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Review

Trends in muco-adhesive analysis☆

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The last two decades has seen progress in the development of potential mucoadhesive carriers for assisting with the oral and nasal administration of drugs based around the polysaccharide chitosan. This progress has been underpinned by the development of molecular assays for mucoadhesiveness focusing on the mucin component of mucus. We review the developments based around molecular or biophysical analyses and consider how the issue of product stability is now being addressed. Although the targets are pharmaceutical, the technology could be extended to the encapsulation and release of nutrients.

Introduction

The alimentary tract is not only the route for digestion of food but is also the most popular route for the administration of drugs. The efficiency of the latter can be low because the drug may not stay at the site of absorption for long enough and has presented a fascinating physiological challenge for over two decades (see Davis, 1985; Gurny, Meyer, & Peppas, 1984; Park & Robinson, 1984; Peppas & Buri, 1985). The mucoadhesive properties of certain types of non-toxic biopolymer can in principle be used to help address this problem by increasing the residence time of drugs as they pass through the stomach and small intestine. A combination of molecular hydrodynamics (analytical ultra-centrifugation, size exclusion chromatography and multi-angle laser light scattering) together with imaging procedures (electron microscopy, atomic force microscopy) reinforced by macroscopic observations (e.g. tensiometry) can be used to help select the appropriate biopolymer carrier in terms of adhesiveness. In this regard chitosans have proved very attractive although these molecules present problems concerning solubility and stability.

The physical form of the carrier (gel/microsphere/capsule etc.) has then to be designed which has to take account of the large change in solvent environment from the mouth to the optimum sites of absorption: in this regard the nasal route has proved an attractive alternative and absorption data particularly with powder as opposed to solution forms have been promising.

Although the main function of the gut is the digestion and absorption of food it also provides a route for the delivery of drugs that is still the most popular with medical staff and patients alike (Fig. 1). The major site for drug absorption by this route is the small intestine, which offers $\approx 100\ m^2$ of surface epithelia across which transfer can at least in principle take place (Booth, 1967). However, the clearance time through the entire alimentary tract and in particular past the ideal absorption site can be too short (4–12 h), thus rendering oral drug administration a rather inefficient process (Davis, 1989). In principle, this problem can be reduced by using biopolymer carriers with the appropriate characteristics as 'macromolecular brakes', and this provides a fascinating physiological challenge particularly with regard the large variation in pH and other conditions in the route from the mouth to the small intestine. We now review some of the developments in molecular analysis of mucoadhesion. We avoid detailed consideration of the more macroscopic aspects, admirably covered for example in a collection of papers in a volume edited by Mathiowitz, Chickering, and Lehr (1999), and aspects of vascular delivery very recently reviewed by Dziubla and Muzykantov (2005).

Mucus and mucin

The adherent mucus gel lining the human alimentary tract has a thickness between 50 and 300 μm (see, e.g. Allen, 1989; Kerss, Allen, & Garner, 1982). Although most of mucus is water (≈95–99% by weight) the key macromolecular components are a class of glycoprotein known as the mucins. Mucins are large molecules with molecular weights (molar masses) ranging from 0.5 to over 20 million Da (g/mol). These glycoproteins contain large amounts of O-linked carbohydrate (for gastrointestinal mucins 70–80% carbohydrate, 15–25% protein and up to

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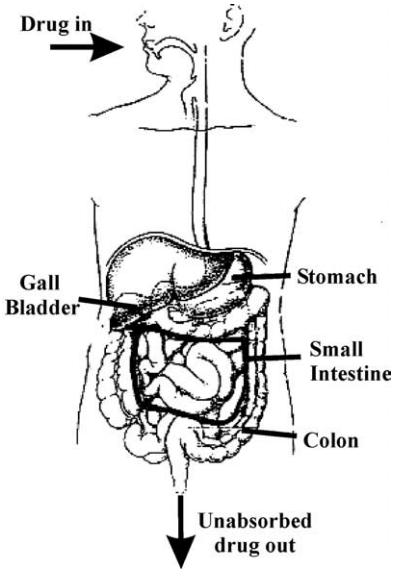

Fig. 1. The human alimentary tract.

≈5% ester sulphate). Undegraded mucins from a variety of sources are made up of multiples of a basic unit ≈400–500 kDa), linked together into linear arrays (Fig. 2) as shown first in 1983 by Creeth *et al.* (Harding, Creeth, & Rowe, 1983; Harding, Rowe, & Creeth, 1983) to give the macroscopic mucins. Molecular weights as high as ≈50 MDa have been reported (Carlstedt & Sheehan, 1984). The basic units are linked together by regions of low or no glycosylation which can be attacked by for example trypsin: the ≈400 kDa digestion products that result are thus commonly referred to as 'T-domains' (see Sheehan & Carlstedt, 1989). Every third or fourth T-domain is linked by a disulphide bridge and these are susceptible to reductive disruption by thiols. The thiol reduction products (of molecular weight between 1.5 and 2.5 MDa) are commonly referred to as 'subunits'. One of the later examples of such architecture discovered in a mucin is that of colonic mucin (Jumel *et al.*, 1997) (Fig. 3).

Fig. 2. Human mucin visualized by transmission electron microscopy (from Harding, Rowe, *et al.*, 1983).

Mucin glycoproteins are characterized not only by large molecular weights but by large molecular weight distributions, as demonstrated by analytical ultracentrifugation (Harding, 1984, 1989) and by the powerful technique of SEC-MALLs (size-exclusion chromatography coupled to multi-angle laser light scattering) as shown, for the first time, by Jumel *et al.* (1995, 1997) (Fig. 4). Even mucins produced externally by cell lines have been shown to adopt this architecture, although they appear to be only up to one or two subunits in length (<5 MDa) (Dodd, Place, Hall, & Harding, 1998). In solution, mucins adopt a random-coil like conformation (see Sheehan & Carlstedt, 1989) occupying a time-averaged spheroidal domain as shown by hydrodynamics (Harding, Creeth, *et al.*, 1983) critical-point-drying electron microscopy (Hallett, Rowe, & Harding, 1984) and atomic force microscopy (Deacon *et al.*, 2000). There is one exception: a class of mucins which do not conform to this structural model are the submaxillary mucins, with a lower carbohydrate content (Gottschalk, Bhargava, & Murty, 1972): these are not, however, so relevant in terms of gastrointestinal or nasal adhesion strategies.

Although direct sequencing of the protein chain has been virtually impossible because of the insolubility of mucins stripped of their carbohydrate, 14 *MUC* genes coding for mucin production have now been identified and several have been sequenced (see, e.g. Campbell, 1999; Hounsell, Young, & Davies, 1997; Ringel & Lohr, 2003): the key ones as far as mucoadhesion are concerned are located on a cluster on chromosome 11p15.5 (Pigny *et al.*, 1997), namely *MUC2* which codes for mucins secreted into the small intestine and colon, and *MUC5AC*, *MUC5B* and *MUC6* for mucins secreted into the stomach. The characteristic features are tandem repeats (e.g. 22 residues for *MUC2*),

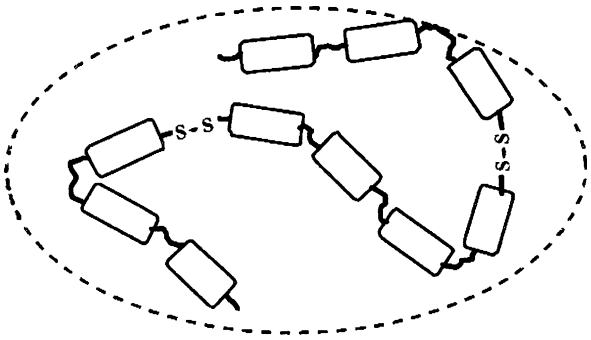

Fig. 3. Highly expanded linear random coil model for colonic mucin (from Jumel *et al.*, 1997). The macroscopic mucin consists of a single polypeptide chain with regions of high glycosylation and no/low glycosylation, with ≈ 1 in 4–5 of the latter regions containing a disulphide link.

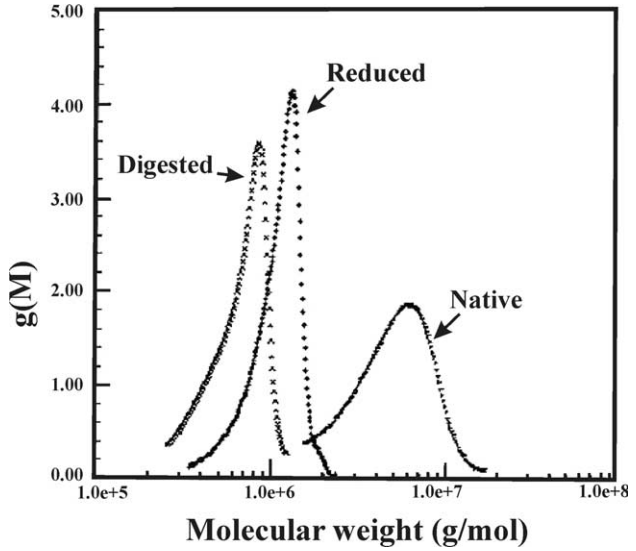

Fig. 4. Molecular weight distribution of colonic mucin (from Jumel *et al.*, 1997). Distributions are shown for native, 'reduced' (dilsulphide bond disrupted) and 'digested' (after disruption of the no/low glycosylation regions).

and high levels of serine, threonine and proline. Serine and threonine are the sites for O-glycosylation and the high levels of proline help to coil the molecule up (see, e.g. Harding, Creeth, *et al.*, 1983; Harding, Rowe, *et al.*, 1983). The key sites for mucoadhesive interactions appear to be on the carbohydrate residues, either of the electrostatic type through terminal sialic acid residues or any sulphonated residues, or of the hydrophobic type through possible clusters of fucose residues, which possess a methyl group.

Unfortunately, mucin of the required purity from human alimentary tract is very difficult to obtain in any useful quantity. Pig gastric mucin has, therefore, been commonly chosen as the model mucin system, although it has been possible to perform mucoadhesive assays on highly purified mucin from different regions of the human stomach as considered below. Unless otherwise stated, the mucin we subsequently refer to in the mucoadhesive assays is highly purified pig gastric mucin whose molecular weight M_w, depending on the success of the purification procedure, is $\approx$10 MDa.

Macroscopic and biomolecular mucoadhesive assays

The mucoadhesive should be non-toxic, not expensive, with a high drug-loading capability. The toxicity issue means that, compared with synthetic polymers, polysaccharides are a very attractive option, since these, or at least the vast majority of these, are non-toxic; indeed many are used widely in food products as thickeners and stabilizers. The polymer needs of course to be adhesive towards the mucus layer, and also not only to have a high drug-loading capacity but also high unloading capacity in the small intestine or thereabouts (see, e.g. Fiebrig, Davis, & Harding, 1995; Harding, Deacon, Fiebrig, & Davis, 1999).

The simplest 'macroscopic' test for adhesiveness is to perform a tensiometry experiment which involves the force required to detach two surfaces. Lehr and colleagues at the Universities of Saarbrucken and Ghent coated one surface with mucus and the other with a selection of candidate mucoadhesive polysaccharides (Lehr, Bouwstra, Schacht, & Junginger, 1992). In this way they were able to show that neutral polysaccharides such as HP-cellulose, HE-starch and scleroglucan, and polyanionic polysaccharides such as pectin, xanthan and carboxymethyl cellulose gave virtually no interaction. A series of polycationic chitosans showed strong interactions whereas two other polycationics, namely DEAE-dextran and amino-dextran showed no significant interaction. Other macroscopic probes using mucus, such as flow-through methods (flow rate required to dislodge a mucoadhesive-coated sphere), colloidal gold staining (measurement of the so-called 'adhesion number') and *in vivo* methods (endoscopy and radioisotope imaging), have also proved useful (see, e.g. Fiebrig, Davis, *et al.*, 1995; Fiebrig, Harding, *et al.*, 1995; Harding *et al.*, 1999).

Methods involving raw mucus are, however, not rigorous because of its variable nature from batch to batch and also with time because of the presence of degradative enzymes: in the alimentary tract mucins are constantly being degraded and replenished. In order to understand the fundamental molecular processes involved complementary molecular mucin-based analyses are needed. A selection of potential probes are available: the hydrodynamic-based techniques of viscometry/rheology, surface plasmon resonance, dynamic light scattering, turbidity, SEC-MALLs and the analytical ultracentrifuge, together with the imaging techniques of electron microscopy and atomic force microscopy. At Nottingham, we have found analytical ultracentrifugation particularly useful because of the huge range of particle sizes analysable (from a small sucrose molecule of 342 Da to particles of $\approx 10^9$ Da) and its ability to separate and analyse solutions of macromolecules without the need for separation media or inherent assumptions of inertness. Electron microscopy and atomic force microscopy have proved powerful complementary probes. SEC-MALLs has proved particularly useful for checking the molecular integrity of the mucins (Fig. 4) and mucoadhesives and viscometry for assaying the stability of mucoadhesive formulations as we shall see below.

The cornerstone probe for investigating mucin–mucoadhesive interactions in solution has thus been the analytical ultracentrifuge, reinforced with measurements by electron microscopy and atomic force microscopy. The analytical ultracentrifuge is a pure solution technique: molecules do not require fixing to a surface, embedding in a gel, passing down a chromatography column or through or across a membrane.

Analytical ultracentrifuge assays

There are two principal approaches. We could assay for change in molecular weight using sedimentation

equilibrium, but this has an upper limit of ≈50 MDa (unless we start turning the rotor by hand!). Since, complexes can be very large, a more sensible assay procedure (using the same equipment) is to use sedimentation velocity (which can cope, as we have already noted, with complexes as large as 10^9 Da) with change in sedimentation coefficient, *s*, as our marker for mucoadhesion. If we so wish we can then convert this to a change in molecular mass if we assume a conformation ($s \sim M_w^b$ where *b* is 0.67, 0.15 or 0.4–0.5 for a sphere, rod or coil, respectively). We choose though simply to use *s* directly as our size criterion (as used in, for example, ribosome size representations, 30S, 50S, etc. or in seed globulins, the 7S, 11S soya bean globulins, etc.). Where a mucin is available in only miniscule amounts (e.g. from different regions of the human stomach), we can use a special procedure known as sedimentation fingerprinting where we assay for its effect on the mucoadhesive.

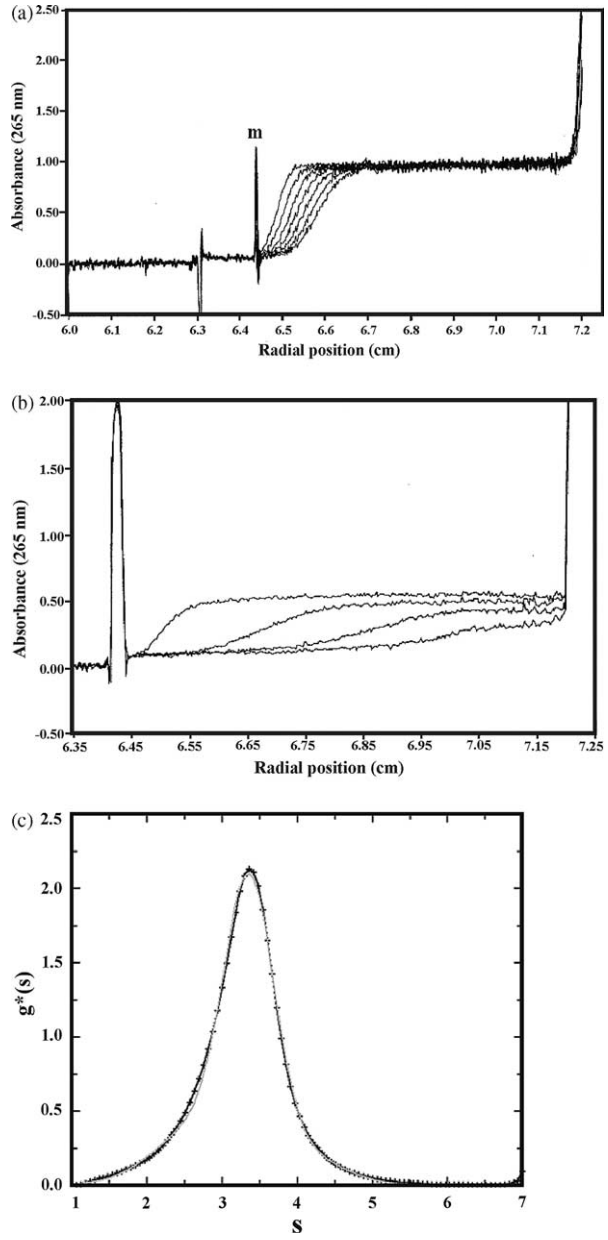

Fig. 5. Analytical ultracentrifuge assays. (a) Sedimentation velocity concentration profiles (recorded using UV absorption optics) for the mussel glue protein mefp1. Rotor speed = 40,000 rpm, solvent: phosphate-chloride buffer, $I = 0.10$, loading protein concentration = 0.8 mg/ml. Concentration scans recorded every 10 min, with the direction of sedimentation from left to right. The position of the meniscus is shown by the letter m. The sedimentation coefficient *s* at 20 °C = 2.3 S). (b) Same as (a) but with 0.1 mg/ml pig gastric mucin added and a speed of 2000 rpm, with scans recorded every 10 min, $s \sim 7000$ S. (c) Sedimentation velocity apparent sedimentation coefficient distribution plot for guar: g(*s*) vs *s*. Loading concentration = 0.75 mg/ml, rotor speed = 40,000 rpm. A Gaussian fit to the data (lighter line) is also shown. Rotor speed was 40,000 rpm at 20.0 °C. (a) and (b) From Deacon *et al.* (1998). (c) From Patel *et al.* (2005).

Mucoadhesion involving a protein: the mussel glue protein mefp1

Before we look at polysaccharide mucoadhesion, as a simple illustration consider the mussel glue protein mefp1. This is an unusual protein of ≈110 kDa that consists of a globular region with a long protruding tail of alternating flexible and rigid regions (Deacon, Davis, Waite, & Harding, 1998). This tail is rich in lysine—the molecule at neutral pH and below, therefore, behaves as a polycation. In free solution at 20 °C mefp1 sediments at ≈2.3S, as shown by a sedimentation velocity experiment in an XL-A ultracentrifuge (using UV absorption optics as the detection system) at 40,000 rpm at a protein concentration of 0.8 mg/ml (Fig. 5(a)) This is quite typical for a highly asymmetric protein of ≈110 kDa. If we repeat the experiment but with the protein in the presence of some highly purified mucin (at 0.1 mg/ml, too low to be picked up by the absorption optical system), the effect on the protein is spectacular, with the whole quantity of mefp1 now sedimenting at ≈7000 S (Fig. 5(b)). Despite this impressive demonstration mefp1 would be of limited practical use since protein-based mucoadhesives would be rapidly eaten away by the enzymes of the digestive tract. 7000 S also represents too strong an interaction with little opportunity for control. Also the reaction is far too strong and gives little room for manipulation. However, this provides the stepping stone for consideration of the use of polysaccharides that are not attacked by the digestive system and can be readily manipulated to control the extent of complexation. It is worth also pointing out that with modern instrumentation we do not just follow the sedimenting boundary but we can look at the change with time of the whole concentration distribution which allows us to obtain a sedimentation coefficient for a distribution, important for the much more molecularly polydispersed nature of polysaccharides: an example is given in Fig. 5(c) for guar (Patel *et al.*, 2005).

Mucoadhesive experiments on polysaccharides

UV absorption optics are used as the optical detection system. However, in this case the mucoadhesive is invisible (most polysaccharides do not absorb in the near-UV,

Table 1. Mucoadhesive analysis. The sedimentation coefficient ratio ($s_{complex}/s_{mucin}$) as an index of adhesiveness (from Fiebrig, Harding, Davis, 1994; Fiebrig, Harding, Stokke, *et al.*, 1994; Fiebrig, Davis, *et al.*, 1995; Fiebrig, Harding, 1995; Anderson *et al.*, 1989)

Mucoadhesive	$s_{complex}/s_{mucin}$	Conditions
DEAE-dextran	1.1–1.9[a]	pH 6.8, 20 °C
	1.2–1.4[a]	pH 6.8, 37 °C
Chitosan ($F_A \approx 0.11$)	48	pH 6.5, 20 °C
	15	pH 4.5, 20 °C
	22	pH 2.0, 20 °C
	12	pH 2.0, 37 °C
	18	pH 4.5, 20 °C + 6 mM bile salt
Chitosan ($F_A \approx 0.42$)	31	pH 4.5, 20 °C

[a] Depending on the mixing ratio.

≈280 nm), but the pig gastric mucin at the concentrations normally employed is visible. The sedimentation ratio ($s_{complex}/s_{mucin}$), the ratio of the sedimentation coefficient of any complex involving the mucin to that of pure mucin itself, is used as the measure for mucoadhesion.

Experiments on a series of neutral polysaccharides—such as guar—and polyanionic polysaccharides were performed. No significant change in the sedimentation coefficient of the mucin was seen (sedimentation ratio, $s_{complex}/s_{mucin}, \approx 1$), reinforcing the macroscopic observations using tensiometry that were considered above (see Table 1). The polycationic dextran derivative DEAE-dextran gave sedimentation ratios of 1.1–1.9 depending on the mixing ratio and temperature (Anderson, Harding, & Davis, 1989). This is extremely modest considering the high charge density on the polymer with lots of potential sites for interaction with the fully deionized sialic acid groups on the mucin. This disappointment also reflects the disappointing result from the tensiometry analyses (Lehr *et al.*, 1992). The $\alpha(1 \rightarrow 3)$ branches of the dextran appear to be responsible for considerable steric hindrance, preventing access to the charged mucin groups.

Mucoadhesion experiments involving chitosans

A contrasting picture is seen for chitosans. Chitosans are derivatives of chitin (after an alkali-extraction procedure) and hence are available in large quantities from the shells of crabs, lobsters and other crustaceans. Pure chitin is poly-*N*-acetyl glucosamine. The *N*-acetyl groups are de-acetylated in chitosan to an extent represented by the degree of acetylation F_A, with $F_A = 1$ being pure chitin and $F_A = 0$–0.6 representing the range of soluble chitosans. We stress here that chitosans are only readily soluble at pH values of 6.5 or less, and this factor has to be borne in mind in the formulation of any mucoadhesive product involving these substances. Interestingly, whereas mucins present two types of residue for potential mucoadhesive interaction (the charged acidic groups on sialic acid and any sulphonated residues, and the hydrophobic methyl groups on fucose residues) chitosans present similar opportunity (the charged NH_3^+ groups on deacetylated *N*-acetyl groups and also the hydrophobic acetyls on non-deacetylated residues). The results are quite spectacular (Table 1). A highly charged chitosan ('sea-cure' 210+) of $F_A \approx 0.11$ has impressive sedimentation ratios of 15–48 depending on the temperature, pH and salt environment, suggesting the interaction may hold up irrespective of the local conditions in the alimentary tract. Interestingly for a lower-charged chitosan of $F_A \approx 0.42$, high values were still returned, reinforcing the view that both electrostatic and hydrophobic effects are important.

The demonstration of large-size interaction products by the analytical ultracentrifuge used in this manner is reinforced by images from the powerful imaging techniques of electron microscopy and atomic force microscopy. Conventional transmission electron microscopy clearly demonstrates large complexes of the order of ≈1 (m in size (Fiebrig, Davis, *et al.*, 1995; Fiebrig, Harding, *et al.*, 1995) and if we label the chitosan with gold we can see that the chitosan is distributed throughout the complex with 'hot spots' in the interior (Fiebrig, *et al.* 1997). Images from atomic force microscopy, visualized in topographic and phase modes, again shows complexes of this size (Fig. 6): contrasting with individual images for mucin and chitosan, which appear as random coils and more stiffish structures, respectively (Deacon *et al.*, 2000).

We would dearly love to perform these types of experiments on human small intestinal mucin if we could only get them in sufficient quantities in purified form. We have, however, been successful in performing experiments on human mucin extracted from different parts of the stomach, namely the cardia, corpus and antrum regions. Although available in miniscule quantities we can assay mucoadhesiveness of chitosan on these by using a modification of the approach using the analytical ultracentrifuge described above, called sedimentation fingerprinting (Deacon *et al.*, 1999). Although the mucins from human stomach are at too low a concentration to be detected we can assay for interaction from the loss of the chitosan from optical registration caused by interaction. In this way it was possible to demonstrate significant differences between different regions of the stomach.

Chitosan based systems for the alimentary tract and nose

Having found an appropriate mucoadhesive material, the problem, which follows, is the construction of encapsulation systems based on chitosan, which are sufficiently stable. Tripolyphosphate appears to have been a popular choice for crosslinking chitosan into sphere-like nanoparticles (see, e.g. He, Davis, & Illum, 1998; Zengshuan, Yeoh & Lim, 2002), but much work is still required because of the large variation in pH that any encapsulation system has to

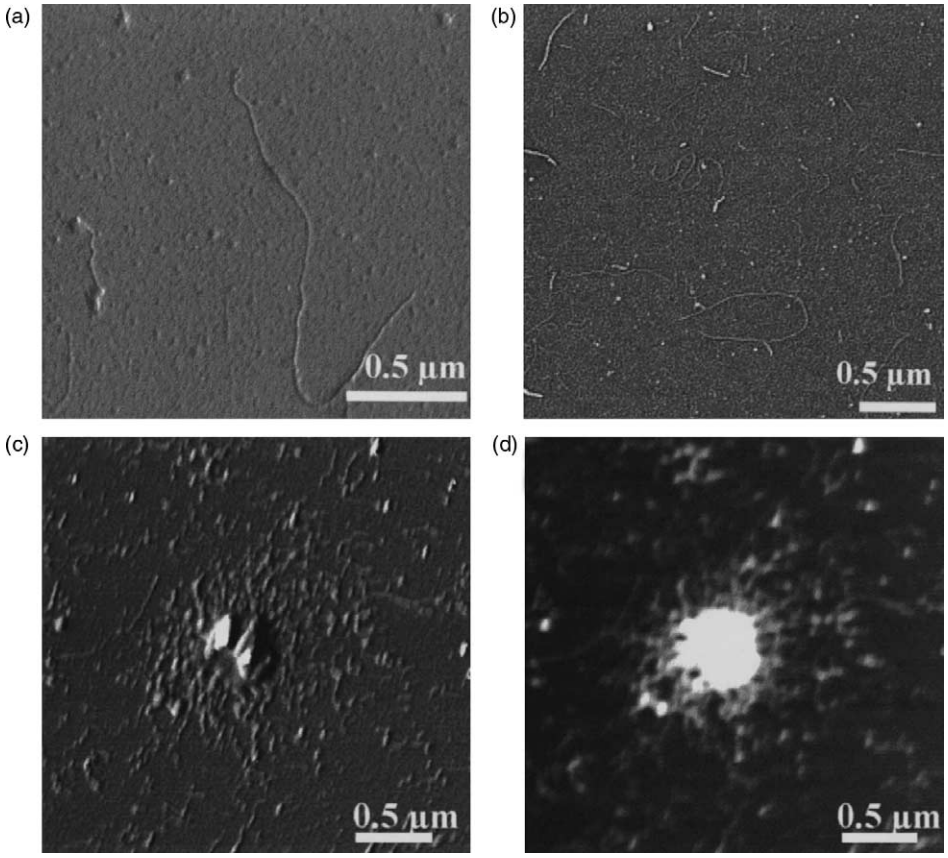

Fig. 6. Atomic force microscopy image of mucin, chitosan and chitosan–mucin complex. (a) Mucin by itself; (b) chitosan; (c) and (d) chitosan–mucin complex. All images taken in 'phase' mode apart from (d) which was taken in 'tapping' mode. From Deacon *et al.* (2000).

encounter from mouth to stomach—and the insolubility of chitosan at pH$>\sim$6.5. There are further problems:

There is evidence to suggest that tripolyphosphate can block the functional groups relevant for mucoadhesion.
Soluble mucin in the lumen will compete with the mucin in the adherent mucus gel layer.
There is also a relatively short turnover of the mucus layer, with some estimates suggesting that the turnover time is as low as a few hours (Lehr, Poelma, Junginger, & Tukker, 1991).

To bypass these problems the nasal route offers a most attractive alternative. The benefits of mucoadhesion of chitosan can be employed (without the need for a complicated encapsulation system) and chitosans offer the added benefit of apparently enhancing the absorption of drug through the surface epithelia. Illum (2002) and her group (see, e.g. He *et al.*, 1998) have shown the mucoadhesiveness of chitosan solutions by demonstrating longer clearance times from the nose compared with normal saline. Chitosan powder formulations are even more impressive. The Illum team have also shown enhanced delivery of insulin from chitosan solutions and especially chitosan powder formulations (see, e.g. Illum, 2002).

Current work: development of stable chitosan delivery systems

This is focused on the generation of stable encapsulation (oral delivery) and nasal delivery forms. I have already alluded to the problems through the oral route. Although the nasal route using chitosan is extremely attractive, there are some problems here. The production of acceptable delivery systems requires a thorough and rigorous investigation of the stability of chitosan-based systems. Chitosans are only readily soluble below pH 6.5. Derivatization can render them soluble at higher pH values, but are they still viable? Will a chitosan formulation remain stable on the shelf of a pharmacist's shop in tropical conditions or will it need to be kept in a refrigerator? Here, use of viscometry is proving a particularly valuable probe and some early observations have been very revealing (Fee, 2005). For example in studies on one chitosan of high degree of de-acetylation, it is stable under cold storage at 4 °C (Fig. 7(a)), but not at higher temperatures. There appeared greater stability at low pH (Fig. 7(b)) although change in ionic strength did not seem to have any dramatic effect. Once stability issues have been thoroughly researched and properly resolved we may not be too far off seeing a chitosan based pharmaceutical product reach phase III clinical trials and onto the shelves of a Dispensary.

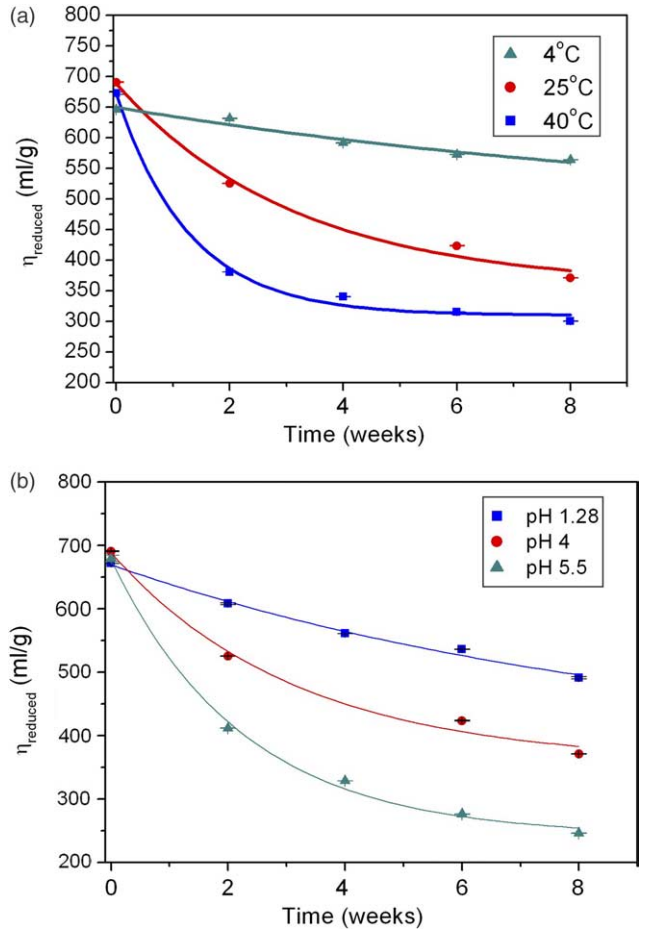

Fig. 7. Reduced specific viscosity as a measure of the stability of a chitosan solution of high degree of acetylation as a function of (a) temperature (b) pH (adapted from Fee, 2005).

Concluding remarks

The considerations above have referred exclusively to mucus, mucin and mucoadhesion in relation to more efficient drug delivery formulations. The principal role of the alimentary tract is, however, the delivery and absorption of food and nutrients, but one particular area in food science where this research may have strong resonances is in the encapsulation and release of flavour in relation to the mucus lining of the nose and gut. It may also have resonances in the treatment of allergic responses such as the proposed use of polysaccharides in the treatment of celiac disease (Seifert, Heinevetter, Cölfen & Harding, 1995).

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References

Allen, A. (1989). In J. G. Forte (Vol. Ed.), *Handbook of physiology—The gastrointestinal physiology salivary, gastric and hepatobiliary secretions, section 6* (Vol. III) (pp. 359–382). Bethesda, MD: American Physiological Society.

Anderson, M. T., Harding, S. E., & Davis, S. S. (1989). *Biochemical Society Transactions, 17*, 1101–1102.

Booth, C. C. (1967). *Federation Proceedings, 28*, 1583–1588.

Campbell, J. B. (1999). In E. Mathiowitz, D. E. Chickering, & C.-M. Lehr (Eds.), *Bioadhesive drug delivery systems* (pp. 85–130). New York: Marcel Dekker.

Carlstedt, I., & Sheehan, J. K. (1984). *Biochemical Society Transactions, 12*, 615–617.

Davis, S. S. (1985). *Journal of Controlled Release, 2*, 27–38.

Davis, S. S. (1989). In J. G. Hardy, S. S. Davis, & C. G. Wilson (Eds.), *Drug delivery to the gastrointestinal tract* (pp. 49–61). Chichester: Ellis Horwood.

Deacon, M. P., Davis, S. S., Waite, J. H., & Harding, S. E. (1998). *Biochemistry, 37*, 14108–14112.

Deacon, M. P., Davis, S. S., White, R. J., Nordman, H., Carlstedt, I., Errington, N., et al. (1999). *Carbohydrate Polymers, 38*, 235–238.

Deacon, M. P., McGurk, S., Roberts, C. J., Williams, P. M., Tendler, S. J. B., Davies, M. C., et al. (2000). *Biochemical Journal, 348*, 557–563.

Dodd, S., Place, G. A., Hall, R. L., & Harding, S. E. (1998). *European Biophysics Journal, 28*, 38–47.

Dziubla, T. D., & Muzykantov, V. R. (2005). *Biotechnology and Genetic Engineering Reviews, 22*, 267–298.

Fee, M. (2005). PhD dissertation, University of Nottingham, England.

Fiebrig, I., Davis, S. S., & Harding, S. E. (1995). In S. E. Harding, S. E. Hill, & J. R. Mitchell (Eds.), *Biopolymer mixtures* (pp. 373–419). Nottingham: Nottingham University Press.

Fiebrig, I., Harding, S. E., & Davis, S. S. (1994). *Progress in Colloid and Polymer Science, 93*, 66–73.

Fiebrig, I., Harding, S. E., Rowe, A. J., Hyman, S. C., & Davis, S. S. (1995). *Carbohydrate Polymers, 28*, 239–244.

Fiebrig, I., Harding, S. E., Stokke, B. T., Vårum, K. M., Jordan, D., & Davis, S. S. (1994). *European Journal of Pharmaceutical Sciences, 2*, 185.

Fiebrig, I., Vårum, K. M., Harding, S. E., Davis, S. S., & Stokke, B. T. (1997). *Carbohydrate Polymers, 33*, 91–99.

Gottschalk, A., Bhargava, A. S., & Murty, V. L. N. (1972). In A. Gottschalk (Ed.), *Glycoproteins, their composition, structure and function* (pp. 810–829). Amsterdam: Elsevier.

Gurny, R., Meyer, J.-M., & Peppas, N. A. (1984). *Biomaterials, 5*, 336–340.

Hallett, P., Rowe, A. J., & Harding, S. E. (1984). *Biochemical Society Transactions, 12*, 878–879.

Harding, S. E. (1984). *Biochemical Journal, 219*, 1061–1064.

Harding, S. E. (1989). *Advances in Carbohydrate Chemistry and Biochemistry, 47*, 345–381.

Harding, S. E., Creeth, J. M., & Rowe, A. J. (1983). In A. Chester, D. Heinegard, A. Lundblad, & S. Svensson (Eds.), *Proceedings of the seventh international glucoconjugates conference* (pp. 558–559). Sweden: Olsson-Reklambyra.

Harding, S. E., Deacon, M. P., Fiebrig, I., & Davis, S. S. (1999). *Biotechnology and Genetic Engineering Reviews, 16*, 41–86.

Harding, S. E., Rowe, A. J., & Creeth, J. M. (1983). *Biochemical Journal, 209*, 893–896.

He, P., Davis, S. S., & Illum, L. (1998). *International Journal of Pharmaceutics, 166*, 75–88.

Hounsell, E. F., Young, M., & Davies, M. J. (1997). *Clinical Science, 93*, 287–300.

Illum, L. (2002). *Drug Discovery Today, 7*, 1184–1189.

Jumel, K., Fiebrig, I., & Harding, S. E. (1995). *International Journal of Biological Macromolecules, 18*, 133–139.
Jumel, K., Fogg, F. J. J., Hutton, D. A., Pearson, J. P., Allen, A., & Harding, S. E. (1997). *European Biophysics Journal, 25*, 477–480.
Kerss, S., Allen, A., & Garner, A. (1982). *Clinical Science, 63*, 187–195.
Lehr, C.-M., Bouwstra, J. A., Schacht, E. H., & Junginger, H. E. (1992) . *International Journal of Pharmaceutics, 78*, 43–48.
Lehr, C.-M., Poelma, F. G. J., Junginger, H. E., & Tukker, J. J. (1991). *International Journal of Pharmaceutics, 70*, 235–240.
Mathiowitz, E., Chickering, D. E., & Lehr, C.-M. (Eds.). (1999). *Bioadhesive drug delivery systems*. New York: Marcel Dekker.
Park, K., & Robinson, J. R. (1984). *International Journal of Pharmaceutics, 19*, 107–217.
Patel, T. R., Picout, D. R., Pavlov, G., Garcia de la Torre, J., Ross-Murphy, S. B., & Harding, S. E. (submitted for publication).
Peppas, N. A., & Buri, P. A. (1985). *Journal of Controlled Release, 2*, 257–275.
Pigny, P., Guyonnet-Duperat, V., Hill, A. S., Pratt, W. S., Galiegue-Zouitina, S., D'Hooge, M. C., et al. (1997). *Genomics, 38*, 340–352.
Ringel, J., & Lohr, M. (2003). *Molecular Cancer, 2*, 9–17.
Seifert, A., Heinevetter, L., Cölfen, H., & Harding, S. E. (1995). *Carbohydrate Polymers, 28*, 325–332.
Sheehan, J. K., & Carlstedt, I. (1989). In S. E. Harding, & A. J. Rowe (Eds.), *Dynamic properties of biomolecular assemblies* (pp. 256–275). Cambridge: Royal Society of Chemistry.
Zengshuan, M. A., Yeoh, H. H., & Lim, L. Y. (2002). *Journal of Pharmaceutical Sciences, 91*, 1396–1404.