Polymer Bulletin 56, 239–246 (2006)
DOI 10.1007/s00289-005-0489-8

Polymer Bulletin

Rheological properties of pH-induced association and gelation of pectin

Petter Stang Holst[a,b], Anna-Lena Kjøniksen[a] (✉), Huaitian Bu[a], Sverre Arne Sande[b], Bo Nyström[a]

[a] Department of Chemistry, University of Oslo, P.O. Box 1033, Blindern, N-0315 Oslo, Norway
[b] Department of Pharmaceutics, School of Pharmacy, University of Oslo, P.O. Box 1068, Blindern, N-0316 Oslo, Norway

E-mail: a.l.kjoniksen@kjemi.uio.no, Fax: +47 22855441

Received: 24 August 2005 / Revised version: 9 December 2005 / Accepted: 13 December 2005
Published online: 22 December 2005 –

Summary

Pectin is an important polysaccharide with applications in food industry, cosmetics and pharmacy. In recent years, there has been an increased use of hydrogels in pharmaceutical formulations in connection with drug-delivery administration. In this study of aqueous pectin systems, association and gelation have been accomplished by changing pH of the samples to an acidic pH. This is carried out by adding glucono-δ-lactone (GDL), which will gradually reduce the pH of the system. As the pH is reduced, the carboxyl acid groups on the pectin chains are neutralized, and the polyelectrolyte character of the polymer is reduced promoting association and gelation of the system. The viscoelastic properties of the system are monitored by oscillatory shear measurements as a function of time after the addition of GDL. The system exhibits very long gelation times and a pronounced viscosity increase in the vicinity of the gel points.

Keywords

Pectin, hydrogel, pH, rheology, GDL

Introduction

The polysaccharide pectin is found in plant cell walls of higher plants. Citrus peel and apple pomace are usual sources for commercial pectins [1]. Pectin consists mainly of linear chains of α-D galacturonic acid residues together with a small fraction of rhamnose with small side chains of other sugars ("hairy regions") [1,2]. The properties of pectin are dependent on the degree of methylation (DM) of the carboxyl groups, and they are divided into high-methoxy (HM) pectin (DM > 50 %) and low-methoxy (LM) pectins (DM < 50 %). The ability of pectin to act as a thickener and gelling agent in an aqueous environment has made it a useful additive in foods and cosmetics.

Its non-toxicity and biocompatibility also render it interesting for pharmaceutical controlled drug delivery applications. Pectin gels can be used as a carries for drugs, e.g., for oral administration of colon-specific drug delivery [3].
Pectin and alginate gels are often produced by using divalent cations, usually Ca^{2+} ions [1,3-5]. However, it has recently been shown that both alginate [6-8], HM-pectin [5] and LM-pectin [4,9] exhibit acid-induced gelation. By adding a strong acid, large-scale inhomogeneities are often formed during the gelation process, therefore glucono-δ-lactone (GDL) is often used as the acidifying agent [4,6-8]. In this paper we wish to scrutinize the rheological behavior and the gel forming properties of LM-pectin/GDL systems.

Experimental

Materials and solution preparation

The pectin used in this study (Pectin classic CU 701; Lot-nr. 00306088) is obtained from Herbstreith & Fox KG, Pektin Fabrik, Neuenbürg, Germany. According to the manufacturer, the degree of methoxylation is 35 %. Glucono-δ-lactone (GDL) was obtained from Calbiochem® (Lot-nr B32713), and was used without further purification. Pectin solutions (1.0 wt %) were centrifuged at 3800 rpm for 2.5 h to remove non water-soluble impurities, and then dialyzed against pure water for 5 days to remove low molecular weight components (impurity from manufacturing), and were thereafter freeze-dried. As the dialyzing membrane, regenerated cellulose with a molecular weight cutoff of 8000 (Spectrum Medical Industries) was utilized. After being freeze-dried, the polymer was redissolved in Millipore water, and GDL was added to the pectin/water system shortly before the commencement of experiments. The pectin concentration was kept constant at 3.0 wt % throughout this study.

pH measurements

A PHM 210 standard pH-meter, MeterLab®, from Radiometer analytical was employed for the pH-measurements. All pH-measurements were conducted at room temperature (ca 25 ^{o}C).

Rheology

Oscillatory shear measurements of the samples were carried out on a Paar-Physica MCR 300 rheometer using a cone-and-plate geometry, with a cone angle of 1^{o} and a diameter of 75 mm. The samples were introduced onto the plate, and to prevent evaporation of the solvent, the free surface of the sample was always covered with a layer of low viscosity silicone oil (the viscoelastic response of the samples is not observed to be affected by this layer). The values of the strain amplitude were checked to ensure that all oscillatory shear measurements were performed within the linear viscoelastic regime, where the dynamic storage modulus (G') and loss modulus (G'') are independent of the strain amplitude. The measurements were conducted in the angular frequency (ω) domain 0.5-5 rad/s. All experiments were conducted at 25.0 ^{o}C.

Results and discussion

pH measurements

As can be seen from Figure 1, the addition of GDL to pectin solutions gradually reduces the pH of the samples; this is due to the hydrolyzation of GDL to gluconic acid. The pH reaches a constant value after a couple of hours, and the end pH decreases as the GDL concentration is raised (see inset plot in Figure 1). Since pectin contains carboxyl acid groups, it is charged at high pH-values and neutral at low pH values (the pK_a value of galacturonic acid is 3.5 [2]). A lowering of the pH will reduce the amount of charged groups and thereby the electrostatic repulsion between the polymer chains, causing the attractive forces (hydrogen bonds and hydrophobic interactions) to become more dominant.

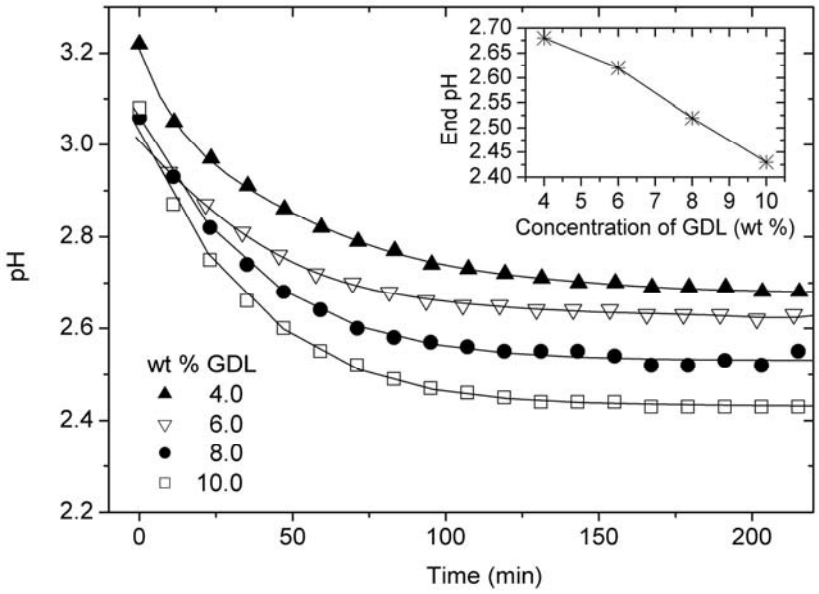

Figure 1. The change in pH of 3.0 wt % pectin samples as a function of time after the addition of GDL. The inset plot shows the GDL concentration dependence of the pH at long times.

Rheology

The rheological properties of the system after the addition of GDL were scrutinized as a function time by oscillatory shear measurements. By using the theory of Winter and Chambon [10] the gel points (GP) of the samples can be determined as the time where tan δ (= G"/G') is independent of frequency, see Figure 2. An alternative method to determine the gel point [11] is to plot the "apparent" viscoelastic exponents n' and n" ($G' \sim \omega^{n'}$, $G'' \sim \omega^{n''}$) versus time, and observing the crossover where $n' = n'' = n$ (see the upper inset of Figure 2). These two methods yield the same gel points for all the considered systems. At the gel point $G' \sim G'' \sim \omega^{n}$, where n is the relaxation exponent (see the lower inset of Figure 2).

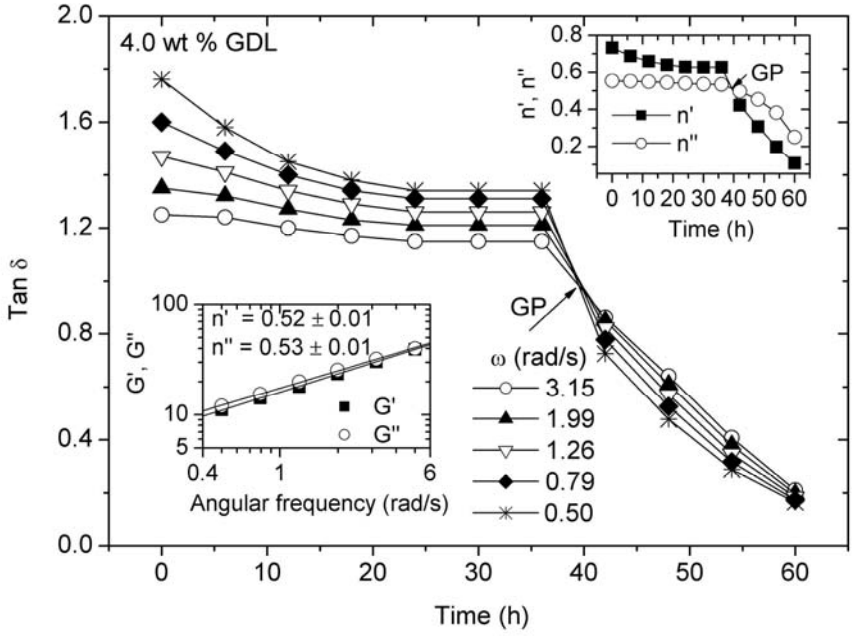

Figure 2. Illustration of determination of the GP for the sample containing 3.0 wt % pectin and 4.0 wt % GDL.

The gel point as a function of GDL concentration is depicted in Figure 3a. The gel point exhibits a minimum as the GDL concentration is increased. A faster gel formation with increasing GDL addition has been observed for Alginate/GDL gels [8]. This was explained by the more acid conditions achieved at high levels of GDL addition, causing a neutralization of the charges of the polymer chains, whereby the attractive forces like hydrogen bonds and hydrophobic associations becomes more dominant. However, the subsequent raise in the gelation time was not observed for the Alginate/GDL systems (the highest GDL concentration used here was 7 wt %). The anomalous longer gel times at high levels of GDL addition is discussed below in connection with Figure 4.

If one examine the gelation times they are extremely long, especially compared to the much shorter times it takes the samples to reach their final pH values (Figure 1). The long times indicate a very slow formation of associations in the system. The gel times observed is much longer than what is found in earlier studies of both pectin and alginate gels formed by addition of GDL [4,8]. Draget et al. [6,7] observed that homo-polymeric regions, especially glucoronic acid blocks, is important in the formation of association in alginate/GDL gels. Toft et al. [12] observed the same for alginate/pectin/GDL gels. Löfgren et al. [5] showed that the viscosification of acid induced HM-pectin gels is stronger for pectins where the methyl esters are concentrated into a block structure, and according to Ralet et al. LM-pectin with a blockwise distribution of free carboxyl groups are especially sensitive to low calcium levels [2]. This suggests that the gel-forming associations in these kinds of systems are built up of aggregates between block structures in the polymer chain. Our long gelation times might therefore indicate that the pectin sample used here has short and

few blocks-like structures. This can also explain why this pectin, unlike another pectin sample with the same degree of methoxylation [13-15], does not exhibit shear-induced viscosification and gelation.

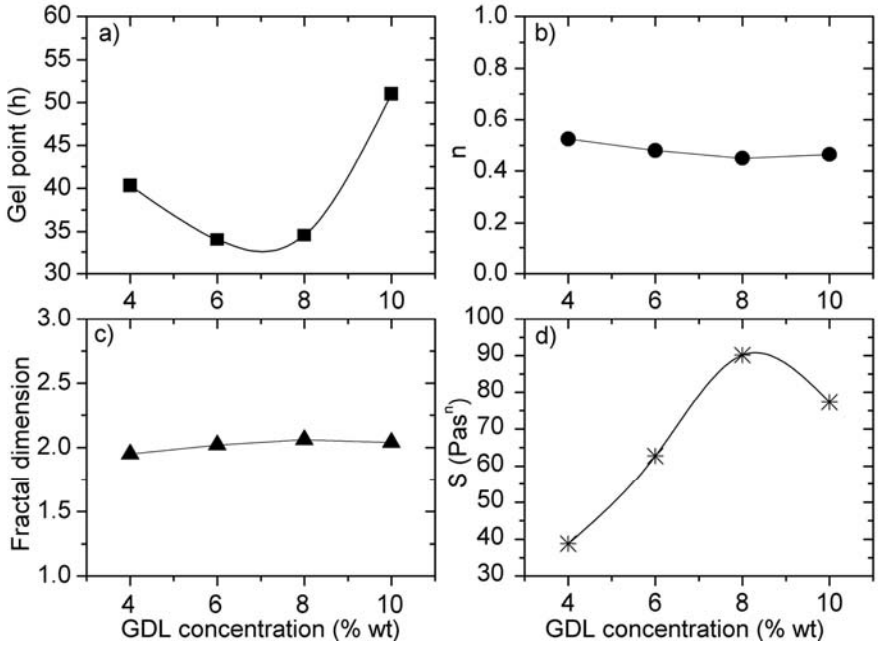

Figure 3. The GDL concentration dependency of a) the gel point, b) the relaxation exponent, c) the fractal dimension, and d) the gel strength parameter.

The relaxation exponent (Figure 3b) is near 0.5 for all GDL concentrations. According to Muthukumar [16], when excluded-volume effects are screened out the fractal dimension, d_f, can be calculated from the relaxation exponent:

$$n = \frac{d(d+2-2d_f)}{2(d+2-d_f)} \qquad (\mathbf{1})$$

where d (d = 3) is the spatial dimension. As can be seen from Figure 3c, the fractal dimension at the gel point is about 2 independent of the GDL concentration. GDL independent values of d_f have also been observed for the alginate/GDL system [8]. However, the alginate gels have a more open structure with a d_f of only 1.4. A fractal dimension near 2 has also been observed for chemical cross-linked gels of the same pectin sample that was used in this study [17]. Axelos and Kolb [18] observed n = 0.71 ($\Rightarrow$ d_f = 1.72) for a Pectin/Ca^{2+}gel. A fractal dimension of about 2.4 has been observed for pectin/chitosan gels [19], and for shear induced pectin gels [13,15] using the same pectin batch as reference 19, while a d_f of 1.6 was observed for HM-pectin/sucrose gels [20]. This suggests that the fractal dimension of pectin gels are more dependent on the kind of pectin used (degree of methylation, the amount and distribution of block-like structures and "hairy regions" on the polymer chain etc.), than it is on the gel-forming process used.

The gel strength parameter, S, can be calculated for the incipient gels by [21]:

$$G' = G''/\tan\delta = S\omega^n\Gamma(1-n)\cos\delta \qquad (2)$$

where $\Gamma(1-n)$ is the Legendre gamma function. The gel strength parameter is observed to be dependent on the strand length between cross-links in the gel network [22], and decreasing the strand length (higher cross-link density) gives higher values of S. This suggests (see Figure 3d) that increasing the GDL concentration gives a higher cross-link density (shorter distance between the cross-linking junctions) and therefore a stronger incipient gel network. The exception from this is the highest GDL concentration where the gel strength parameter starts to decline. This is probably caused by the same phenomena that induce the longer gelation times of this sample, see discussion below.

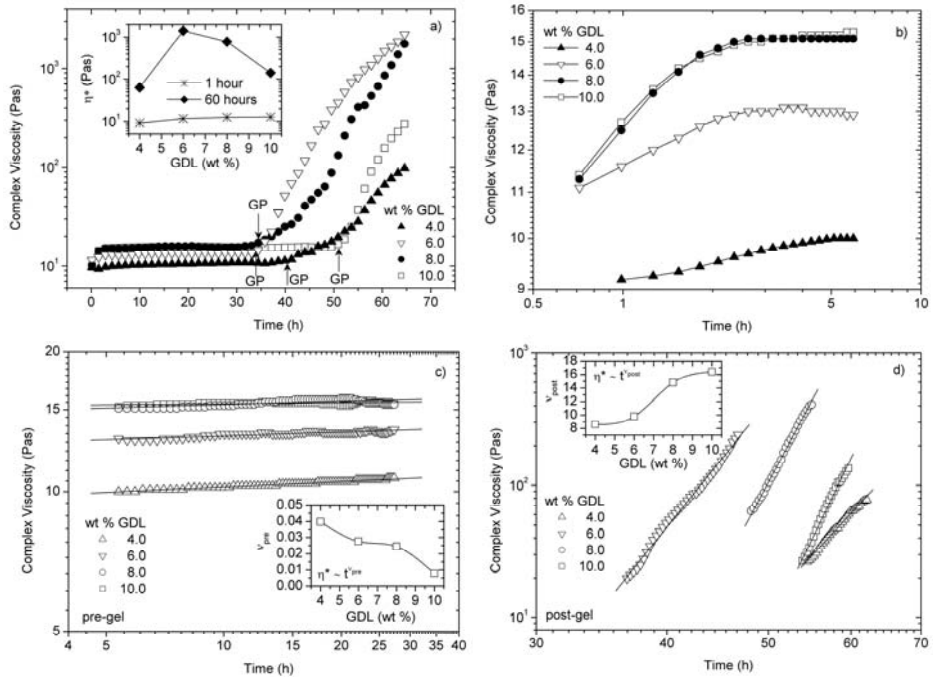

Figure 4. Evolution of the complex viscosity (ω = 5 rad/s) of 3.0 wt % pectin samples as a function of time after the addition of GDL. a) The evolution of η^* during the whole gelation process. The gel points (GP) are indicated with arrows. The inset plot shows the complex viscosity after 1 and 60 hours as a function of the GDL concentration. b) The time dependency of η^* at short times after the addition of GDL. c) The time dependency of η^* in the pre-gel regime. The lines represent a power-law fit to the data, and the power-law exponent (ν_{pre}) is displayed in the inset. d) The time dependency of η^* in the post-gel regime. The lines represent a power-law fit to the data, and the power-law exponent (ν_{post}) is displayed in the inset.

The evolution of the complex viscosity (η^*) during the gelation process is depicted in Figure 4a. The gel points are indicated by arrows and, as usually observed for gelling systems, there is a marked increase of the complex viscosity in the vicinity of the gel

point. The inset plot in Figure 4a shows the complex viscosity as a function of GDL concentration at 1 and 60 hours after the addition of GDL. In the pre-gel regime (after 1 hour), the complex viscosity rises slightly with increasing GDL concentration, whereas in the post-gel regime (after 60 hours) the complex viscosity exhibits a pronounced maximum. To gain a better understanding of the gelation process and why the dynamic viscosity in the post gel regime, the gel point, and the gel strength parameter all exhibit either a maximum or a minimum, each stage of this process need to be examined closely.

In Figure 4b the complex viscosity at short times after the addition of GDL is displayed. Initially, an increase in the complex viscosity is observed before it starts to flatten out at longer times. This raise in η^* is due to the simultaneously decrease in pH as displayed in Figure 1. This process continues for some time after the end pH is reduced, because of the kinetics of the association process. As expected, the viscosification is strongest for the highest GDL concentrations. This is because of the increased associations (hydrophobic interactions and hydrogen bonds) as the ionic repulsion between the polymer chains weakens when the pH is reduced. This also causes the viscosity increase observed after one hour in the inset of Figure 4a.

The complex viscosity in the pre-gel regime after the initial stage is displayed in Figure 4c. At this stage there is a very slow increase of the viscosity. This slow build-up of the complex viscosity is probably due to a rearrangement of the association zones in the samples. In order to quantify the viscosity change, the curves are fitted to a power-law. The power-law exponent, ν_{pre}, is displayed in the inset of Figure 4c. The power-law exponent decreases as the GDL concentration is raised, indicating that the rearrangement of the association zones are faster for the lowest GDL concentration because of fewer initial associations giving rise to a better mobility and therefore a faster rearrangement.

The gel point will be affected by both the initial stage where the viscosification is strongest for the highest amount of added GDL, and the subsequent very slow increase of the complex viscosity where the largest effect is observed for the lowest GDL concentration. The first process favors gel points that decrease with GDL concentration, while the second process promotes the opposite trend. Together the two processes give rise to the GP minimum that is observed in Figure 3a. The gel strength parameter will also be affected by this, and the lower value of S for the highest GDL concentration (Figure 3d) is probably due to the very slow re-arrangement of the associations in this sample (Figure 4b) causing a decrease in the effective cross-linking density at the GP.

The complex viscosity in the post-gel regime is shown in Figure 4d. At this stage, there is a very strong viscosification of the systems. The data have been fitted to a power-law, and the power-law exponent, ν_{post}, is displayed in the inset of Figure 4d. In this case, the power-law exponent increases with the GDL concentration, indicating that after the gel point is reached and an interconnected network is established, the build-up of the network is again favored at low pH values.

The viscosity maximum observed after 60 hours (post-gel regime) in the inset of Figure 4a is therefore an accumulation of all three stages displayed in Figure 4b-d. Since the viscosification in the post-gel regime is much stronger for the highest GDL concentration (Figure 4d), one would expect that at very long times the complex viscosity would again rise with increasing GDL concentration.

Conclusions

The gelation process studied for the acid-induced pectin/GDL systems can be divided into four stages:

i. Initially a viscosification of the samples occurs as a result of the gradual lowering of the pH of the samples. At this stage, the complex viscosity (η^*) is observed to increase with increasing level of GDL addition.

ii. The pre-gel region where η^* raises very slowly due to re-organization of the association zones in the sample. This effect is strongest for the solution with the lowest GDL concentration.

iii. The value of the gel point passes through a minimum as the level of GDL addition increases because of the opposite trends at stages i and ii.

iv. In the post-gel region a strong viscosity enhancement occurs. As in the initial stage, this effect becomes more pronounced as the GDL concentration increases.

The overall picture that emerges from this study is that the viscoelastic features of a semidilute pectin solution and its gelling ability can be tuned by changing the level of GDL addition and thereby the pH of the sample.

Acknowledgement. B.N., H.B., and S.A.S. gratefully acknowledge support from the Norwegian Research Council through a NANOMAT Project (158550/431).

References

1. Thakur BR, Singh RK, Hanada AK (1997) critical Reviews in Food Science and Nutrition 37:47.
2. Ralet, MC, Bonnin E, Thibault JF (2002) Pectins. In: Steinbüchel A (ed) Biopolymers. Wiley-VCH, Weinheim. pp 345-380.
3. Liu LS, Fishman ML, Kost J, Hicks KB (2003) Biomaterials 24:3333.
4 Lootens D, Capel F, Durand D, Nicolai T, Boulenguer P, Langendorff V (2003) Food Hydrocolloids 17:237.
5. Löfgren C, Guillotin S, Evenbratt H, Schols H, Hermansson AM (2005) Biomacromolecules 6:646.
6. Draget KI, Skjåk-Bræk G, Smidsrød O (1994) Carbohydrate Polymers 25:31.
7. Draget KI, Stokke BT, Yuguchi Y, Urakawa H, Kajiwara K (2003) Biomacromolecules 4:1661.
8. Baldursdóttir S, Kjøniksen AL (2005) European Journal of Pharmaceutics and Biopharmaceutics 59:501.
9. Gilsenan PM, Richardson RK, Morris ER (2000) Carbohydrate Polymers 41:339.
10. Winter HH, Chambon F (1986) Journal of Rheology 30:367.
11. Hodgson DF, Amis EJ (1991) Journal of Non-Crystalline Solids 131-133:913.
12. Toft K, Grasdalen H, Smidsrød O (1986) ACS Symposium Series 310:117.
13. Kjøniksen AL, Nordby M, Roots J, Nyström B (2003) Journal of Physical Chemistry B 107: 6324.
14. Kjøniksen AL, Hiorth M, Nyström B (2005) European Polymer Journal 41: 761.
15. Tho I, Kjøniksen AL, Nyström B, Roots J (2003) Biomacromolecules 4: 1623.
16. Muthukumar M (1989) Macromolecules 22:4656.
17. Werner B, Bu H, Kjøniksen AL, Sande SA, Nyström B Submitted to Polymer Bulletin.
18. Axelos MAV, Kolb M (1990) Physical Review Letters 64:1457.
19. Nordby MH, Kjøniksen AK, Nyström B, Roots J (2003) Biomacromolecules 4:337.
20. Bulone D, Martorana V, Xiao C, San Biago PL (2002) Macromolecules 35:8147.
21. Izuka A, Winter HH, Hashimoto T (1992) Macromolecules 25:2422.
22. Chambon F, Petrovic ZS, MacKnight WJ, Winter HH (1986) Macromolecules 19:2146.