8
STABILITY OF PECTIN-BASED DRUG DELIVERY SYSTEMS

G.A. Morris[1*], G.G. Adams[2], S.E. Harding[2], J.D. Castile[3] and A. Smith[3]

[1]Department of Chemical & Biological Sciences, School of Applied Sciences, University of Huddersfield, Queensgate, Huddersfield, HD1 3DH, UK
[2]National Centre for Macromolecular Hydrodynamics, School of Biosciences, University of Nottingham, Sutton Bonington, LE12 5RD, UK
[3]Archimedes Development Limited, Albert Einstein Centre, Nottingham Science Park, Nottingham, NG7 2TN, UK
*G.Morris@hud.ac.uk

1 INTRODUCTION

The delivery of drugs through the alimentary tract following oral administration via the mouth (the "oral delivery route") remains the most popular method of drug administration for both medical staff and patients alike largely due to convenience, capacity and ease of administration. However, oral drug delivery can be inefficient due to poor absorption, hostile conditions e.g. due to acidic conditions present in the stomach or degradation by digestive enzymes and particularly due to metabolism in the liver (widely known as the "hepatic first pass effect"). A relatively slow onset of action can also be an issue following oral administration even for well absorbed drugs. Recent research has therefore focused on drug absorption through nasal epithelia, although rapid clearance can be an issue for simple nasal formulations. To address these problems there has been significant interest in macromolecular-based carrier and mucoadhesive systems; these approaches are of growing importance. An outstanding example, one particularly successful system of delivery using pectin – a dietary fibre polysaccharide from the cell walls of fruit - is now utilised (PecSys[®]) in a nasal product recently approved for commercial sale (Fentanyl Nasal Spray marketed as PecFent[®] / Lazanda[®] by Archimedes Pharma).
     In this chapter we review some of the recent work that has been conducted to characterise the physical properties of pectins that have been used for such purposes, focusing in particular on aspects of stability as reflected in the hydrodynamic and other physico-chemical properties.

2 PHYSICO-CHEMICAL PROPERTIES

Pectins are a complex family of heteropolysaccharides that constitute a large proportion of plant primary cell walls.[1-3] Pectins are made of several structural elements the most important of which are the homogalacturonan (HG) and type I rhamnogalacturonan (RG-I)
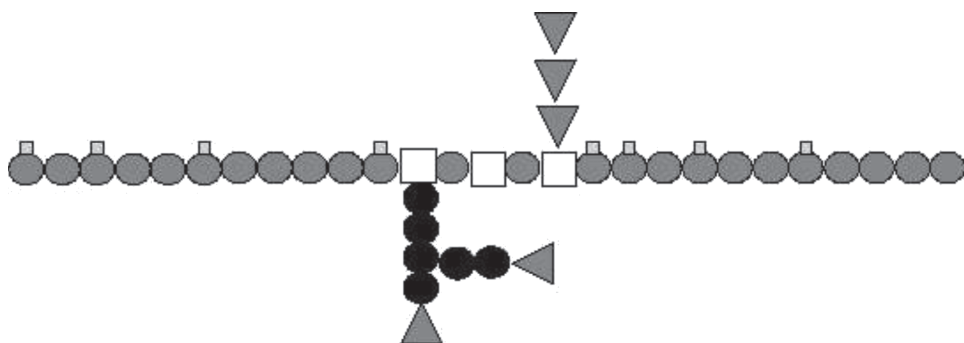
Figure 1 *Schematic structure for pectin: galacturonic acid (◉); galactose (●); arabinose (▼); rhamnose (□) and methyl groups (▫)*

regions often described in simplified terms as the "smooth" and "hairy" regions respectively[4] (Figure 1). The HG region is composed of (1→4) linked α-D-GalpA residues that can be partially methylated at C-6 and possibly partially acetyl-esterified at O-2 and/or O-3.[5,6] 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%. The RG-I region consists of disaccharide repeating unit [→4)-α-D-GalpA-(1→2)-α-L-Rhap-(1→]n with a variety side chains consisting of L-arbinosyl and D-galactosyl residues.[7] It has been reported that GalA residues in the RG-I region are partially acetylated[8,9] but not methylated.[9,10]

The degree of esterification and therefore the charge on a pectin molecule is important to the functional properties in the plant cell wall, as considered in detail in the chapter earlier in this volume by G. Tucker. It also significantly affects their commercial use as gelling and thickening agents. In some ways this is analogous to the critical effect the degree of acetylation (or deaceytlation) has on the properties of chitosans, as considered in the following chapter by Schütz, Käuper and Wandrey.

HM pectins (lower charge) form gels at low pH (< 4.0) and in the presence of a high amounts (> 55 %) of soluble solids, usually sucrose and are stabilised by hydrogen-bonding and hydrophobic interactions.[11] Conversely, LM pectins (higher charge) form electrostatically stabilised gel networks with divalent metal cations, usually calcium, in the so-called "egg-box" model, which also depends on the distribution of negative carboxylate groups and structure breaking rhamnose side chains[12,13], analogous to the alginate systems, again considered by Schütz, Käuper and Wandrey.

Solution properties such as viscosity also depend on degree of esterification, molar mass, conformation, solvent environment (e.g. salt concentration, sugar concentration and pH) and temperature (Tables 1 and 2). Hydrodynamic studies based on intrinsic viscosity ([η]), sedimentation coefficient ($s^o_{20,w}$), radius of gyration (R_g) and weight average molar mass (M_w) have suggested a semi-flexible conformation (Figure 2) for pectins irrespective of degree of esterification (and charge).[14-27] Pectin molar mass and chain flexibility are also important in mucoadhesive interactions.[28]

Table 1 *Representative physical properties of pectin in dilute solution*[14-27]

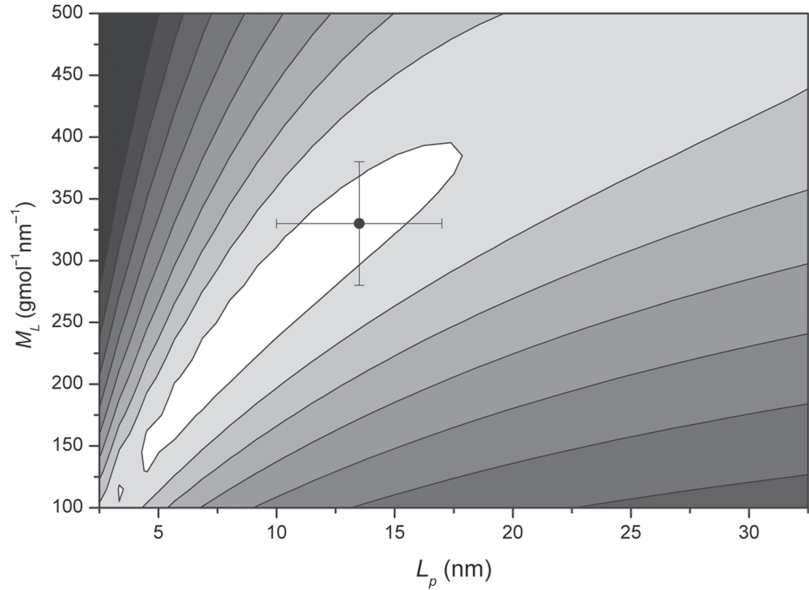| Physical Property	Values
Weight-average molar mass, M_w (g/mol)	30000 - 350000
Intrinsic viscosity, $[\eta]$ (ml/g)	60 - 1000
Sedimentation coefficient, $s^o_{20,w}$ (S)	1.6 – 2.3
Radius of gyration, R_g (nm)	13 - 45

Figure 2 *Combined analysis method – HYDFIT – for pectin which estimates the best values (or best range of values of persistence length L_p and mass per unit length M_L) based on minimisation of a target function Δ. The x-axis and y-axis represent L_p (nm) and M_L (g.mol^{-1}.nm^{-1}) respectively. In these representations, the values of Δ are represented in grey shading from black to white, where for white ($\Delta = 0.2$) and black ($\Delta \geq 1$). The range of the target function minimum is typical of a semi-flexible coil where $L_p/M_L \sim 0.03 – 0.05$ nm^2.mol.g^{-1} and an estimation of the minima has been indicated*[24]

Table 2 *Representative conformation parameters of pectin in dilute solution*[14-27]

Conformational Property	Representative Values	Comments
"Power law" parameters: a b c	 0.62 – 0.94 0.17 0.57	a, b and c are Mark-Houwink-Kuhn-Sakurada (MHKS) power law relations for the molar mass dependency for intrinsic viscosity, sedimentation coefficient and root-mean-square radius respectively. For spheres, non-draining coils and rigid rods respectively (cf. the previous chapter in this volume): $a \sim 0$, 0.5-0.8 and 1.8 $b \sim 0.67$, 0.4-0.5 and 0.2 $c \sim 0.33$, 0.5-0.6 and 1.0
Frictional ratio f/f_o	7 – 10	The translational frictional ratio, f/f_o depends on conformation and molecular expansion through hydration effects. It has a value of 1 for an anhydrous sphere and increases with solvent association and with chain asymmetry.[28]
Wales-van Holde ratio $k_s/[\eta]$	0.10 – 0.85	The Wales-van Holde ratio provides a hydration independent *estimation* of conformation and has a value of ~ 1.6 for spheres and random coils and < 1.6 for asymmetric structures, approaching a lower limit of ~ 0.1 for a rigid rod.
Conformational Zone	A/B/C/D/E	A "sedimentation conformation zoning plot"[29] involving a combination of k_s with the sedimentation coefficient s and the mass per unit length M_L enables an estimate of the "overall" solution conformation of a macromolecule in solution. Zone A: rigid rod; Zone B: rod with some degree of flebility; Zone C: semi-flexible coil; Zone D: random coil; Zone E: globular sphere.[29]
Persistence length L_p (nm)	10 - 17	The linear flexibility of a polysaccharide chain can be represented quantitatively in terms of the persistence length L_p of the equivalent worm-like chain where the L_p is defined as the average projection length along the initial direction of the polymer chain (in the limit of infinite chain length).[30] Practical limits are ~ 1 nm for random coils and ~ 200 nm for a rigid rod.

3   USE IN DRUG DELIVERY

Pectins are a major natural constituent of the diet and thus are demonstrably safe. Consequently they have been used as a gelling agent (for example) in the food industry for many years. Recently there has also been significant interest in the gelling properties of pectins in controlled drug delivery.[32-35] For example, LM pectin may be used to prolong the residence of an incorporated drug substance at mucosal surfaces and thereby modulate its rate of systemic absorption. LM pectins do not act primarily as a mucoadhesive agent or absorption enhancer, instead they may alter retention and drug release characteristics due to the chelation of calcium present in nasal secretions; this results in the formation of a gel in which pectin chains are linked in an ordered three-dimensional network cross-linked by calcium ions.[36] Pharmaceutical interest in pectins is also in part due to their long standing reputation of their GRAS (Generally Regarded As Safe) status,[33,34,37] their relatively low production costs[32] and high availability[38]. It is proposed that pectin could be used to deliver drugs orally, nasally, topically and vaginally[32,33,39-43] which are routes that are generally well accepted by patients.[33,34,44] Pectin gels are pseudoplastic[32,42,43] and drug release is diffusion-controlled at low pectin concentrations[33,42] and determined by gel dissolution at higher pectin concentration.[33] The use of pectins in modulating drug absorption is particularly noteworthy in the field of nasal drug delivery. The utility of nasal route is limited by the small sample volume of up to 200 μl that can be delivered,[45] which is important in drug formulations especially if the drug is sparingly soluble or if a drug has to be delivered over a prolonged period. Even at these low volumes run-off from the nose and/or dripping into the throat can be an issue for a simple nasal spray solution.[45,46] PecSys®, a gelling technology based on LM pectin has been developed to optimise nasal delivery by addressing problems such as this. The utility of PecSys® for enhanced nasal delivery has been demonstrated in the clinic with a number of molecules including fentanyl and buprenorphine.[37]

3.1 Case study – Fentanyl Nasal Spray

Fentanyl Nasal Spray (marketed as PecFent® [EU] and Lazanda® [US] by Archimedes Pharma) is indicated for the management of breakthrough pain in adults receiving maintenance opioid therapy for chronic cancer pain. Breakthrough pain in cancer (BTPc) is a transitory exacerbation of pain that occurs on a background of otherwise controlled persistent pain. It is an intense, sudden pain that is often unpredictable and debilitating and occurs despite otherwise appropriate opioid therapy for background pain. BTPc often has high intensity, a rapid onset, usually reaching maximum intensity within five minutes, and a short duration, lasting between 30 to 60 minutes per episode. On average, BTPc affects more than half of patients with cancer and often interferes with patients' health and ability to engage in daily living activities. Given the profile of such a BTPc episode, onset of action is of primary importance in managing the condition. Fentanyl is the gold standard opioid for management of BTPc since it acts rapidly once it enters the systemic circulation and its duration of action closely matches the duration of a typical pain episode.

In addition to modulating absorption to provide rapid but controlled delivery of fentanyl, [37,47,48] PecSys® technology is employed to help avoid run-off from the nose and/or dripping into the throat[45] that can be associated with conventional nasal spray formulations and thus further optimise efficiency of drug absorption. The LM pectin element of PecSys® causes the product to gel *in situ* when sprayed into the nose due to the interaction of LM pectin with calcium in endogenous nasal secretions. *In situ* gelling

ensures that the product can be sprayed readily as a finely dispersed plume before forming a gel on contact with the mucosal surface.

4 STABILITY

Pectins are not only subject to enzymatic degradation – as we have seen already in the previous chapters in this volume by Tucker and Grassby *et al.* – but also to thermal and other degradative effects of bioprocessing. In solution, depending on conditions, they can degrade by de-esterification and depolymerisation. The extent and rate of degradation depends on factors such as pH, water activity and temperature[45], so choice of the optimum storage conditions is essential in commercial products. In general, the maximum stability is found at pH ~ 4.[49] The presence of sugar also has a certain protective effect.[49] At low pH-values and elevated temperatures degradation is due to depolymerisation whilst de-esterification is also favoured. At pH 6, HM-pectin is stable is relatively stable at moderate temperatures (~20 °C), however as temperature or pH increases β-elimination will occur which results in depolymerisation and a loss of viscosity[23] and gelling properties.[32,50] LM-pectins are more stable with respect to depolymerisation under these conditions (Figure 3).[51] HM-pectins, as a solid form, lose their ability to form gels if stored in humid conditions whilst LM-pectins are again more stable and loss is not expected to be significant after one year storage at room temperature[49], an observation that is consistent with the use of LM pectin in pharmaceutical products that have remained demonstrably stable for up to 3 years.
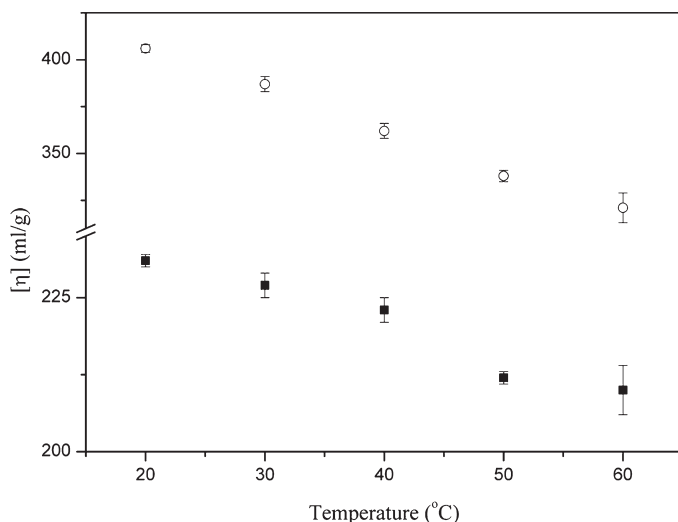

Figure 3 *The effect of increased temperature on the intrinsic viscosity, [η], for LM (■) and HM pectins (○) in phosphate-chloride buffer (pH ~ 6.8; I = 0.1 M) adapted from Figures 4 and 2 in references 50 and 23, respectively. In the case of the LM pectin, the decrease in intrinsic viscosity is a least partially due to change in conformation to a less rigid structure*

The stability (shelf-life) of pectin in terms of viscosity and gel strength is highly relevant to its commercial uses as these properties can play an important role in the function of pectin.[33,34] It is, therefore, fundamentally important to have the means available with which to measure the effects of and understand the relationships between storage conditions and stability.

   We have previously looked at the stability of pectin solutions, in terms of viscosity, across a range of different temperature conditions: 4°C, 25°C and 40°C and the consequent effect on molar mass and gel strength.[52,53] The viscosity of pectin solutions decreases marginally after 6 months storage at 25 °C and significantly after 6 months at 40 °C (Table 3). A correlation between decrease in viscosity and gel strength upon addition of calcium ions has been demonstrated (Figure 4; Table 3)[53], observations which can be ascribed to a significant depolymerisation of the pectin over time (Figure 5).

Table 3 *Solution viscosities, intrinsic viscosities, gel strengths and molecular weights for pectin of degree esterification 19% (P_{19}) stored at different temperatures (4°C, 25°C or 40°C). Adapted from Table 2 in ref. 53*

| Storage time (days)	Storage temperature (°C)	Viscosity (mPas)	Intrinsic viscosity (mL/g)	Weight-average molar mass (g/mol)	Gel strength area (g.sec)
0	-	4.41 ± 0.01	396 ± 1	154000 ± 1000	1645 ± 105
30	4	4.52 ± 0.07	405 ± 6	158000 ± 3000	1305 ± 200
90	4	4.48 ± 0.01	402 ± 1	156000 ± 1000	1400 ± 235
180	4	4.45 ± 0.02	399 ± 2	155000 ± 1000	1320 ± 340
30	25	4.40 ± 0.05	395 ± 4	153000 ± 2000	1340 ± 20
90	25	4.24 ± 0.02	383 ± 1	147000 ± 1000	1340 ± 380
180	25	4.05 ± 0.02	367 ± 2	140000 ± 1000	1000 ± 265
30	40	3.75 ± 0.06	341 ± 5	128000 ± 2000	935 ± 25
90	40	3.12 ± 0.01	282 ± 1	103000 ± 1000	1035 ± 25
180	40	2.45 ± 0.01	212 ± 1	73000 ± 1000	860 ± 80
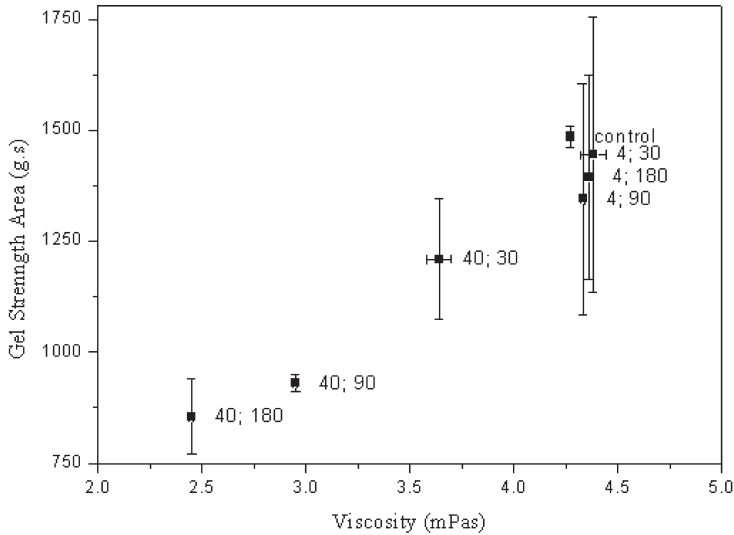
Figure 4 *The relationship between gel strength and viscosity for LM pectins of DM 21 % (labels indicate the temperature (^oC); and the time (days) at which a measurement was taken)*
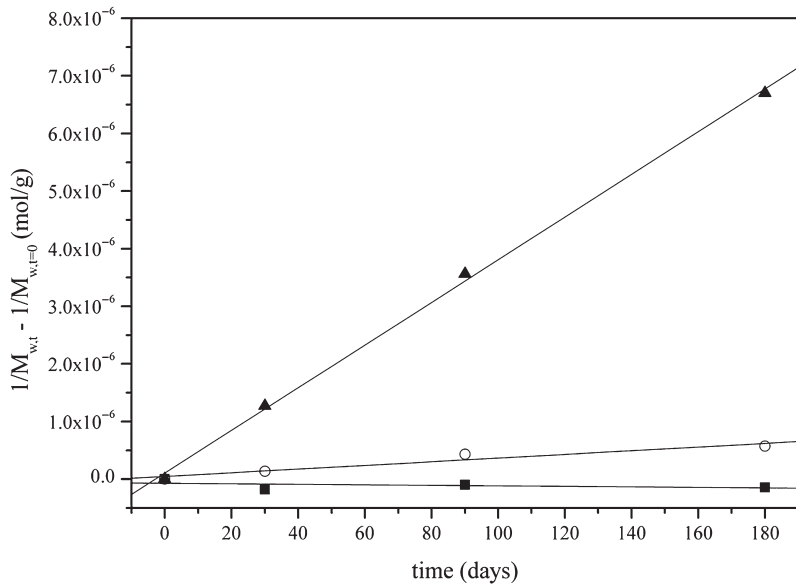

Figure 5 *First order kinetic plots of (mol/g) vs. time (days) for pectin of DM ~ 19%, where closed symbols represent molar masses estimated from viscometry at 4 ^oC (■), 25 ^oC (○) and 40 ^oC (▲) (adapted from Figure 3 in 35). The kinetic rate constants (day^{-1}) are (-0.8 ± 1.1) x 10^{-7}, (5.7 ± 1.1) x 10^{-7} and (6.7 ± 0.2) x 10^{-6} at 4 ^oC, 25 ^oC and 40 ^oC, respectively*

It has been shown that drug release rates from pectin gels *in vitro*[36,42] are relatively unaffected by a decrease in absolute viscosity from approximately 5 – 2 mPas[52,53] which suggests that a decrease in pectin molar mass from 175000 – 50000 g/mol would have no significant effect on drug release rates from pectin gels (Figure 6). This therefore implies that even at 40 °C there will be no significant effect on drug release upon storage of up to 1 year. 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.[32] Similarly diffusion profiles of fentanyl from LM pectin-based (PecSys®) nasal liquid formulations have been studied,[37] and have been shown to remain stable throughout product shelf-life.
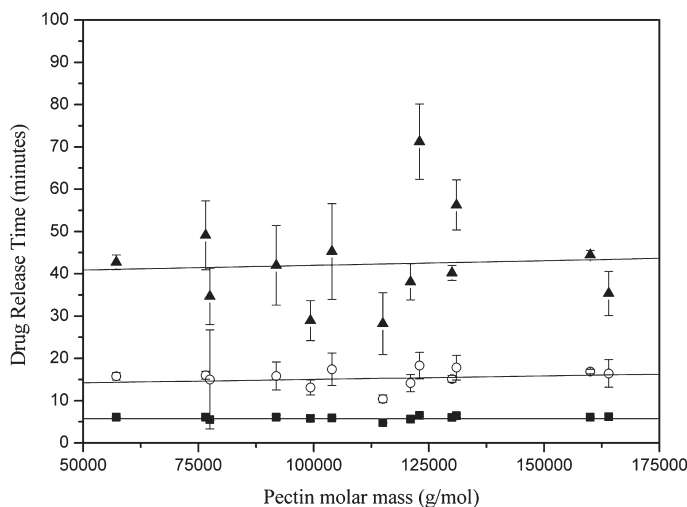

Figure 6 *Effect of pectin molar mass on model drug (paracetamol) release from pectin gel systems. 10% drug release (■), 50% drug release (○) and 90% drug release (▲)*

5   CONCLUSIONS

Over the last few decades there has been significant interest in the use of polysaccharides such as pectin in drug delivery systems, with the realisation of some successful and demonstrably stable products (*e.g.* PecFent® / Lazanda® Nasal Spray). However, in view of the polydisperse nature of the biopolymer itself it remains necessary to evaluate, refine and optimise any pectin-based drugs delivery systems for improvement and refinement for a particular application with respect to issues such as optimal molar mass and molar mass distribution, macromolecular conformation[54], degree of esterification and distribution of methyl groups[4]. Further appreciation of these parameters will provide a better understanding of molecular interactions[55] and improved ways of optimising stability (in terms of molar mass/viscosity/gel strength) and the effect of temperature and pH.[23,35,50] Another fruitful area of further study would appear to be an exploration of interactions with other polysaccharides *e.g.* cationically charged chitosan[55] and the effect of mechanical[56], chemical[56,57] or enzymatic[2,58] modification on the any of the above issues.

Acknowledgements

References

1   W. G. T. Willats, L. McCartney, W. Mackie and J. P. Knox, *Plant Mol. Biol.*, 2001, **47**, 9.
2   G. A. Morris, M-C. Ralet, E. Bonnin, J-F. Thibault and S. E. Harding, *Carbohyd. Polym.*, 2010, **82**, 1161.
3   G. A. Morris and M-C. Ralet, *Carbohyd. Polym.*, 2012, **87**, 1139.
4   M. A. K. Williams, A. Cucheval, A. Ström and M-C. Ralet*, Biomacromolecules*, 2009, **10**, 1523.
5   W. Pilnik and A. G. J. Voragen in *Biochemistry of Fruits and their Products*, ed. A. C. Hulme, Academic Press, New York, 1970, 53.
6   F. M. Rombouts and J-F. Thibault in *The Chemistry and Function of Pectins,* ed. M. L. Fishman and J. J. Jen, American Chemical Society, Washington DC, 1986, 49.
7   A. G. J. Voragen, W. Pilnik, J-F. Thibault, M. A. V. Axelos and C. M. G. C. Renard in *Food Polysaccharides and their Interactions* ed. A. M. Stephen, Marcel Dekker, New York, 1995, 287.
8   T. Ishii, *Plant Physiol.*, 1997, **113**, 1265.
9   P. Perrone, H. C. Hewage, H. R. Thomson, K. Bailey, I. H. Sadler and S. C. Fry, *Phytochemistry*, 2002, **60**, 67.
10 P. Komalavilas and A. J. Mort, *Carbohyd. Res.*, 1989,**189**, 261.
11 J. A. Lopes da Silva and M. P. Gonçalves, *Carbohyd. Polym.*, 1994, **24**, 235.
12 E. R. Morris, *Food Chem.*, 1980, **6**, 15.
13 E. R. Morris, D. A. Powell, M. J. Gidley and D. A. Rees, *J. Mol. Biol.*, 1982, **155**, 507.
14 H. Anger and G. Berth, *Carbohyd. Polym.*, 1985, **5**, 241.
15 M. A. V. Axelos, J. Lefebvre and J-F. Thibault, *Food Hydrocolloid.*, **1**, 1987, 569.
16 G. Berth, H. Anger and F. Linow, *Nahrung-Food*, 1977, **31**, 939.
17 S. E. Harding, G. Berth, A. Ball, J. R. Mitchell and J. Garcìa de la Torre, *Carbohyd. Polym.*, 1991, **16**, 1.
18 C. Garnier, M. A. V. Axelos and J-F. Thibault, *Carbohyd. Res.*, 1993, **240**, 219.
19 A. Malovikova, M. Rinaudo and M. Milas, *Carbohyd. Polym.*, 1993, **22**, 87.
20 S. C. Cros, C. Garnier, M. A. V. Axelos, A. Imbery and S. Perez, *Biopolymers*, 1996, **39**, 339.
21 I. Braccini, R. P. Grasso and S. Perez, *Carbohyd. Res.*, 1999, **317**, 119.
22 G. A. Morris, T. J. Foster and S. E. Harding, *Food Hydrocolloid.*, 2000, **14**, 227.
23 G. A. Morris, T. J. Foster and S. E. Harding, *Carbohyd. Polym.*, 2002, **48**, 361.
24 G. A. Morris, J. García de la Torre, A. Ortega, J. Castile, A. Smith and S. E. Harding, *Food Hydrocolloid.*, 2008, **22**, 1435.
25 M. L. Fishman, H. K. Chau, P. D. Hoagland and A. T. Hotchkiss, *Food Hydrocolloid.*, 2006, **20**, 1170.
26 M. L. Fishman, H. K. Chau, F. Kolpak and J. Brady, *J. Agr. Food Chem.*, 2001, **49**, 4494.
27 R. Noto, V. Martorana, D. Bulone and B. L. San Biagio, *Biomacromolecules*, 2005, **6**, 2555.

28 C. Tanford. *Physical Chemistry of Macromolecules*. John Wiley and Sons, New York, 1961.

29 G. M. Pavlov, A. J. Rowe, and S. E. Harding, *Trends Anal. Chem.*, 1997, **16**, 401.

30 O. Kratky, and G. Porod, *Recl. Trav. Chim. Pay-B.*, 1949, **68**, 1106.

31 N. A. Nafee, F. A. Ismail, N. A. Boraie and L. M. Mortada, *Drug Dev. Ind. Pharm.*, 2004, **30**, 985.

32 S. Sungthongjeen, P. Sriamornsak, T. Pitaksuteepong, A. Somsiri and S. Puttipipatkhachorn, *AAPS PharmSciTech*, 2004, **5**, 1.

33 L. Lui, M. L. Fishman and K. B. Hicks, *Cellulose*, 2007, **14**, 15.

34 L. Lui, M. L. Fishman, J. Kost and K. B. Hicks, *Biomaterials*, 2003, **24**, 3333.

35 G. A. Morris, J. Castile, A. Smith, G. G. Adams and S. E. Harding, *Polym. Degrad. Stabil.*, 2010, **94**, 1344.

36 S. T. Charlton, S. S. Davis and L. Illum, *J. Control. Release*, 2007, **118**, 225.

37 P. Watts and A. Smith, *Expert Opin. Drug Del.*, 2009, **6**, 543.

38 C. M. Beneke, A. M. Viljoen and J. H. Hamman, *Molecules*, 2009, **14**, 2602.

39 N. A. Peppas, P. Bures, W. Leobandung and H. Ichikawa, *Eur. J. Pharm. Biopharm.*, 2000, **50**, 27.

40 V. R. Sinha and R. Kumria, *Int. J. Pharm.*, 2001, **224**, 19.

41 C. Valenta, *Adv. Drug Deliver. Rev*, 2005, **57**, 1692.

42 S. Chelladurai, M. Mishra and B. Mishra, *Chem. Pharm. Bull.*, 2008, **56**, 1596.

43 N. Thirawong, R. A. Kennedy and P. Sriamornsak, *Carbohyd. Polym.*, 2008, **71**, 170.

44 N. Yadav, G. A. Morris, S. E., Harding, S. Ang and G. G. Adams, *Endocr., Metab. Immune Disord.: Drug Targets*, 2009, **9**, 1.

45 J. Castile, Y.-H. Cheng, B. Simmons, M. Perelman, A. Smith and P. Watts, *Drug Dev. Ind. Pharm.* 2012, (in press)

46 E. O. Meltzer, J. E. Stahlman, J. Leflein, S. Meltzer, J. Lim , A. A. Dalal, B. A. Prillaman and E. E. Philpot, *Clin. Therap.* 2008, **30**, 271.

47 A. N. Fisher, M. Watling, A. Smith and A. Knight, *Int. J. Clin. Pharmacol. Ther.,* 2010, **48**, 138.

48 A. N. Fisher, M. Watling, A. Smith and A. Knight, *Int. J. Clin. Pharmacol. Ther.* 2010, **48**, 860.

49 P. Sriamornsak, *Silpakorn University International Journal*, 2003, **3**, 206.

50 G. A. Morris, T. J. Foster and S. E. Harding, *Prog. Coll. Pol. Sci.,* 1999, **113**, 205.

51 C. Rolin, In *Industrial Gums*, ed. R. L. Whistler and J. N. BeMiller, Academic Press, New York, 1993, 257.

52 G. A. Morris, M. S. Kök, S. E. Harding and G. G. Adams, *Biotechnol. Genet. Eng. Rev.*, 2010, **27**, 257.

53 G. A. Morris, J. D. Castile, A. Smith, G. G. Adams and S. E. Harding, *Polym. Degrad. Stabil.,* 2011, **95**, 2670.

54 G. A. Morris and M-C. Ralet, *Carbohyd. Polym.*, 2012, **88**, 1488.

55 S. E. Harding, *Trends Food Sci. Tech.* 2006, **17**, 255.

56 U. Einhorn-Stoll, H. Kunzek, and G. Dongowski, *Food Hydrocolloid.*, 2007, **21**, 1101.

57 G. A. Morris, Z. Hromadkova, A. Ebringerova, A. Malikova, J. Alfoldi and S. E. Harding, *Carbohyd. Polym.*, 2002, **48**, 351.

58 K. T. Inngjerdingen, T. Patel, X. Chen, L. Kenne, S. Allen, G. A. Morris, S. E. Harding, T. Matsumoto, D. Diallo, H. Yamada, T. E. Michaelsen, M. Inngjerdingen and B. S. Paulsen, *Glycobiology*, 2007, **17**, 1299.