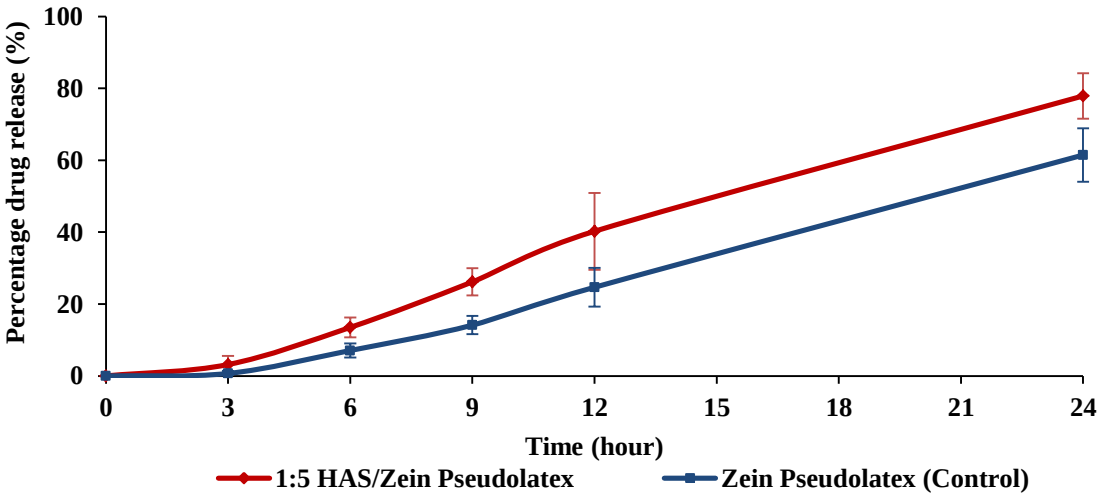


Figure 6.9 Dissolution profiles of paracetamol tablets coated (TWG = 20%) with 1:5 HAS/Zein pseudolatex and zein alone pseudolatex under Protocol I colonic conditions. Each point represent the mean ± SD (n = 3).

Table 6.3 displays the average percentage drug release from tablets coated with zein alone and 1:5 HAS/Zein pseudolatexes in colonic conditions, and the [1:5 HAS/Zein] release: zein alone release ratio. Upon the incorporation of HAS to the film coatings, the average % drug release was increased by a factor between 1.27 to 4.6.

Table 6.3 The average % of drug release from tablets coated with zein alone and 1:5 HAS/Zein pseudolatex in Protocol I colonic conditions, and the [1:5 HAS/Zein]: zein alone release ratio.

Time (hour)	**Zein alone**	**1:5 HAS/Zein**	**[1:5 HAS/Zein]: zein alone release ratio**
	Average % released $_1$ ± SD	Average % released $_2$ ± SD	Average $_2$/Average $_1$
0	0.00 ± 0.00	0.00 ± 0.00	--
3	0.69 ± 0.73	3.17 ± 2.39	4.60
6	7.10 ± 1.96	13.55 ± 2.74	1.91
9	14.22 ± 2.56	26.33 ± 3.80	1.85
12	24.93 ± 5.44	40.63 ± 10.82	1.63
24	62.46 ± 7.56	79.04 ± 6.34	1.27

Both zein alone and 1:5 HAS/Zein pseudolatex coated tablets remained intact throughout the whole 24 hours test in both colonic and control conditions; optical microscopy images are shown in Figure 6.10.

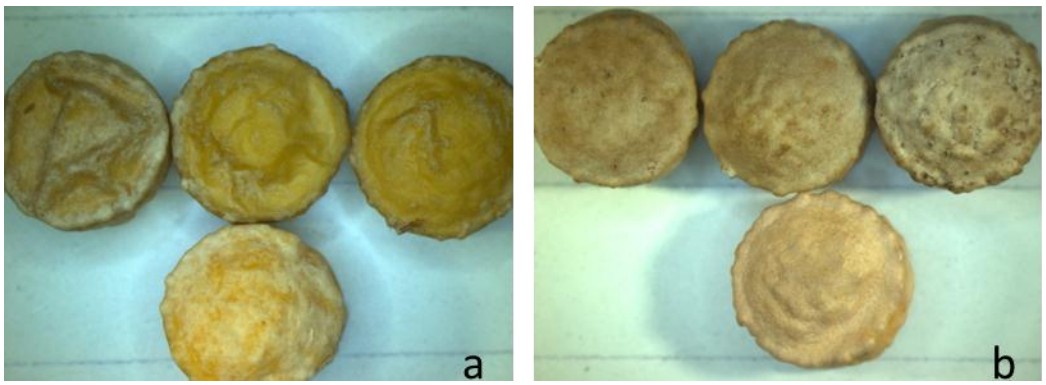


Figure 6.10 Optical microscope images (4x) of paracetamol tablets coated (TWG = 20%) with zein alone (a) and 1:5 HAS/Zein pseudolatex (b) after 24 hours immersion in colonic (upper row) and control (lower row) conditions.

The SEM microscopic images correlate well with the dissolution profiles obtained. Figure 6.11 presents SEM images of tablets coated with zein alone pseudolatex prior to any tests (a and b) and after a 24-hour immersion in control (c and d) and colonic (e and f) conditions. The images representing the control conditions show a more porous tablet surface compared to the one in colonic conditions, and this matches the higher release profile obtained under control conditions. Similarly, a tablet coated with 1:5 HAS/Zein pseudolatex showed porous surfaces under both conditions with more signs of digestion in colonic media. Comparing the surface of zein alone and 1:5 HAS/Zein pseudolatex coated tablets under colonic conditions (e and f) in Figure 6.11 and Figure 6.12, the HAS containing coatings show a porous structure along with some signs of digestion, whereas zein alone coatings are only porous in appearance.

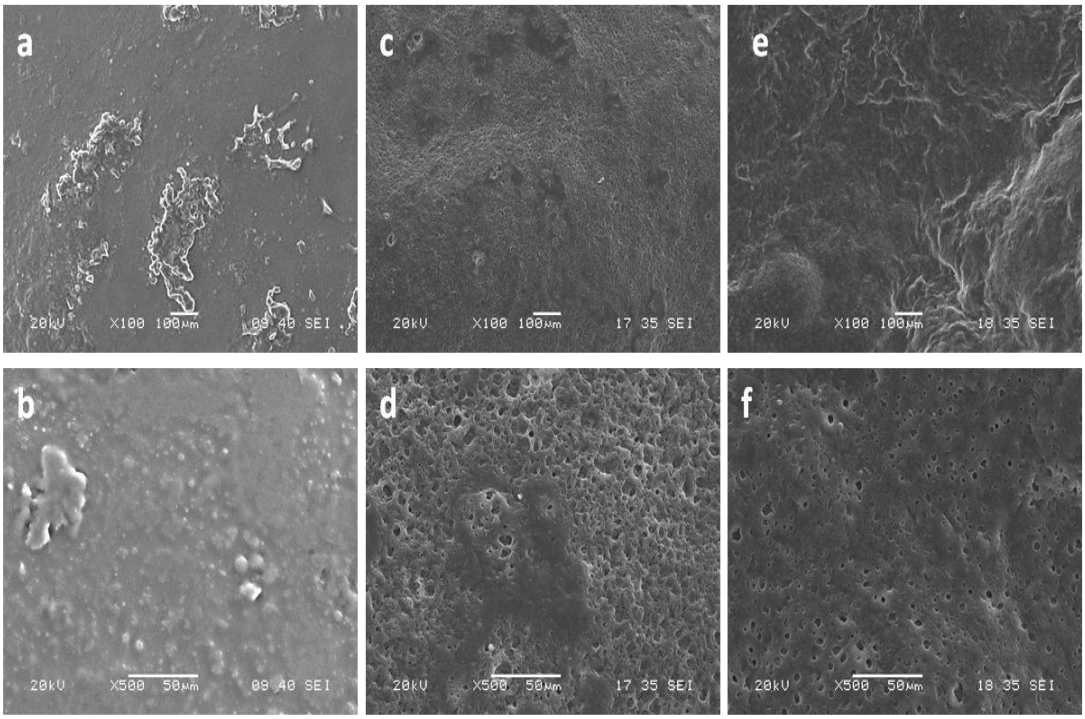


Figure 6.11 SEM images (100 and 500x) of paracetamol tablets coated (TWG = 20%) with zein alone pseudolatex prior to any treatment (a and b) and after 24 hours immersion in Protocol I control (c and d) and Protocol I colonic (e and f) conditions.

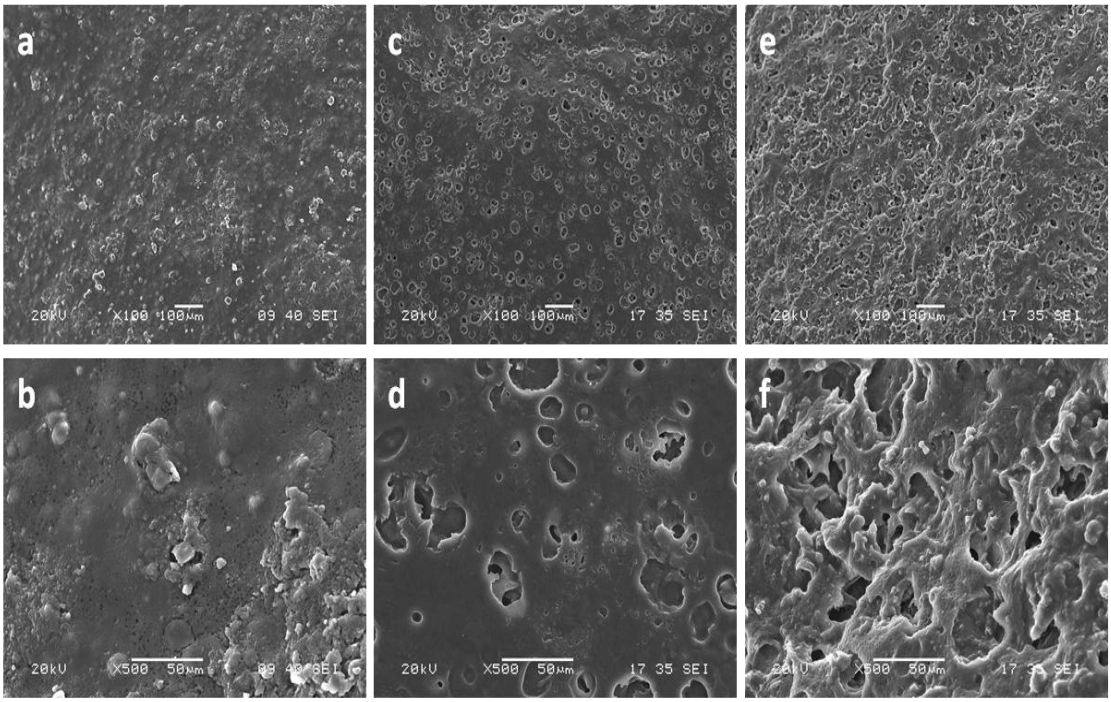


Figure 6.12 SEM images (100 and 500x) of paracetamol tablets coated (TWG = 20%) with 1:5 HAS/Zein pseudolatex: prior to any treatment (a and b) and after 24 hours immersion in Protocol I control (c and d) and Protocol I colonic (e and f) conditions.

6.3.4 Faecal Fermentation of Zein Alone and HAS/Zein Pseudolatex Coated Tablets, Protocol II

This section presents similar faecal fermentation studies to those performed in Section 6.3.3 on paracetamol tablets coated with zein alone and HAS/Zein aqueous dispersions (formulations 3 and 4, Table 6.1); however, protocol II was used.

These coatings i.e. zein alone and HAS/Zein pseudolatex films were demonstrated to confer release protection through the upper GIT, with only 19% and 21%, respectively, of the drug released after a total of 8 hours in SGF with pepsin and SIF with pancreatin (Figure 6.6). This result makes zein alone and HAS/Zein coatings good candidates for colonic targetting. Figure 6.13 shows the drug release from paracetamol tablets coated (TWG = 20%) with zein alone aqueous dispersion under colonic (with faecal content), control (with autoclaved faecal content) conditions and in phosphate buffer (pH 6.8) over 24-hour test. Figure 6.14 shows the drug release from paracetamol tablets coated (TWG = 20%) with 1:5 HAS/Zein aqueous dispersion under same conditions.

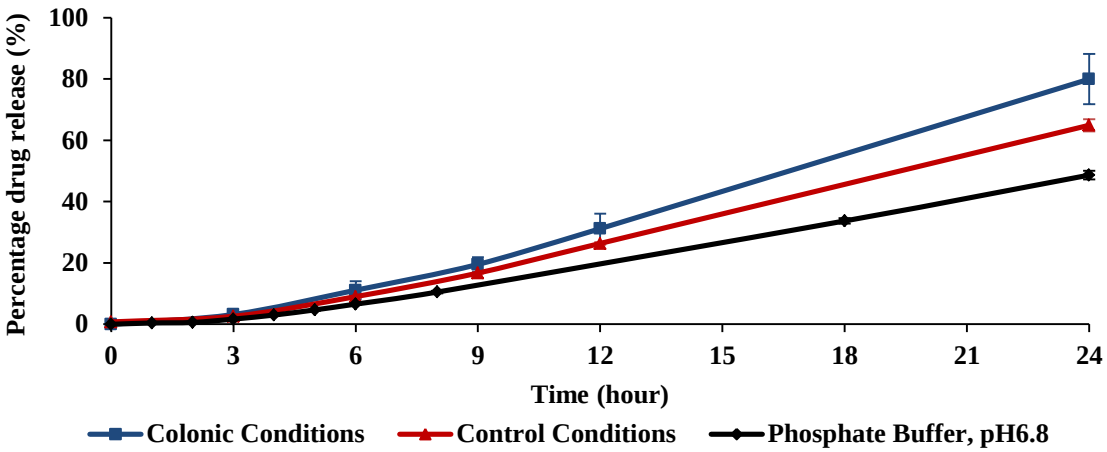


Figure 6.13 Dissolution profiles of paracetamol tablets coated (TWG = 20%) with zein alone pseudolatex under Protocol II colonic conditions, Protocol II control conditions and phosphate buffer (pH 6.8). Each point represents the mean ± SD (n = 3).

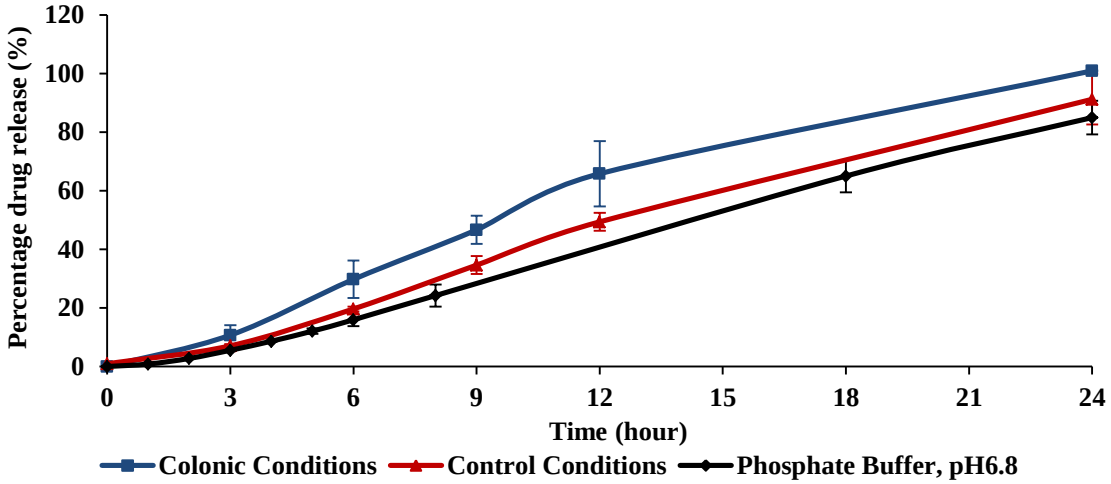


Figure 6.14 Dissolution profiles of paracetamol tablets coated (TWG = 20%) with 1:5 HAS/Zein pseudolatex under Protocol II colonic conditions, Protocol II control conditions and phosphate buffer (pH 6.8). Each point represents the mean ± SD (n = 3).

Under colonic conditions the release from both coatings i.e. zein alone and HAS/Zein is faster in the presence of the colonic microbiota. For example, at 12 hours the percentage drug release from both systems was increased by a factor of 1.2 and 1.3, respectively. Using a 2-tailed T-test (2 samples) to compare the AUC of the two curves, i.e. under Protocol II colonic and control conditions, the total drug release under colonic conditions was greater than under control conditions ($p = 0.049$ for zein alone and $p = 0.04$ for HAS/Zein coatings). The release in phosphate buffer (pH 6.8) was; however, lower than in

the control conditions suggesting that the release is not simply a response to the more alkaline conditions of the colon. It further suggests that the medium ingredients which closely represent the colonic conditions enhance the drug release through the film coatings possibly by solubilising the film components or by modifying the swelling behaviour of the zein as explained in Section 6.3.1.

Figure 6.15 compares the drug release from zein alone and HAS/Zein mixed coatings under colonic and control conditions. In comparison with the control conditions (dashed-lines), an increase in the drug release extent under colonic conditions (solid lines) is observed after approximately 9 and 3 hours for zein alone and mixed HAS/Zein coatings, respectively. The higher drug release in the presence of the colonic microbiota suggests that both polymers are being digested by the microbiota, albeit to a different extent. Comparing the release profile of zein alone and 1:5 HAS/Zein coated tablets under colonic conditions (solid lines) reveals that zein itself is degraded to some extent by the microbiota; however, the incorporation of HAS accelerated the film degradation process resulting in a significant ($p < 0.05$) increase in the rate and extent of paracetamol release.

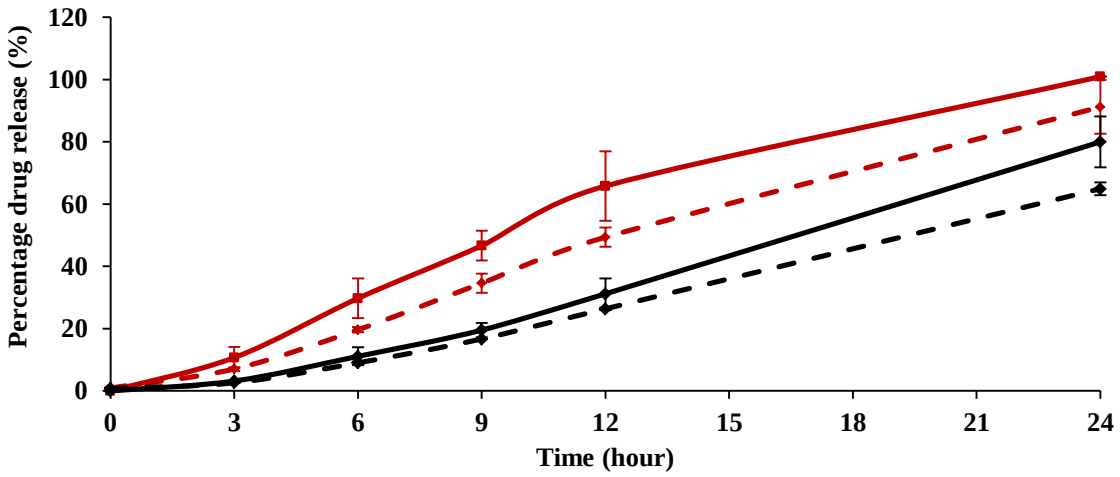


Figure 6.15 Release profile of paracetamol from tablets coated (TWG = 20%) with zein alone (black lines) and 1:5 HAS/Zein dispersions (red lines) in Protocol II colonic (solid lines) and Protocol II control (dashed lines) conditions. Each point represents the mean ± SD (n = 3).

In HAS/Zein mixed coating systems, the HAS is expected to serve as a substrate to the colonic enzymes and the drug release is expected to occur following the digestion of starch domains within the film and the subsequent pore formation. This enzymatic digestion and the drug release from tablets coated with 1:5 HAS/Zein films in colonic conditions can be

assumed to follow the Michaelis–Menten mechanism, as explained by Freire et al. (2010b). It is thus time-, enzyme concentration- and substrate concentration- dependent. The transit time in the colon is long enough to allow sufficient contact time between the starch and the colonic enzymes. The composition of colonic microbiota and enzymes can vary according to different factors such as age, diet, diseases, medications and geographic region (Yang, 2008). Studying the enzymes activity, particularly the amylase activity, was beyond the objectives of this project, although it can be hypothesized that enzyme activity may play an important role in the determination of the release rate of the drug. Nevertheless, consistent test conditions were used throughout the studies. However, many formulation factors can influence the substrate (HAS) concentration and its susceptibility to the colonic microbiota within a film coat including the coating thickness, HAS/Zein ratio, surface area of the dosage form, and starch type.

The relatively small difference in the percentage drug release from tablets coated with 1:5 HAS/Zein aqueous dispersion between colonic (with faecal content) and control (with autoclaved faecal content) conditions, as shown in Figure 6.15, can be attributed to different reasons including the low HAS/Zein ratio (1:5) and limited amount of amylose in the film which would prevent the formation of a continuous amylose network and thus render amylose less accessible to the colonic enzymes. This was supported by the AFM results (discussed in Chapter 5) which suggested a miscible and homogenous surface with no signs of phase separation of the zein and HAS film components. The low surface area provided by single dosage forms such as tablets compared to that provided by the muliparticulate systems such as pellets is another reason responsible for slowing down the digestion of starch. Moreover, the high coat thickness can slow the bacterial action and finally the chemical modification, i.e. the acetylation of starch, although minimal (DS = 0.06), might have altered the susceptibility of this starch type to the colonic enzymes. A similar result was reported by Freire et al. (2010b). However, the optimization of the HAS/Zein mixed coatings (described in Chapter 5) intended for colon-specific delivery revealed that only the formulation with 1:5 HAS to zein ratio and film-coating thickness of 20% TWG was able to suppress drug release in 0.1 M HCl. Increasing the HAS/Zein ratio up to 1:3 at a coating level of 20% TWG or reducing the coating thickness of 1:5 HAS/Zein coatings from 20% to 10% TWG, resulted in faster drug release accompanied by film cracking in 0.1 M HCl. Thus a premature drug release in the upper GIT is expected

to occur, indicating that zein could not control the swelling of HAS at a high level (1:3 HAS/Zein) or at a low coating thickness (TWG = 10%).

The difference in the extent of drug released from tablets coated with zein alone and 1:5 HAS/Zein films under colonic conditions (with faecal content), as shown in Figure 6.15, can be explained by a combination of both enzymatic digestion of the starch domains within the film and the pH-dependent swelling properties of this starch type. As demonstrated in Chapter 5, the dissolution results of HAS-containing coatings suggested a pH-dependent swelling release. The drug release from 1:5 HAS/Zein pseudolatex coatings was faster than that from zein alone pseudolatex coatings in phosphate buffer pH 6.8. However, the drug release rate and extent in 0.1 M HCl were essentially the same for both coatings with an average of 64 % of the drug being released over 24-hours test. The pH-dependent swelling of different starch types has been reported in the literature (Freire et al., 2009b).

Table 6.4 displays the average percentage drug release from tablets coated with zein alone and 1:5 HAS/Zein pseudolatex in Protocol II colonic conditions, and the [1:5 HAS/Zein] release: zein alone release ratio. It reveals that the average % release from tablets coated with 1:5 HAS/Zein pseudolatex is 2 to 3 times higher than from those tablets coated with zein alone aqueous dispersion, specifically for the first four sampling points (3, 6, 9 and 12 hours). The decrease in the average % released $_2$ to average % released $_1$ ratio towards the end of the test can be explained by the fact that a 24 hours long dissolution test would eventually compromise the film integrity of both systems minimizing the difference in the % released by the continuous expansion of the coating as the tablet core material and the water-soluble film ingredients dissolve.

Table 6.4 The average % of drug release from tablets coated with zein alone and 1:5 HAS/Zein pseudolatex in Protocol II colonic conditions, and the [1:5 HAS/Zein]: zein alone release ratio.

Time (hour)	Zein alone	1:5 HAS/Zein	[1:5 HAS/Zein]: zein alone release ratio
	Average % released $_1$ ± SD	Average % released $_2$ ± SD	Average $_2$/Average $_1$
0	0.00 ± 0.00	0.00 ± 0.00	--
3	3.20 ± 0.08	10.71 ± 3.41	3.35
6	11.12 ± 2.89	29.74 ± 6.38	2.67
9	19.53 ± 2.35	46.63 ± 4.79	2.39
12	31.19 ± 4.91	65.79 ± 11.16	2.11
24	79.95 ± 8.18	100.90 ± 0.16	1.26

Optical and SEM microscopic imaging were performed to examine the tablets prior and after the dissolution and Protocol II fermentation studies. Figure 6.16 shows some optical microscopy images of 1:5 HAS/Zein (a to c) and zein alone (d to f) pseudolatex coated paracetamol tablets after 24 hours immersion in phosphate buffer (pH 6.8) and Protocol II colonic and control conditions. Zein alone coated tablets remained intact in the three media, whereas HAS containing coatings ruptured into two halves at the end of the test in the Protocol II colonic medium (b and b´). These observations are different from those obtained with protocol I where both tablet types remained intact throughout the 24 hours test in phosphate buffer pH 6.8, Protocol I control and Protocol I colonic media. Figure 6.16 shows a profile view of tablets coated with zein alone aqueous dispersion tested in Protocol II control conditions (g) and phosphate buffer pH 6.8 (h) for 24 hours. This microscopy image shows that the tablet swells to a higher degree in the control medium suggesting that the film integrity and hence the coating barrier properties were more compromised in the Protocol II control conditions than in phosphate buffer. The increase in coating permeability could result in higher levels of medium diffusion into the tablet which leads to the expansion of the tablet core. The tablet expansion is expected to further decrease the film integrity allowing more medium to pass through the coatings into the tablet core.

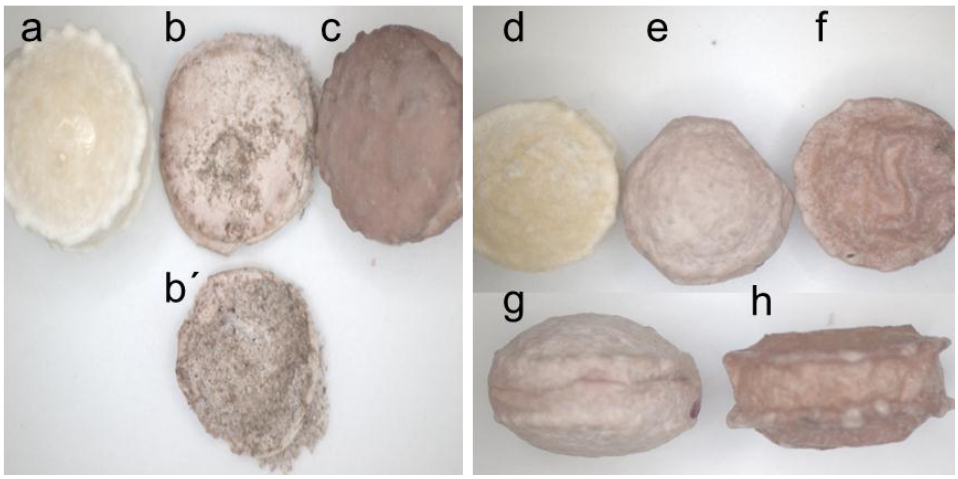


Figure 6.16 Optical microscopy images of paracetamol tablets coated (TWG = 20%) with 1:5 HAS/Zein after 24 hours immersion in phosphate buffer pH 6.8 (a), Protocol II colonic (b, b´, where b and b´ represent the two halves of the ruptured coating) and Protocol II control (c) conditions. Images d, e and f are paracetamol tablets coated (TWG = 20%) with zein alone pseudolatex after 24 hours immersion in the same conditions as a to c. Images g and h are profile views of tablets coated with zein alone pseudolatex after 24 hours immersion in Protocol II control (g) and phosphate buffer (h) conditions.

The results of the release studies are confirmed by the SEM images of the surface of coated tablets. SEM images before and after the Protocol II fermentation study from zein alone and 1:5 HAS/Zein pseudolatex coated tablets are shown in Figure 6.17 and Figure 6.18, respectively. Figure 6.17 shows that the coated tablet had a fairly smooth surface before the release (a and b) but showed signs of extensive digestion under Protocol II colonic conditions (e and f) compared to the Protocol II control (c and d) conditions. More significantly, SEM images of paracetamol tablets coated with HAS-containing dispersions (Figure 6.18) revealed a complete loss of coating structure and integrity under Protocol II colonic conditions (e and f), whereas the coating became highly porous under Protocol II control conditions (c and d) in comparison with the fairly smooth surface prior to the study (a and b). However, the surface of these HAS containing coatings was more porous and altered compared to the zein alone coatings under Protocol II control conditions (d in Figure 6.17 and Figure 6.18) which suggests that the HAS preferentially swells and dissolves in this medium and under a basic pH. This result is also shown in SEM images of both tablet types prior and after 24 hours immersion in phosphate buffer, pH 6.8 (Figure 6.19). The images display a more porous surface of the 1:5 HAS/Zein coatings compared to the zein alone ones.

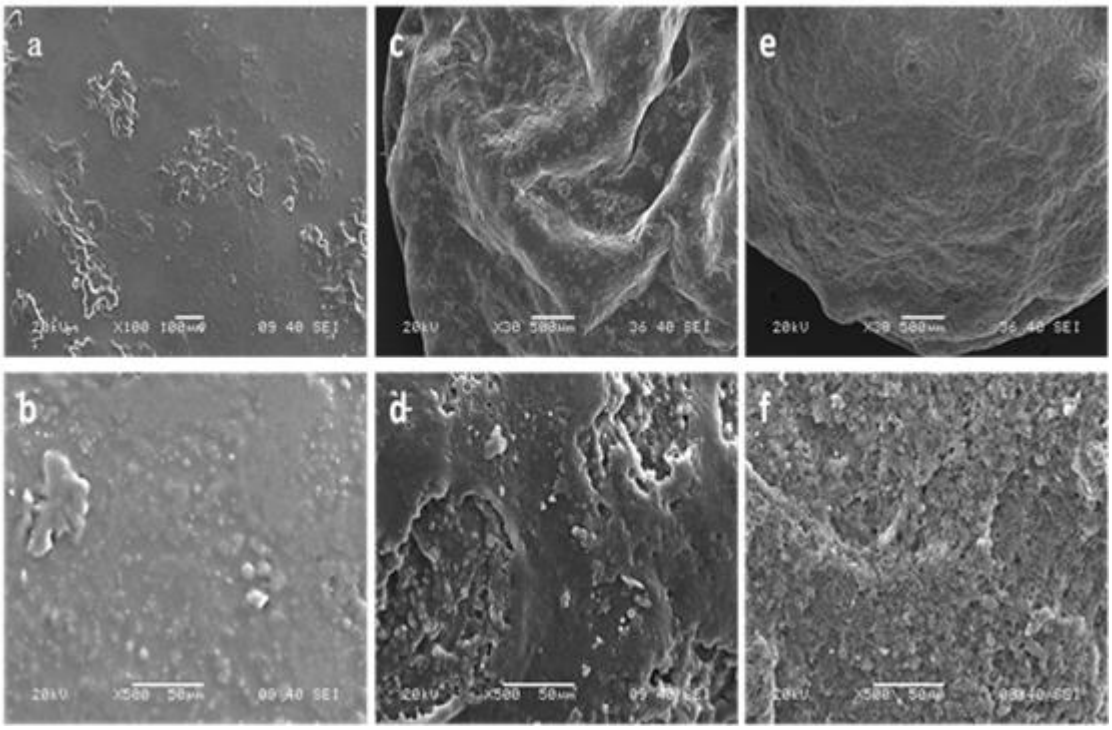


Figure 6.17 SEM images of paracetamol tablets coated (TWG = 20%) with zein alone pseudolatex prior to any treatment (a and b) and after 24 hours immersion in Protocol II control (c and d) and Protocol II colonic (e and f) conditions.

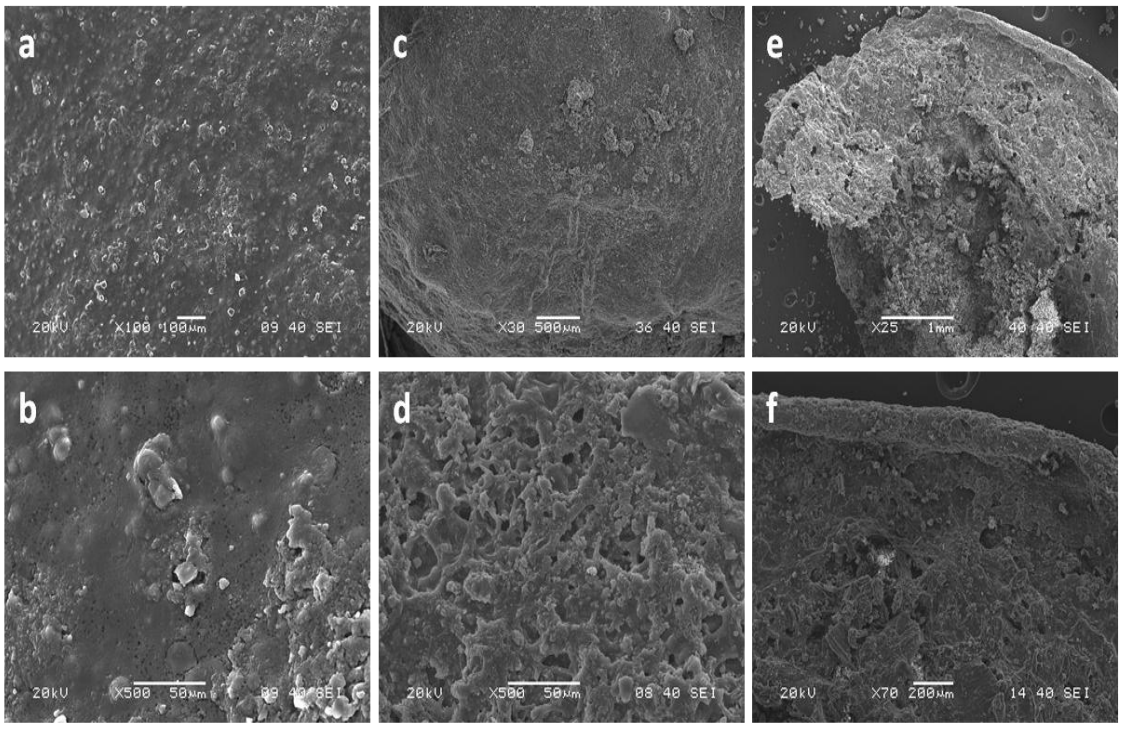


Figure 6.18 SEM images of paracetamol tablets coated (TWG = 20%) with 1:5 HAS/Zein pseudolatex prior to any treatment (a and b) and after 24 hours immersion in Protocol II control (c and d) and Protocol II colonic (e and f) conditions.

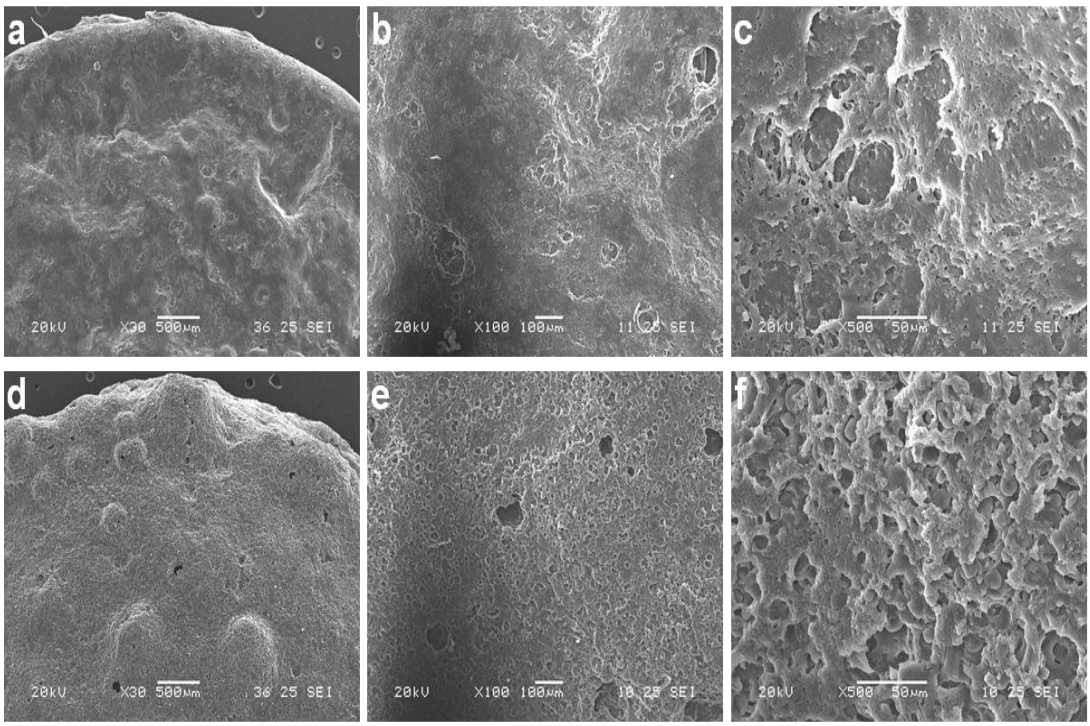


Figure 6.19 SEM images of paracetamol tablets coated (TWG = 20%) with zein alone (a, b and c) and 1:5 HAS/Zein pseudolatex (d, e and f) after 24 hours immersion in phosphate buffer pH 6.8.

6.3.5 A Comparison between Protocol I and II

Protocol II induced a faster drug release from both zein alone and 1:5 HAS/Zein pseudolatex coated tablets in comparison with protocol I under colonic conditions, as illustrated in Figure 6.20 and Figure 6.22, respectively. Differences in the two protocols can be summarized as follow: protocol II had a two-fold higher faecal content along with a lower PBS content. In addition, the basal medium used in the second protocol was fully reduced and hence the absence of oxygen would be expected to support the inoculated bacterial population and its growth to a better extent compared to protocol I. Consequently, the bacteria concentration would be expected to be higher using protocol II and hence a faster digestion of starch domains within film coatings would also be expected.

On the other hand, the release of paracetamol under control conditions was slower when protocol II was followed with both zein alone (Figure 6.21) and 1:5 HAS/Zein (Figure 6.23) pseudolatex coated tablets. This can be attributed to, as explained in Section 6.3.3, differences in the density, viscosity and composition of the basal growth medium used as the control condition in protocol I compared to the basal medium inoculated with

autoclaved faecal material used in protocol II. In protocol II, the density and viscosity of the control medium was therefore more comparable to the colonic medium (inoculated with fresh faecal material), whereas the density and viscosity of the control medium used in protocol I were much less than those of the colonic medium. As concluded earlier, the control conditions used in the second protocol offered a better and more relevant control environment.

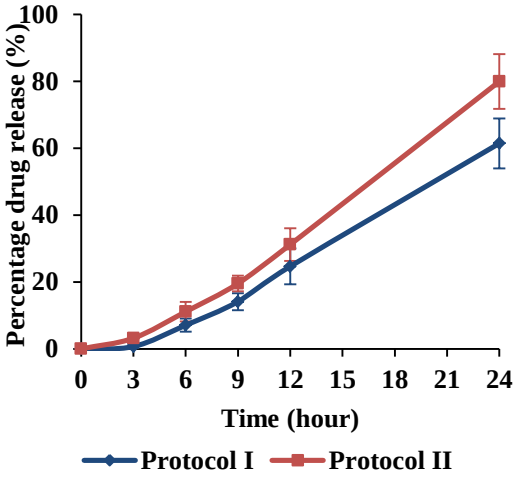


Figure 6.20 Dissolution profiles of paracetamol tablets coated with zein alone pseudolatex under colonic conditions. A comparison between protocol I and II (n = 3).

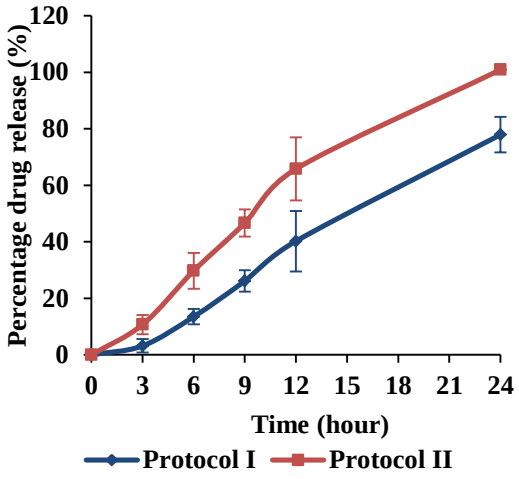


Figure 6.22 Dissolution profiles of paracetamol tablets coated with 1:5 HAS/Zein pseudolatex under colonic conditions. A comparison between protocol I and II (n = 3).

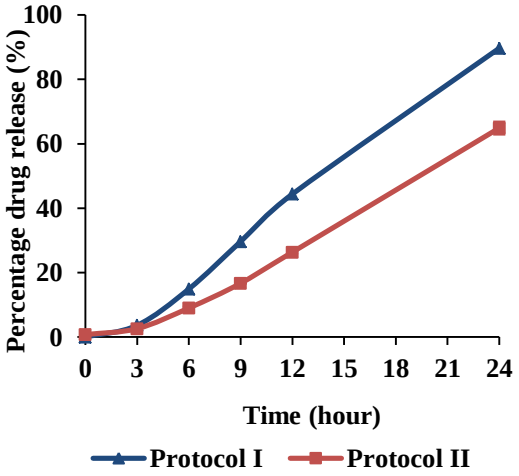


Figure 6.21 Dissolution profiles of paracetamol tablets coated with zein alone pseudolatex under control conditions. A comparison between protocol I (n=1) and II (n = 3).

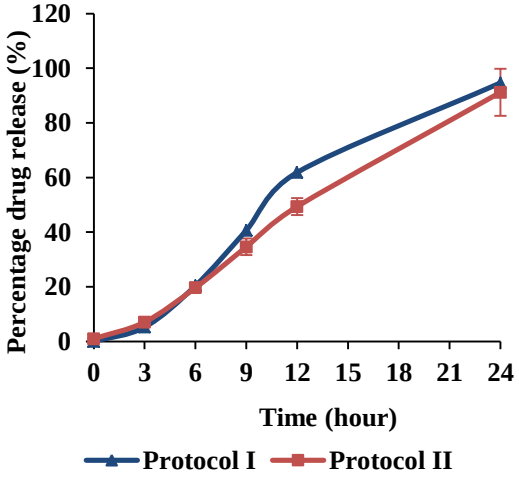


Figure 6.23 Dissolution profiles of paracetamol tablets coated with 1:5 HAS/Zein pseudolatex under control conditions. A comparison between protocol I (n=1) and II (n = 3).

6.3.6 Analysis of Release Data: Drug Release Kinetics and Mechanism of Drug Release

As explained in Chapter 1, the release rate can be either controlled by diffusion- or dissolution-controlled release mechanisms (Sah et al., 2001). Both diffusion- and dissolution-controlled systems can be further divided into: reservoir devices, in which the drug is surrounded by a polymeric membrane; and matrix devices, where the drug is distributed in a polymeric matrix. Various mathematical models have been used to describe the release rate from both systems. For diffusion-controlled reservoir-type non-degradable systems, the release rate is described primarily by Fick's law, which can be ultimately simplified to a zero-order rate equation (described in Chapter 1). However, diffusion-controlled matrix-type non-degradable systems can be described by the Higuchi model (Higuchi, 1963) or the Korsmeyer-Peppas model (Korsmeyer et al., 1983). Moreover, the release rate from biodegradable polymeric systems is governed by the degradation of the polymer membrane or matrix. Degradation can occur via: bioerosion, which involves material loss from the polymer bulk; or biodegradation, which refers to chemical or enzymatic degradation of the polymer structure. However, the degradation rate can be slow compared to the rate of drug diffusion, and hence the drug release mechanisms and kinetics in these cases can be considered to be analogous to those obtained with non-degradable systems. In cases where drug release occurs in parallel with polymer degradation, the drug release mechanism is complicated and occurs by drug diffusion, polymer degradation and/or polymer dissolution. In such systems, the permeability of the polymer to the drug increases with time as the polymeric matrix or membrane is being gradually degraded by enzymatic/chemical action.

The zero order equation, Eq. (6.1), describes the systems where the drug release rate is independent of its concentration, whereas the drug release is concentration dependent in systems that follow first order kinetics, Eq. (6.2). The Higuchi (1963) model, Eq. (6.3), describes a linear relationship between the drug release from an insoluble matrix and the square root of time based on Fickian diffusion processes.

$$C = k_0 t \qquad \text{Eq. (6.1)}$$

where C is drug concentration, k_0 is the zero-order rate constant expressed in units of

concentration/time and *t* is the time.

$$\log C = \log C_0 - k_1 t / 2.303 \qquad \text{Eq. (6.2)}$$

where C_0 is the initial concentration of drug, C is the drug concentration at time t, and k_1 is the first order constant.

$$Q = k_H t^{1/2} \qquad \text{Eq. (6.3)}$$

where Q is the amount of drug released, t is the time and k_H is a constant reflecting the design variables of the system.

The mechanism of drug release can be determined by fitting the first 60% of the drug release data in Eq. (6.4); this is the Korsmeyer-Peppas model (Korsmeyer et al., 1983):

$$\frac{M_t}{M_\infty} = k_{kp} t^n \qquad \text{Eq. (6.4)}$$

where M_t and M_∞ are the absolute cumulative amount of drug released at time t and infinite time, respectively; k_{kp} is a constant incorporating structural and geometric characteristics of the drug dosage form, and n is the release exponent, indicative of the mechanism of drug release. For "very thin polymeric films", the exponent n has a value of 0.5 when the drug release is diffusion-controlled (Fickian release). Case II transport or relaxation controlled delivery gives an n value of 1 (zero order process). Peppas et al. (1989) defines case II transport as the drug transport mechanism associated with stress and state-transition in hydrophilic glassy polymers which swell in water or biological fluids. When the n value lies in between these two values ($0.5 < n > 1$), it is in the case of anomalous or non-Fickian release which involves a coupling of diffusion and polymer relaxation. In addition, Super Case II kinetics is regarded when the values of $n > 1$ and it is mainly due to erosion of polymeric chains.

Mockel et al. (1993) use the mean dissolution time (*MDT*) as an expression for drug release rate which reflects the polymer's ability to retard the drug release; a higher *MDT*

value indicates great drug-retarding ability. *MDT* is estimated by Eq. (6.5):

$$MDT = \frac{n}{n+1} \cdot k_{kp}^{-1/n} \qquad \text{Eq. (6.5)}$$

where n is the release exponent and k_{kp} is the release rate constant derived from Korsmeyer-Peppas equation, Eq. (6.4).

In this work, zein alone and mixed HAS/Zein aqueous dispersions were used to coat the paracetamol tablets. Zein proteins are water insoluble which might suggest that drug release is driven mainly by diffusion. In addition, the polymer(s) surround(s) the drug compartment in coated systems and therefore such systems represent a good example of diffusion-controlled reservoir-type systems and a zero-order release is expected. However, a more complicated mechanism is expected if polymer biodegradation occurred in the presence of the colonic microbiota. A simple visual analysis of the dissolution profiles tends to suggest that the release rate can be described by a zero order process. The exception is when the tablet ruptured and the subsequent profile resembled more a first order process. The release data were fitted to these two models (zero and first order equations) and the calculated parameters are shown in Table 6.5. Table 6.5 reveals that with the exception of HAS containing coatings in colonic medium, the drug release rate relationship with drug concentration exhibited zero order kinetics for both formulations in some media; straight lines (r^2 ranging from 0.9928 to 0.9982) were obtained when the cumulative % drug release vs. time was plotted in accordance with the zero order equation. On the other hand, the drugs release from1:5 HAS/Zein pseudolatex-coated tablets in colonic medium followed first order kinetics (r^2 = 0.9776); which suggests that the drug release was concentration dependent. These results can be explained by the rupture of those tablets in the presence of the colonic microbiota, and the depletion of drug content from tablet core over test period.

The zein alone and HAS/Zein mixed coatings on the tablet are relatively thick (circa 203 μm) but could possibly be viewed as a “thin polymeric film” mathematically in the Korsmeyer-Peppas sense. In this case, the Higuchi model was used and the more in depth analysis of the Korsmeyer-Peppas model may be appropriate, as it could be argued that the molecular dynamics of the polymer may have a significant effect on the release profile of

the drug (data shown in Table 6.5). The release data showed a good fit to the Korsmeyer-Peppas equation, except the HAS containing coatings under both Protocol II colonic and control conditions that showed good fit to both models with approximately similar r^2 values. The values of *n* were > 1 suggesting a Super Case II release where the drug is mainly released by erosion of polymeric chains. The kinetic constant (k_{kp}) is higher for HAS-containing formulations particularly in colonic medium, and this fact suggests that the polymer relaxation rate increases when HAS is incorporated due to HAS swelling properties and its susceptibility to colonic digestive enzymes. Both formulations (zein alone and HAS/Zein coatings) exhibited a similar trend where the k_{kp} value in phosphate buffer pH 6.8 < k_{kp} value in control conditions < k_{kp} value in Protocol II colonic conditions. The values of the kinetic constant correlate well with the drug release profiles in these media. However, HAS-containing coatings exhibited higher values owing to the fact that HAS sped up the release of drug. Similarly, 1:5 HAS/Zein films had the lowest *MDT* value and hence the lowest ability to retard the drug release in colonic conditions, which is explained by the digestion of HAS domains by the colonic microbiota. In conclusion, the faster drug release of HAS containing coatings under colonic conditions along with the lower *MDT* values suggests that the extent of polymer relaxation was promoted by the colonic microbiota. Furthermore, the *MDT* values of HAS containing films in phosphate buffer (pH 6.8) were lower compared to zein alone coatings owing to the swelling properties HAS under more basic conditions.

Zein formulations showed the highest k_{kp} value in 0.1 M HCl due to the higher solubility of the tablet core at low pH values (as explained in Chapter 4), whereas 1:5 HAS/Zein coatings had the highest k_{kp} value in colonic conditions. Zein alone coatings had the highest *MDT* in phosphate buffer indicating that the polymer retarded the drug release in this medium to a higher extent compared to the other release media whose pH or enzymatic activity compromised the release retarding ability of the polymer. The release data in SGF with pepsin/ SIF with pancreatin was far below 60% (was circa 20% at the end of the experiment) and therefore the data points on the release profiles may be insufficient to provide accurate fitting to Korsmeyer-Peppas equation.

From the literature, one would expect the drug release from coated tablets to occur via diffusion through the spaces between the polymeric chains that form aqueous channels,

which would be enlarged as the polymer is being plasticized by the aqueous media and as the tablet core expands upon core dissolution. Oh et al. (2003) studied zein as a particular example and revealed that zein membranes hydrate and swell forming aqueous channels that are the dominant diffusional pathways for the drug rather than the drug partitioning into the membrane (which occurs by the dissolution of the solute into the membrane material itself). Diffusion can also occur through the pores formed upon the dissolution of the water-soluble ingredients of the film such as plasticizers, surfactants and the polysaccharides. Moreover, the digestive enzymes in SGF, SIF and particularly the colonic media can result in polymer erosion (dissolution) and/or degradation, which would ultimately speed up the drug release process.

In this study, the release of paracetamol from tablets coated with either zein alone or HAS/Zein pseudolatex is postulated to occur mainly via drug diffusion through the aqueous channels formed either by the spaces between the polymeric chains or by the dissolution of some film ingredients along with some contribution of erosion and/or degradation in the presence of digestive enzymes. Although macroscopically zein alone coatings remained intact after 24 hours exposure to the colonic media, SEM images indicated a degree of surface erosion. The fact that the polymeric carrier retained its structural integrity further confirms that these coated systems are primarily diffusion controlled systems (Ravivarapu, 2006), with the exception of tablets coated with HAS/Zein pseudolatex that showed a complete loss of the coating structure and integrity under colonic conditions. Accordingly, the *n* values obtained from Korsmeyer-Peppas equation might not be relevant to these coated systems. The Higuchi model and Korsmeyer-Peppas equation are essentially used to describe the drug release from polymeric matrices and thus viewing zein coatings as "thin polymeric film" might not have been applicable. In addition, the power law (Eq. 6.4) can give limited insight to the exact release mechanism as the values of *n* can be beyond the limits of Korsmeyer-Peppas model ($n < 0.45$) or the *n* values might not necessarily refer to a valid release mechanism for certain systems, as discussed by Shoaib et al. (2006).

Table 6.5 Mathematic modelling and kinetics of paracetamol release from tablets coated with zein alone and 1:5 HAS/Zein pseudolatex.

Formulation	Zero order		First order		Higuchi model		Korsmeyer-Peppas model				
	r^2	k_0 (h^{-1})	r^2	k_1 (h^{-1})	r^2	k_H ($h^{-1/2}$)	r^2	*n* value	K_{kp} (h^{-n})	Order of release	*MDT* (h)
In colonic conditions											
Zein alone	0.9928	3.727	0.9465	0.0338	0.9443	24.739	0.9970	1.5836	0.597	Super case II	0.85
1:5 HAS/Zein	0.9597	4.326	0.9776	0.0456	0.9937	29.005	0.9954	1.3078	2.644	Super case II	0.27
In control conditions											
Zein alone	0.9954	3.024	0.9692	0.0218	0.9520	20.126	0.9937	1.5418	0.531	Super case II	0.91
1:5 HAS/Zein	0.9928	3.999	0.9645	0.0504	0.9911	27.200	0.9892	1.2914	1.830	Super case II	0.35
In phosphate buffer(pH 6.8)											
Zein alone	0.9906	2.169	0.9765	0.0126	0.9246	12.817	0.9822	1.614	0.316	Super case II	1.26
1:5 HAS/Zein	0.9976	3.800	0.9612	0.0347	0.9545	22.735	0.9934	1.519	0.974	Super case II	0.61
In 0.1M HCl*											
Zein alone	0.9970	2.871	0.9695	0.0198	0.9431	17.081	0.9839	1.463	0.788	Super case II	0.70
1:5 HAS/Zein	0.9982	2.698	0.9774	0.0179	0.9471	16.075	0.9864	1.396	0.892	Super case II	0.63
In SGF/SIF*											
Zein alone	0.9957	2.690	0.9921	0.0129	0.9652	10.198	0.9834	1.748	0.543	Super case II	0.9
1:5 HAS/Zein	0.9937	2.957	0.9883	0.0143	0.9573	11.176	0.9738	2.159	0.299	Super case II	1.2

(*The data have been shown in Chapter 5).

6.4 Conclusions

Zein alone coatings were able to prevent extensive drug release in the upper GIT while under colonic conditions the release was considerably more rapid, particularly in the presence of the colonic microbiota. These data therefore suggest that zein can be potentially used for colon-specific drug delivery.

The extent of drug release was, however, modified by the incorporation of polysaccharides including pectin and acetylated high amylose starch. Zein could not control the swelling and dissolution of pectin within the pectin/zein polymeric coat even at a very low pectin/zein ratio (1:30). These systems were associated with premature drug release in the upper GIT occurred making them inappropriate candidates for colon-specific delivery. On the other hand, 1:5 HAS/Zein coatings were able to prevent extensive drug release in the upper GIT. The extent and rate of drug release from tablets coated with zein alone and HAS/Zein films were essentially the same in enzyme containing simulated gastric and simulated intestinal fluids. This implies that the digestive enzymes of the upper GIT are not being particularly selective to either zein or HAS polymers, at least over the test duration. However, divergence in the drug release profiles obtained with zein and HAS/Zein coated systems was found out under colonic conditions (with faecal content). The incorporation of HAS to zein coatings resulted in a significant increase ($p < 0.05$) in release rate and extent under colonic conditions. These data therefore indicate that zein can be potentially mixed with starch for colonic targeting purposes.

The drug release kinetics from both coating types (zein alone and HAS/Zein coatings) exhibited zero-order kinetics in various test media, with the exception being the 1:5 HAS/Zein coatings that ruptured when tested in colonic media and they therefore exhibited first order kinetics. Diffusion is expected to be responsible primarily for the drug release from these coated systems along with some contribution of the biodegradation of the polymer in the presence of digestive enzymes to the total release process.

This work also revealed the importance of the model used to represent the colonic and control conditions. The faecal content in the batch culture fermenters and the viscosity of the medium had noticeable influence on the drug release from the coated tablets under study.

7 General Conclusions

Zein proteins are the major storage proteins of corn. Zein has several interesting properties, potentially making it a good candidate for being used as a pharmaceutical excipient. It is soluble in aqueous alcohol solutions but not in water and possesses good film forming properties (Shukla et al., 2001). It is also biodegradable and biocompatible. The use of zein in the food industry is well established; however, its use as a pharmaceutical excipient needs to be more fully explored. Establishing such information is the overall aim of this study, with particular emphasis on the use of these proteins in modified-release formulations and specifically in drug delivery systems targetted to the colon. As part of this work, the potential use of zein as a carrier in a matrix system prepared using extrusion/spheronization was investigated. However, the use of organic solutions or aqueous dispersions (pseudolatexes) of zein as a film coating material was mainly studied.

Zein pellets of good quality were produced over a wide range of zein content (15 to 65% w/w on a dry basis), suggesting that zein could be used to reduce the level of the commonly used extrusion aid, i.e. MCC. Dissolution studies on different formulations of pellets were carried out in order to determine the factors which had an influence on the level of drug release obtained. The drug release from zein-based pellets followed first order kinetics but the release did not comply with the Biopharmaceutics Classification System (BCS) guidelines for immediate release products. At the same time, they showed no real evidence of a sustained-release profile. It was suggested that extrusion/spheronization did not permit a sufficiently intimate mixing of the drug and the matrix to occur.

Dissolution studies on the organic-based zein coated substrates were performed using several media and testing conditions in order to determine the factors that could change the drug release kinetics. SEM was used to observe the changes in the surface morphology of the coated tablets prior to and after the dissolution studies. From the dissolution studies, it was found that the organic-based zein coatings were quite resistant to degradation in simple buffers of acidic (pH 1) and more basic (pH 6.8) pH. The release pattern was found, as determined by a pH change method, to remain constant regardless of the pH of the medium. Zein was, however, susceptible to the digestive action of pepsin and rupture of the film coat was observed. The time needed for the coat to break was thought to be a

function of the coating thickness, i.e. thicker coatings showed higher stability to digestion. The drug release from tablets coated at 20% TWG was very low (circa 2%) prior to rupture and over the residence time of a solid dosage form (2 hours) in the stomach. Digestion was not observed to the same extent in the presence of pancreatin and the film coats had not broken even at the end of the experiment (5 hours). Given these results, zein-based coated substrates could be potentially designed to escape the digestion in the stomach, with the use of higher coating levels. Along with the low susceptibility of zein to the intestinal pancreatic enzymes, zein-based coated substrates might be expected to enter the colon intact, potentially making them good candidates for colon-specific drug delivery.

Another set of dissolution tests was carried out on zein alone and mixed polysaccharide/zein pseudolatex-based coated tablets in order to determine the factors that had an influence on the level of drug release attained. The results of the dissolution tests were of particular importance in the determination of the ability of the water insoluble protein (zein) to work as a potential carrier for the water-soluble and/or hydrophilic excipients, i.e. polysaccharides. The optimization of the formulation with regard to the polysaccharide/zein ratio and coating thickness was carried out on the basis of these results. SEM was also used to observe the changes in the surface morphology of the coated tablets prior to and after the dissolution studies. At the level of formulation, the drug release from pseudolatex-based zein alone coated paracetamol tablets was sensitive to the plasticization and coating level. The drug release rate remained constant when the pH of the medium was changed and was sufficiently controlled in enzyme-containing media resembling the upper gastrointestinal tract.

The incorporation of polysaccharides into zein coatings is expected to alter the performance of the coated substrates in the dissolution media as a result of their dissolution and/or swelling and the associated disruption of the film structure (Siew et al., 2000). The ability of zein to withstand these changes and maintain the physical integrity of the membrane surrounding the substrate core was assessed. Pectin/zein polymeric coatings were not able to suppress the release of the drug even at a very low pectin/zein ratio (1:30) and premature drug release within the transit time of the coated substrate in the upper GIT is expected to occur. Thus, pectin/zein coatings did not prove ideally suitable for colonic delivery.

On the other hand, the addition of heat-treated acetylated high amylose maize starch (HAS) into zein coatings allowed sufficient control of the drug release in the upper GIT. The drug release was, however, dependent on the HAS/Zein ratio and the coating level. Only 1:5 HAS/Zein coatings (TWG = 20%) were able to prevent extensive drug release in the upper GIT. Premature drug release was seen with lower coating levels or higher HAS/Zein ratios. It is interesting to note that the extent and rate of drug release from tablets coated with zein alone and 1:5 HAS/Zein pseudolatex-based films were essentially the same in enzyme containing simulated gastric and simulated intestinal fluids, suggesting that the digestive enzymes of the upper GIT are not being particularly selective to either zein or HAS polymers, at least over the test duration (chosen to reflect the average transit time of solid dosage forms in the stomach and small intestine).

The heat treatment of the starch fraction prior to mixing with the water-insoluble polymer was thought to be essential to form a more resistant starch to enzymatic attacks by pancreatic α-amylases, yet susceptible to those present in the colon, known as retrograded starch (Miles et al., 1985; Freire et al., 2009a). The investigation of the influence of moisture-heat treatment during the preparation of HAS samples on their physicochemical properties was evaluated using X-ray powder diffraction (XRPD) and FT-IR techniques. From the XRPD studies, it was found that retrogradation has not occurred in the samples in this study. Nevertheless, the heat-treated acetylated high amylose maize starch was resistant to digestion by the pancreatic enzymes (having α-amylase activity) in the Simulated Intestinal Fluid (SIF). Thus, 1:5 HAS/Zein coatings (TWG = 20%) were ideal candidates for colon-specific drug delivery.

As a prerequisite for the use of these mixed films in colon-specific delivery, the two polymers (in this case HAS and zein) should be immiscible, so that the starch domains in the film are readily accessible to the colonic microbiota (Freire et al., 2009b). A number of characterization techniques were used to study the pseudolatex-based zein alone and HAS/Zein free films and these include scanning electron microscopy (SEM), modulated temperature differential scanning calorimetry (MTDSC), thermogravimetric analysis (TGA), nano-thermal analysis (n-TA) and attenuated total reflectance-Fourier transform infra-red (ATR-FTIR) spectroscopy. The mixed system of zein and HAS proved difficult to be analysed using MTDSC and the MTDSC results were, therefore, not conclusive and

gave no evidence for phase separation. The FT-IR spectra of the HAS/Zein films suggested a lack of chemical interaction between the two polymers. Although both SEM and the topographical images of n-TA showed some structural differences based on HAS/Zein ratio, the LTA data and the tapping mode AFM images suggested that the surfaces are homogenous and that these structural differences are not compositional differences or phase-separated systems.

In the final experimental chapter, the susceptibility of pseudolatex-based zein alone and HAS/Zein coatings to digestion by the colonic microbiota was investigated using batch culture fermentation. Zein alone coatings were able to prevent extensive drug release in the upper GIT while under colonic conditions the release was considerably more rapid, particularly in the presence of the colonic microbiota. The incorporation of HAS to zein coatings resulted in a significant increase ($p < 0.05$) in release rate and extent under colonic conditions, as compared to the zein alone coatings, with essentially no difference being observed in conditions reflecting the upper GIT. These data therefore indicate that zein can be potentially mixed with starch for colonic targeting purposes.

This work also revealed the importance of the model used to represent the colonic and control conditions in the batch culture fermentation studies. The faecal content in the batch culture fermenters and the viscosity of the medium had noticeable influence on the drug release from the coated tablets under study.

Drug release data were also fitted to several models to try and establish a particular mechanism of release. The drug release from zein coatings was essentially linear following zero-order kinetics, with the exception being zein-coated tablets which ruptured during the dissolution test and thus exhibited first-order kinetics. The Higuchi model and Korsmeyer-Peppas equation are essentially used to describe the drug release from polymeric matrices and thus proved to be not relevant to these coated systems. The release of paracetamol from tablets coated with zein is postulated to occur mainly via drug diffusion through the aqueous channels formed either by the spaces between the polymeric chains or by the dissolution of some film ingredients along with some contribution of erosion and/or degradation in the presence of digestive enzymes.

To conclude, zein can be viewed as an "attractive" excipient with multifaceted applications. Zein was successfully used as an extrusion aid forming sufficiently plastic yet brittle extrudates which broke into spheres on spheronization. Zein could thus be used to reduce the level of the commonly used MCC which has been reported not to be the excipient of choice in several cases such as chemical incompatibility (Dukic-Ott et al., 2009). However, zein might not be able to completely replace MCC due to its low water uptake properties compared to the large water absorption and retention capacity of the excipients generally used for producing pellets via extrusion/spheronization. The drug release from zein-based pellets was not fast enough for these pellets to be considered a true immediate-release product. At the same time, they showed no real evidence of a sustained-release profile. Assumptions including the ability of extrusion/spheronization to provide means of intimate mixing between the drug and polymer, the high specific surface area provided by multiparticulates and drug migration during the drying step, were made to account for the level of drug release attained. However, further studies would be necessary to fully understand the reasons for the fast release obtained with these devices and to possibly manipulate the formulation and/or process parameters that could ultimately retard the drug release. One of these suggestions includes changing the granulation fluid; i.e. the use of zein solution in aqueous ethanol which was mentioned in this part of the work. However, the ethanol/water ratio in these solutions could be adjusted so that a good compromise between zein solubility and pellet mechanical strength could be achieved. The influence of adding a plasticizer to the granulation fluid is another attribute that is worth investigation with possible usage of different types and/or levels of plasticizers. Zein was thought to possess binding properties; however, the addition of a binder such as polyvinyl pyrrolidone might be favourable and necessary to reduce cracking observed at the end of the dissolution study on zein-based pellets. Finally, it might be interesting to investigate the use of zein-based pellets to enhance the release of poorly water-soluble drugs as the drug would be molecularly dispersed in the matrix prior to dissolution.

Zein proteins offer an alternative to semi-synthetic excipients used in the pharmaceutical formulations, with particular reference to coating polymers. Importantly, zein is a natural by-product of starch production and is a "generally recognized as safe" (GRAS) material (Georget et al., 2008), with no evidence of its allergenicity being reported (Takagi et al., 2003). Pure zein-based coatings, applied either as organic solutions or aqueous dispersions,

demonstrated low solubility in the upper gastrointestinal tract (GIT). These film coatings showed an effectively zero-order release all the way through the GIT. Thus, zein proteins can be potentially used in oral controlled-release pharmaceutical formulations. Organic or pseudolatex-based zein coatings can be applied either to single dosage forms such as tablets or to multiparticulates such as pellets. The desired drug release patterns from zein-coated dosage forms can be adjusted by the addition of water-soluble ingredients to the films. In this work, the influence of the plasticizer level on the drug release was obvious with a trend of increasing the drug release with increasing the plasticization level. In addition, water-soluble polymers could be potentially mixed with zein to accelerate the drug release. This could be particularly essential for drugs with low permeability in this polymer. Thus, the influence of drug type and its physico-chemical properties on the release from zein-based coatings is yet to be investigated. Zein coatings, being insoluble and insensitive to pH changes in the upper GIT, can be also employed in time-controlled drug release systems where zein would serve as a barrier surrounding an active substrate core. The drug release would eventually be induced after a certain lag time by the swelling properties or the osmotic activity of the core material. The lag time can be controlled by the thickness of the polymeric coating. These systems can be essentially used to delay the drug to various sites in the GIT to achieve local treatment of diseases. In this work, the influence of the coating thickness on the drug release from zein-coated tablets was highlighted with abrupt release being attained at the lower coating level. The influence of the core material was also revealed in this study; however, a further investigation might be necessary to determine the effectiveness of such systems.

In addition, zein proved to be a potential carrier for polysaccharides that are selectively degraded in the colon, revealing a new application of zein proteins for colonic targetted drug delivery. Further optimization of the pseudolatex-based film coatings might be required with the aim to reduce the coating thickness and possibly increase the polysaccharide (HAS) fraction. The incorporation of different starch types might be necessary to determine their differential susceptibility to the colonic microbiota and enhance the usefulness of these systems for colonic targetting. Additionally, the application of HAS/Zein pseudolatex-based zein coatings onto pellets might be interesting to determine the influence of the surface area in contact with the colonic medium on the rate of drug release.

In short, zein showed a wide spectrum of performances; it worked as a bulking agent and had almost negligible influence on the drug release at one end of the spectrum, as seen in the drug release profiles of zein-based pellets. A zero-order controlled release system providing a constant level of drug for an extended period of time was achieved at other end of the spectrum, when zein was used as a coating material. A release pattern having a lag time followed by a rapid release was achieved when substrates were coated at low coating levels. The performance of zein was also manipulated by the addition of other ingredients; the incorporation of polysaccharides such as HAS proved promising for the use of zein-based film for colonic targetting.

8 Future Work

As discussed earlier, pellets produced via the conventional extrusion/spheronization exhibited immediate release profile. Therefore, the investigation of the potential uses of zein in hot melt extrusion is quite necessary to determine the influence of the physical vs. molecular mixing offered by the two techniques, respectively, on the drug release profile.

Based on the colonic studies discussed in Chapter 6, further optimization of the pseudolatex-based film coatings might be required with the aim to reduce the coating thickness and possibly increase the polysaccharide (HAS) fraction. The incorporation of different starch types might be necessary to determine their differential susceptibility to the colonic microbiota and enhance the usefulness of these systems for colonic targetting. Additionally, the application of HAS/Zein pseudolatex-based zein coatings onto pellets might be interesting to determine the influence of the surface area in contact with the colonic medium on the rate of drug release.

Studying the coating material alone, such as cast free films, is necessary to assess how the coating material would function after application onto a solid dosage form. Thus, the mechanical properties, the permeability to water vapour, hydrogen ions and drugs as well as the digestibility of free films are to be investigated.

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