Polysaccharide Drug Delivery Systems based on Pectin and Chitosan

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Introduction
The route for the delivery of drugs that is still the most popular with medical staff and patients alike is through the mouth and down the alimentary tract: the oral route. The major site for drug absorption by this route is the small intestine which offers $≈ 100 \text{ m}^2$ of surface epithelia across which transfer can at least in principle take place. If the drug is poorly soluble, or is in the form of a controlled release dosage form, significant absorption of the drug may also occur in the large intestine (Davis, 1989). However, the clearance time through the whole alimentary tract is generally too short (4–12 h), rendering oral drug administration a very inefficient process, with much of the drug unabsorbed. More recently interest has focused on drug absorption through nasal epithelia, where again clearance problems are an issue. Consideration is also given to other delivery routes (e.g. vaginal and ocular). Other important issues are the degradation of peptide-based drugs in the gastrointestinal tract and low trans-mucosal permeability. Macromolecular based carrier and mucoadhesive systems have been considered for several years and two polysaccharide based systems have emerged as particularly promising: chitosans (cationic) and low-methoxy pectins (anionic).

Chitosan
Chemical Structure
Chitosan is the generic name for a family of strongly polycationic derivatives of poly-N-acetyl-D-glucosamine (chitin) it is found in the exoskeletons of crustaceans such as crabs and shrimps, but can also be found in the cell wall of fungi and bacteria (Tombs and Harding, 1998; Rinaudo, 2006; Yen and Mau, 2007). In chitosan (Figure 1) the N-acetyl group is replaced either fully or partially by NH$_2$ therefore the degree of acetylation can vary from DA = 0 (fully deacteylated) to DA = 1 (fully acetylated i.e. chitin). Acetylated monomers (GlcNAc; A-unit) and deacteylated monomers (GlcN; D-unit) have been shown to be distributed randomly or block wise (Vårum, et al., 1991a, 1991b).

Chitosan is biodegradable, non-toxic, non-immunogenic and biocompatible (Terbojevich and Muzzarelli, 2000) and as the only naturally occurring polycationic polymer chitosan and its derivatives have received a great deal of attention from, for example, the food,
cosmetic and pharmaceutical industries. Important applications include water and waste treatment, antitumor, antibacterial and anticoagulant properties (Illum, 1998; Rinaudo, 2006; Muzzarelli, 2009).

**Physical properties**

Chitosan is a semi-crystalline polymer (solid), which exhibits a degree of polymorphism (Ogawa and Yui, 1994). In an aqueous acidic environment, chitosan is promptly solubilised, as a result of the removal of the acetyl moieties present in the amine functional groups. This solubility is limited, however, in inorganic acids compared to its solubility in organic acids. Solubilisation occurs as a consequence of the protonation of -NH$_2$ functional groups on the C-2 position of D-glucosamine residues. Chitosan is a weak base with pKa values ranging from 6.2 to 7 and at physiological pH 7.4 or higher, low solubility is shown (Park, *et al.*, 1983). However, chitosan’s solution properties are dependent on the distribution of its acetyl groups and the molecular weight of the polymer (Kubota and Eguchi, 1997). The solubility of chitosan in water increases with increasing DA (Vårum *et al*., 1994). With the addition of electrolytes to the solution, the aqueous solubility of chitosan is affected and salting out of chitosan can be seen as in the case of excessive hydrochloric acid use, and the resulting formation of chitosan chlorhydrate (Rinaudo, 2006). Salting out can also be used to recover chitosan from solution and salting out efficiency of anions follows the Hofmeister series SO$_4^{2-}$ > H$_2$PO$_4$ ≈ HPO$_4^{2-}$ > NO$_3^-$ (LeHoux and Depuis, 2007). With an extended chitosan conformation, due to the repelling effect of each positively charged deacetylated unit, the addition of electrolytes reduces the inter-chain repulsion and induces a more random coil-like conformation in the molecule (Terbojevich and Muzzarelli, 2000).

Molecular weight, pH, ionic strength, and temperature are all factors which affect the viscosity of chitosan. Hydrodynamic studies based on intrinsic viscosity ([η]), sedimentation coefficient ($s_{20,w}^0$), radius of gyration ($r_g$) and weight average molecular weight ($M_w$) have focussed on qualitative/semi-quantitative methods of estimating the conformation based around “power law” Mark-Houwink-Kuhn-Sakurada relations (Tombs and Harding, 1998) which link intrinsic viscosity, sedimentation coefficient and
radius of gyration with molar mass \[ \eta \propto M^a, \] \[ s_{20,w}^0 \propto M^b \] and \[ r_g \propto M^c, \] where \( a, b \) and \( c \) have defined values for specific conformation types (Table 1). The translational frictional ratio, \( f/f_o \) (Tanford, 1961), sedimentation conformation zoning (Pavlov, et al., 1997; 1999), the “Wales-van Holde” ratio, \( k_s/\eta \) (Wales and van Holde, 1954) and the persistence length, \( L_p \) (Kratky and Porod, 1949) have also been used to estimate dilute solution conformation (Table 2).

This has resulted in chitosan being reported to have either a rigid rod-type structure (Terbojevich, et al., 1991; Errington, et al., 1993; Cölfen, et al., 2001; Fee, et al., 2003; Kasai, 2006; Morris, et al., 2009a) or a semi-flexible-coil (Rinaudo, et al., 1993; Berth, et al., 1998; Brugnerotto, et al., 2001; Schatz, et al., 2003; Mazeau and Rinaudo, 2004; Vold, 2004; Lamarque, et al., 2005; Velásquez, et al., 2008). It has also been shown that flexibility (in terms of persistence length) is moderately influenced by DA (Terbojevich, et al., 1991; Mazeau and Rinaudo, 2004).

**Chitosan complexation**

The ability of chitosan to complex with other ligands, metals for example, is well known (Rhazi, et al., 2002a,b). The proclivity for chelation is dependent on physical state, -NH\(_2\) content and distribution of chitosan, degree of polymerization, pH and cation content. It has been shown that following a higher degree of deacetylation, there is a characteristic increase in the degree of chelation. The chelation takes place in chitosan with a degree of polymerization greater than 6 monomeric residues (Rhazi, et al., 2002b). In addition, the intensity of chitosan chelation is governed by nature of cation in solution. Studies have shown that the affinity of chitosan for divalent and trivalent cations of chloride salts shows selectivity in the following order: \( Cu^{2+} \gg Hg^{2+} > Zn^{2+} > Cd^{2+} > Ni^{2+} > Co^{2+} \) > \( Ca^{2+}, \) \( Eu^{3+} > Nd^{3+} > Cr^{3+} \) > \( Pr^{3+} \) (Rhazi, et al., 2002b).

**Usage in Drug Delivery**

Chitosan is of great interest to the pharmaceutical industry in drug delivery and the number of publications on this subject has increased by almost an order of magnitude in the last decade (Figure 2). Many aspects including biodegradation, biodistribution and
toxicity (Kean and Thanou, 2010); formulations for delivery of DNA and siRNA (Mao, et al., 2010); delivery systems for protein therapeutics (Amidi, et al., 2010); hydrogels for controlled, localized drug delivery (Bhattarai, et al., 2010); nanostructures for delivery of ocular therapeutics (de la Fuente, et al., 2010) and the targeted delivery of low molecular drugs (Park, et al., 2010) have been reviewed in the most recent volume of Advanced Drug Delivery Reviews (Volume 62).

The mucoadhesive properties of chitosan play an important role in its usage in oral, nasal and ocular drug delivery (Harding, et al., 1999; Illum, 2002; Harding, 2006).

**Mucoadhesion**

Mucoadhesion is the specific term for adhesion when one of the surfaces is mucus (Harding, et al., 1999). Mucus consists largely of water (> 95 %) and the high molecular weight glycoprotein mucin (Harding, et al., 1999; Harding, 2003; Harding, 2006). The key sugar residues for mucoadhesive interaction are the acidic ones (N-acetyl neuraminic acid or “sialic acid”, and some sulphated galactose) and the hydrophobic methyl containing fucose. Despite the polydispersity of these molecules compared to unglycosylated proteins, their structural hierarchy is also well understood. They consist of M ~ 500 000 g/mol basic units linked linearly into “subunits” of M ~ 2 500 000 g/mol. These subunits are further linearly arrayed into macroscopic structures (M between 5 and 50 000 000 g/mol) seen under the electron microscope (Harding, et al., 1983) or using atomic force microscopy (Deacon, et al., 2000). Chitosan interacts strongly with the negatively sialic acid residues (Fiebrig, et al., 1994a,b; 1995a,b; Anderson, et al., 1989; Deacon, et al., 1999; Rossi, et al., 2000; 2001; Dodou, et al., 2005) although hydrogen bonding and hydrophobic interactions are also important (Deacon, et al., 1999; Qaqish and Amiji, 1999; Dodou, et al., 2005; Sogias, et al., 2008). The different theories explaining mucoadhesion and the properties of mucoadhesives are shown in Figure 3 (Dodou, et al., 2005 and references therein). The chitosan/ mucin interaction depends on the zeta potential of the mucin (Figure 4) (Takeuchi, et al. 2005) and this change in zeta potential is related to the concentration, molecular weight and charge of the chitosan (Figure 5) and to the pH (Takeuchi, et al. 2005; Sogias, et al., 2008). This change in zeta
potential is associated with a change particle size (Fiebrig, et al., 1994a,b; 1995a,b; Anderson, et al., 1989; Takeuchi, et al. 2005; Sogias, et al., 2008) (Table 3). As a consequence the degree of chitosan/ mucin interaction is also dependent on the biological source of mucin (Figure 6). Drug delivery systems involving chitosan, therefore, show great potential and the use of encapsulation technology involving chitosan nano- or microparticles are increasing in popularity.

Nanoparticles
Chitosan has been widely used in the preparation of nanoparticles for drug delivery (Dyer et al., 2002; Fernández-Urrasuno, et al., 1999; Gan and Wang, 2007; Gan, et al., 2005; Luangtana-anan, et al., 2005; Shu and Zhu, 2000; Tsai, et al., 2008; Xu and Du 2005). Chitosan nanoparticles can be prepared by at least three different methods (Kumari, et. al., 2010):

1. Electrostatic interaction and resultant ionotrope gelation between chitosan and the for example tripolyphosphate (TPP) polyanion (He, et al., 1998, 1999; Dyer, et al., 2002; Luangtana-anan, et al., 2005; Janes, et al., 2001; Shu and Zhu, 2000; Gan, et al., 2005; Morris, et al., 2010a) (Figure 7).


3. Polyelectrolyte complex (PEC) formation with for example pectin (MacLeod, et al., 1999; Ofori-Kwakye and Fell, 2001) or hyaluronic acid (Lim, et al., 2000; Kim, et al., 2004; Kujawa, et al., 2007). This is of particular importance when a constant drug release profile is not desired (MacLeod, et al., 1999; Ofori-Kwakye and Fell, 2001).

The size of the nanoparticles depends on the molecular weight of the chitosan polymer and higher molecular weight chitosans produce larger nanoparticles (Luangtana-anan et al., 2005; Morris, et al., 2010a). The method of cross-linking affects the mucoadhesive strength and stability of the nanoparticles (Genta, et. al., 1998; Dhawan, et al., 2004).
Stability

The stability (shelf-life) of chitosan in terms of molar mass, viscosity and conformation is very important to pharmaceutical industry as these properties play an important role in the function of chitosan in formulations (Skaugrud, et al., 1999; Terbojevich and Muzzarelli, 2000). Chitosan storage conditions and particularly temperature may be important but whether or not chitosan depolymerisation will be detrimental to its intended application will depend on the functional significance of the changes that occur. Depolymerisation of chitosan in both the polymeric and nanoparticle form is temperature dependent (Nguyen, et al., 2007; Morris, et al., 2009b; Morris, et al., 2010a). For example it has been reported that low molar mass chitosans can cause more cell damage (Aspden, et al., 1996), although they may also prevent diabetes mellitus progression in mice to a greater extent than high molar chitosans (Kondo, et al., 2000), show greater antibacterial activity compared with high molar mass chitosans (Lui, et al., 2001) and whilst the high viscosities of high molar mass chitosans limit its biological usefulness, low molar mass chitosan is more soluble at neutral pH and therefore potentially more available in vivo (Harish Prashanth and Tharanathan, 2007). However, it has also been reported that high molar mass chitosans show greater antibacterial activity compared with low molar mass chitosans (No, et al., 2006), that nasal insulin delivery (Aspden, et al., 1997; Davis and Illum, 2000) is more effective with chitosan of molar mass greater than 100000 g/mol and the reversibility of transepithelial chemical resistance (TEER) values decrease with decreased chitosan molar mass (Holme, et al., 2000).

Pectin

Chemical Structure

Pectins are a complex family of heteropolysaccharides that constitute a large proportion of the primary cell walls of dicotyledons and play important roles in growth, development and senescence (van Buren, 1991; Tombs and Harding, 1998; Ridley, et. al., 2001; Willats, et. al., 2001). Pectic polysaccharides are made of several structural elements the important of which are the homogalacturonan (HG) and type I rhamnogalacturonan (RG-I) regions often described in simplified terms as the “smooth” and “hairy” regions respectively (Figure 8). The HG region is composed of (1→4) linked α-D-GalpA
residues that can be partially methylated at C-6 (Pilnik and Voragen, 1970) and possibly partially acetyl-esterified at O-2 and/or O-3 (Rombouts and Thibault, 1986). The degree of methylation (DM) and the degree of acetylation (DAc) are defined as the number of moles of methanol or acetic acid per 100 moles of GalA. The degree of methylation in native pectins is generally in the order of DM ≈ 70-80; whereas degree of acetylation is generally much lower e.g. DAc ≈ 35 for sugar beet pectins (Rombouts and Thibault, 1986). Theoretically the degree of methoxyl esterification (DM) can range from 0-100 %. Pectins with a degree of esterification (DM) > 50% are known as high methoxyl (HM) pectins and consequently low methoxyl (LM) pectins have a DM < 50% (Walter, 1991). The RG-I region consists of disaccharide repeating unit \( (\rightarrow 4)-\alpha-D-\text{GalpA-(1}\rightarrow 2)-\alpha-L-\text{RhAp-(1}\rightarrow_n \) with a variety side chains consisting of L-arabinosyl and D-galactosyl residues (Voragen, et. al, 1995). It has been reported that GalA residues in the RG-I region are partially acetylated (Ishii, 1997; Perrone, et. al., 2002) but not methylated (Komalavilas and Mort, 1989; Perrone, et. al., 2002). In the case of sugar beet pectin the neutral side chain sugars are substituted with ferulic acid (Fry, 1982; Rombouts and Thibault, 1986) and there is evidence indicating that pectin chains can be dimerised via diferulic bridges (Levigne, et. al, 2004a,b). There are a number of different ways in which ferulic acid can dimerise the most common being: 5-5’; 8-O-4’; 8-5’ cyclic and 8-5’ non-cyclic dimers (Micard, et. al., 1997).

**Physical Properties**

The degree of esterification and therefore the charge on a pectin molecule is important to the functional properties in the plant cell wall. It also significantly affects their commercial use as gelling and thickening agents (Lapasin and Pricl, 1995; Tombs and Harding, 1998). HM pectins (low charge) form gels at low pH (< 4.0) and in the presence of a high amount (> 55 %) of soluble solids, usually sucrose (Oakenfull, 1991). HM pectin gels are stabilised by hydrogen-bonding and hydrophobic interactions of individually weak but cumulatively strong junction zones (**Figure 9**) (Oakenfull, 1991; Lopes da Silva and Gonçalves 1994; Pilnik, 1990; Morris, 1979). Conversely, LM pectins (high charge) form electrostatically stabilised gel networks with/ or without sugar and with divalent metal cations, usually calcium in the so-called “egg-box” model.
(Figure 10) (Morris, et. al., 1982; Pilnik, 1990; Morris, 1980; Oakenfull and Scott, 1998; Axelos and Thibault, 1991), which also depends on the distribution of negative carboxylate groups and structure breaking rhamnose side chains (Powell, et. al., 1982; Axelos and Thibault, 1991). A similar “egg-box” model has been proposed for alginate gels (Wang, et. al., 1994; Morris, 1980) from the results of circular dichroism (CD), small angle X-ray scattering (SAXS) and X-ray fibre diffraction respectively, it is thought that in both pectin and alginate the “egg-box” is formed in a two-step process – dimerisation followed by aggregation of the preformed “egg-boxes” (Thibault and Rinaudo, 1986).

Solution properties such as viscosity also depend on degree of esterification, solvent environment (i.e. salt concentration, sugar concentration and pH) together with temperature (Oakenfull, 1991). Hydrodynamic studies based on intrinsic viscosity ([η]), sedimentation coefficient (s_{20,w}^0), radius of gyration (r_g) and weight average molecular weight (M_w) have focussed on qualitative/semi-quantitative methods of estimating the conformation based around “power law” Mark-Houwink-Kuhn-Sakurada relations (Tombs and Harding, 1998) which link intrinsic viscosity, sedimentation coefficient and radius of gyration with molar mass [η] \propto M^a, s_{20,w}^0 \propto M^b and r_g \propto M^c, where a, b and c have defined values for specific conformation types (Table 1). The translational frictional ratio, f/f_0 (Tanford, 1961), sedimentation conformation zoning (Pavlov, et al., 1997; 1999), the “Wales-van Holde” ratio, k/[η] (Wales and van Holde, 1954) and the persistence length, L_p (Kratky and Porod, 1949) have also been used to estimate dilute solution conformation (Table 2). A picture of a semi-flexible conformation for pectins irrespective of degree of esterification (and charge) has emerged from these studies (Anger and Berth, 1985; Axelos, et al., 1987; Axelos and Thibault, 1991, Berth, et al., 1977; Harding, et al., 1991; Garnier, et al., 1993; Malovikova, et al., 1993; Cros, et al., 1996; Tombs and Harding, 1998; Braccini, et al., 1999; Morris, et al., 2000, 2002, 2008; Fishman, et al., 2001, 2006; Noto, et al, 2005). Pectin molecular weight and chain flexibility is important in mucoadhesive interactions (Nafee, et al., 2007).
Usage in Drug Delivery

Pectins have been used as a gelling agent for a large number of years, however there has been recent interest in the use of pectin gels in controlled drug delivery (Sungthongjeen, et al., 2004; Lui, et al., 2003; Lui, et al., 2006). This is in part due to their long standing reputation of being non-toxic (GRAS – generally regarded as safe) (Lui, et al., 2003; Lui, et al., 2007; Watts and Smith, 2009), their relatively low production costs (Sungthongjeen, et al., 2004) and high availability (Beneke et al., 2009). It is proposed that pectin could be used to deliver drugs orally, nasally and vaginally (Figure 11) (Peppas, et al., 2000; Sinha and Kumria, 2001; Lui, et al., 2003; Nafee, et al., 2004; Valenta, 2005; Lui, et al., 2007; Chelladurai, et al., 2008; Thirawong, et al., 2008), which are generally well accepted by patients (Lui, et al., 2003; Lui, et al., 2007; Yadav, et al., 2009).

Oral Delivery

The oral route is of particular interest as in general oral drug administration results in less pain, greater convenience, higher compliance and reduced infection risk as compared to subcutaneous injections (Chen and Langer, 1998, Yadav, et al., 2009). However, there are disadvantages associated with this route of administration such as low bioavailability due to relatively low passage of active agents across the mucosal epithelium, rapid polypeptide degradation due to action of digestive enzymes in the GI tract, enzymatic proteolysis and acidic degradation of orally administered drugs in the stomach (Lui, et al., 2003; Lin, et al., 2007). Various approaches have been made to increase the buccal penetration using permeation enhancers (Mesiha, et al., 1994; Carino, et al., 2000), protease inhibitors (Yamamoto, et al., 1994), enteric coatings (Morishita, et. al, 1993) and (bio)polymer micro-/nano-sphere formulations (Sarmento, et al., 2007; Jain, et al., 2005). However, protein drugs are essentially free from enzymatic proteolysis and acidic degradation in the colon which has resulted in a concentrated effort to target their delivery to this organ (Sinha and Kumria, 2001; Lui, et al., 2003; Chambin, et al., 2006). Therefore a number of different polymers including pectin have been identified as protective agents against enzymatic proteolysis (Lui, et al., 2003; Srimarornsak, 2003; Pourjavadi and Barzegar, 2009). The pectin-stabilised polypeptide drug therefore mains
intact in the stomach and small intestine prior to pectin digestion by the colonic microflora resulting in the release of drug molecule (Sinha and Kumria, 2001; Vandamme, et al., 2002). The susceptibility of pectin to enzymatic attack is increased in the presence of calcium ions (Miler and MacMilan, 1970) and decreased by methyl esterification (Ashford, et al., 1993). One problem with pectin formulations is that they can swell under physiological conditions which may result in premature drug release (Semdé, et al., 2000; Lui, et al., 2006), the effect can be minimised by the use of pectin in combination with other polymers: cellulosic or acrylic polymers (Semdé, et al., 2000); chitosan (Macleod, et al., 1999); hydroxypropylmethyl cellulose (Ofori-Kwakye and Fell, 2001) and zein (Lui, et al., 2005; 2007).

Nasal Delivery

However, the clearance time through the whole alimentary tract is generally too short (4–12 h), rendering oral drug administration a very inefficient process, with much of the drug unabsorbed. More recently interest has focused on drug absorption through nasal epithelia, which results in very rapid absorption ~ 15 minutes (Jabbal-Gill, et al., 1998). Other important issues are the degradation of peptide-based drugs in the gastrointestinal tract and low trans-mucosal permeability. Macromolecular based carrier and mucoadhesive systems have been considered for several years and pectin based systems have emerged as particularly promising. Low methoxyl pectins are strongly polyanionic polyuronides from fruit used traditionally in jams and jellies (Rolin, 1993) and can also form weak gels in the presence of Ca\(^{2+}\) ions (8 meq/L), which occur naturally in nasal secretions (Chang and Su, 1989; Illum, 2000), and their texture makes them patient friendly in nasal delivery formulations (Dale, et al., 2002; Yadav, et al., 2009). These gels are pseudoplastic (Sriamornsak, 2004; Thirawong, et al., 2008; Chelladurai, et al., 2008) and drug release is diffusion controlled at low pectin concentrations (Lui, et al., 2007; Chelladurai, et al., 2008) and determined by gel dissolution at higher pectin concentration (Lui, et al., 2007) (Figure 12). In addition, this may hold an incorporated drug substance in the nasal cavity for a prolonged period and thereby modulate its rate of systemic absorption. Pectins do not act as an absorption enhancer, however they cause tight junctions to open and therefore alter drug release characteristics due to the chelation
of calcium (Charlton, et al., 2007; McConaughy, et al., 2009). They are also highly mucoadhesive (Nafee, et al., 2004; Lui, et al., 2007; Thirawong, et al., 2008), although less mucoadhesive than chitosan (Nafee, et al., 2004). Their mucoadhesive power depends on molecular weight, viscosity, the local pH and pectin functional groups (Lui, et al., 2007; Thirawong, et al., 2008).

Nasal drug delivery is limited by the small sample volume that can be delivered ~ 150 µl, which is important in drug formulations especially if the drug is sparingly soluble or if a drug has to be delivered over prolonged period (Lui, et al., 2007).

Vaginal Delivery
Like the nose the vagina is another potential site for drug delivery due to its rich blood supply, large surface area (Vermani and Garg, 2000) and well understood microflora (Valenta, 2005). Drug delivery release rates may vary during the menstrual cycle and this is especially important at the menopause (Valenta, 2005). Drug delivery systems are based on mucoadhesion (Harding, et al., 1999; Harding, 2003; 2006). The vaginal route has been demonstrated to be favourable in the delivery of many drugs e.g. propranolol, human growth hormone, etc. (see Valenta, 2005 and references therein). Furthermore it might be expected the vaginal delivery of hormonal contraception may be more efficient than the oral route (Valenta, 2005). The vaginal route offers many of the advantages of the nasal route with main disadvantage being it is only available to females. Pectin-based formulations have demonstrated highest mucoadhesive strength, highest swelling volume and lowest pH reduction in a trial (Baloglu, et al., 2003; 2006).

Stability
However, as yet pectin has not fulfilled its potential as drug delivery system this is due variability in pectin formulations and question marks over formulation stability (Lui, et al., 2003; Lui, et al., 2006). According to Morris, et al. (2010b) the viscosity of pectin solutions decrease significantly after 6 months storage at 25 ºC and 40 ºC respectively, and this is reflected by a decrease in gel strength upon addition of calcium ions. This is explained by a depolymerisation of pectin over time (Figure 13). However, it has been
shown that decreases in viscosity of this magnitude do not significantly change the drug release rates from pectin gels \textit{in vitro} (Nessa, 2003; Chelladurai, \textit{et al.}, 2008). In calcium pectate based tablet formulations drug release time is increased with lower degree of methyl esterification, but higher levels of calcium ions can lead to disintegration of the tablet and increased drug release (Sungthongjeen, \textit{et al.}, 2004).

\textbf{Conclusions}

In the last decade there has been a great deal of interest in the use of polysaccharides and particularly chitosan and pectin in drug delivery systems. It is clear that both the polysaccharides either individually or together show great potential, however, many important issues still remain to be resolved fully. With chitosans, these include (i) their stability, with the important constraint that they are soluble only at pH < 6. (ii) their construction into microparticles capable of surviving the large environmental variation between mouth and intestine for oral drug delivery. In addressing (i) and (ii) issues concerning the optimal degree of acetylation and molecular weight of the chitosan need to be addressed. With low methoxy-pectin systems issues include: (i) optimal molecular weight and degree of esterification (ii) drug diffusivity (iii) interactions with mucosal tissues, (iv) stability (molecular weight/viscosity/gelation).

\textbf{References}


Table 1. The Mark-Houwink-Kuhn-Sakurada (MHKS) power law exponents (a, b and c), and the Wales – van Holde ($k_s/\eta$) for the conformations described by sedimentation conformation zoning.

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<th>Zone A</th>
<th>Zone B</th>
<th>Zone C</th>
<th>Zone D</th>
<th>Zone E</th>
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<td></td>
<td>Extra-rigid rod</td>
<td>Rigid rod</td>
<td>Semi-flexible coil</td>
<td>Random coil</td>
<td>Spherical</td>
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<tr>
<td>a</td>
<td>&gt; 1.4</td>
<td>0.8 – 1.4</td>
<td>0.5 – 0.8</td>
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<tr>
<td>b</td>
<td>&lt; 0.2</td>
<td>0.2 – 0.4</td>
<td>0.4 – 0.5</td>
<td>0.5 – 0.6</td>
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<tr>
<td>c</td>
<td>&gt; 0.8</td>
<td>0.6 – 0.8</td>
<td>0.5 – 0.6</td>
<td>0.4 – 0.5</td>
<td>0.33</td>
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<tr>
<td>$k_s/\eta$</td>
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<td>0.2 – 0.4</td>
<td>0.4 – 1.0</td>
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Table 2. Estimations of the dilute solution conformation of pectin

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<td>$a$</td>
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<td>0.77 – 1.1</td>
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<td>$b$</td>
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<td>0.24 – 0.25</td>
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<tr>
<td>$c$</td>
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<td>0.55 – 0.56</td>
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<td>11 – 16</td>
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<td>$L_p$ (nm)</td>
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<td>4 – 35</td>
</tr>
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</table>

**Zone**

- A/B/C
- B/C

**References**

- Anger and Berth, 1985
- Axelos, et al., 1987
- Axelos and Thibault, 1991
- Berth, et al., 1977
- Harding, et al., 1991
- Garnier, et al., 1993
- Malovikova, et al., 1993
- Tombs and Harding, 1998
- Terbojevich, et al., 1991
- Errington, et al., 1993
- Ottøy, et al., 1996
- Berth, et al., 1998
- Cölfen, et al., 2001
- Brugnerotto, et al., 2001
- Fee, et al., 2003
- Schatz, et al., 2003
- Mazeau and Rinaudo, 2004
- Vold, 2004
- Lamarque, et al., 2005
- Rinaudo, 2006
- Kasai, 2006
- Velásquez, et al., 2008
- Morris, et al., 2009
Table 3. Mucoadhesive analysis. The sedimentation coefficient ratio ($s_{\text{complex}}/s_{\text{mucin}}$) as an index of (muco)adhesiveness (from Fiebrig, et al., 1994a,b; 1995a,b; Anderson, et al., 1989) (adapted from Table 1 in Harding (2003)).

<table>
<thead>
<tr>
<th>Mucoadhesive</th>
<th>$s_{\text{complex}}/s_{\text{mucin}}$</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEAE-dextran</td>
<td>1.1–1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>pH 6.8, 20 ºC</td>
</tr>
<tr>
<td></td>
<td>1.2–1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>pH 6.8, 37 ºC</td>
</tr>
<tr>
<td>Chitosan (FA ≈ 0.11)</td>
<td>48</td>
<td>pH 6.5, 20 ºC</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>pH 4.5, 20 ºC</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>pH 2.0, 20 ºC</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>pH 2.0, 37 ºC</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>pH 4.5, 20 ºC + 3 mM bile salt</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>pH 4.5, 37 ºC + 3 mM bile salt</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>pH 4.5, 20 ºC + 6 mM bile salt</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>pH 4.5, 37 ºC + 6 mM bile salt</td>
</tr>
<tr>
<td>Chitosan (FA ≈ 0.42)</td>
<td>31</td>
<td>pH 4.5, 20 ºC</td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>pH 4.5, 37 ºC</td>
</tr>
</tbody>
</table>

<sup>a</sup>Depends on the mixing ratio
Figure 1. Schematic representation of the structure repeat units of chitosan, where R = Ac or H depending on the degree of acetylation.
Figure 2. Number of publications on chitosan in drug delivery over the last 10 years (adapted from Figure 1 in Amidi and Hennink (2010)).
Figure 3. Theories of mucoadhesion (red circles) and material properties of mucoadhesives (blue circles). The overlapping areas between the circles of the material properties and the mucoadhesive theories indicate how and to what extent the former are connected to the latter (adapted from Figure 2 in Dodou, et al. (2005)).
Figure 6. Zeta potential of coarse mucin particles in the solutions of chitosan having different molecular weight with various concentrations and different pH. (a) pH 5.0, (b) pH 6.8, (c) pH 7.4, (d) pH 9.0, (e) Change in observed particle size of micronized mucin particles when mixed with the chitosan solutions. Concentration of chitosan solution: 1.5% w/v. pH of solution: 6.8. Molecular weight of chitosan: CS-2 = 20000; CS-5 = 50000 and CS-15 = 150000 g/mol respectively (adapted from Figures 1 and 3 in Takeuchi, et al. (2005)).
Figure 5. Dynamic light scattering size measurements of pig gastric mucin mixed with chitosan at pH 2.0 (1), half acetylated chitosan at pH 7.0 (2), and half acetylated chitosan at pH 2.0 (3) at [polymer]/[mucin] weight ratio = 0.05. Insets: pig gastric mucin at pH 2.0 before (a) and after (b) addition of chitosan (adapted from Figure 3 in Sogias, et al. (2008)).
Figure 6. Comparison of the interaction between ‘‘SC210+’’ chitosan with three mucin populations purified from different regions of the porcine stomach (cardia, corpus-LD and antrum-LD) and one mucin population purified from the whole porcine stomach (PGM-MD). (a) I = 0.1 M, (b) I = 0.2 M (adapted from Figure 2 in Deacon, et al. (1999)).
Figure 7. Transmission electron microscopy image of a chitosan–TPP nanoparticle of diameter 140 – 250 nm (adapted from Figure 10 in Gan, et al. (2003)). Inset: particle size distribution measured by differential sedimentation for TPP-chitosan nanoparticles with a mean particle size of 141 nm (adapted from Figure 3 in Morris, et al. (2010a)).
Figure 8. Schematic structure for pectin: galacturonic acid (●); galactose (●); arabinose (▼); rhamnose (□) and methyl groups (▲) (adapted from Figure 1 in Perez, et al. (2003)).
Figure 9. Representation of gelation mechanism in high methoxyl (HM) pectin gels, where the junction zones are indicated in red.
Figure 10. “Egg box” model for the gelation mechanism for low methoxyl (LM) pectin gels.
Figure 11. Major sites for pectin drug delivery systems (adapted from Peppas, et al., 2000; Harding, 2003; Lui, et al., 2003; Valenta, 2005) with insets showing the nasal cavity, vagina and GI tract.
Figure 12. Mass uptake by nasal cavity tissues from pectin derivative carrying a primary amine gel formulation: Pectin (solid bar), BSA (open bar). Study was performed under standard tissue culture conditions (CO$_2$ 5%, O$_2$ 95%) at 37 °C for 4 h. The amounts of adsorbed pectin and BSA were determined according to Liu, et al. (2005). Nasal cavity tissues were harvested from a freshly slaughtered healthy adult swine from a local slaughterhouse (adapted from Figure 4B in Lui, et al. (2007)).
Figure 13. 1st order kinetic plots of (mol/g) vs. time (days) for pectin of DM ~ 19 %, where closed symbols represent molar masses estimated from viscometry at 4 ºC (4), 25 ºC (5) and 40 ºC (7) (adapted from Figure 3 in Morris, et al. (2010b)). The kinetic rate constants (day⁻¹) are (-0.8 ± 1.1) x 10⁻⁷, (5.7 ± 1.1) x 10⁻⁷ and (6.7 ± 0.2) x 10⁻⁶ at 4 ºC, 25 ºC and 40 ºC, respectively.