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Rheological Characterisation of Acid Pectin Samples in Absence and Presence of Monovalent Ions

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ABSTRACT

Ion induced gelation of pectin has been extensively studied due to its use as thickener and gelling agent in the pharmaceutical and food industry. Pectins are traditionally divided into two groups, high methoxy (HM) and low methoxy (LM), which determines the charge of the pectin and the gelation mechanism. However, the distribution of the charges has an important impact on the gelation as well. The aim of this study is to rheologically characterise the acid gelation of a pectin with a high DB_{abs} and degree of methyl esterification of ~37%, in absence and presence of monovalent ions. The results obtained are interpreted such that the acid pectin in absence of monovalent ions at pH close to pKa exhibit weak gel or entangled solution properties. The addition of monovalent ions changes the rheological properties to resemble those of a strong gel.

INTRODUCTION

Pectin is a compound used mainly for its gelling and thickening abilities in both the food and pharmaceutical industries. Pectin describes a family of oligosaccharides and polysaccharides that have common features but are diverse in their fine structures[1]. Commercial pectin contains a high degree of homogalacturonan and a minor amount of neutral sugars[2]. The homogalacturonan is a negatively charged polymer and consists of 1,4 linked D-galacturonate units. The galacturonate units can carry a methyl ester group on the C-6 and the degree of methylation (DM) of the pectin influences network formation capabilities of pectin. Pectins are traditionally divided into two groups, high methoxy (>50% methyl esters) and low methoxy (<50% methyl esters). The former forms gel under acid condition in combination with a high sugar concentration, while the latter forms gels in the presence of most multivalent ions according to the egg box model[3] but also under acid conditions[4,5].

Although pectins are found in most plant tissues, the main sources of commercial pectins are citrus fruits and apple. These pectins have a high degree of methyl esters in their natural form and therefore de-esterification is used to produce pectins with lower degrees of methyl esters[6]. De-esterification can be performed using acids, alkalis or enzymes where the ester groups on the pectin are removed in a random manner. However, pectins subjected to certain enzymes are de-esterified in a 'blockwise' manner resulting in a blockwise distributed galacturonate residue[7].

The distribution pattern of the ester groups and galacturonate residues is known to affect the gelation behaviour of pectins[8]. Ström and co-workers showed that the distribution of the methyl esters, expressed as the degree of blockiness (DB) or the absolute degree of blockiness (DB_{abs}), has a large impact on the capability of the pectin to form gels[9].

Acid gelation of LM-pectin is related to a change in the pectin conformation from an extended two fold (2_1) form to a more compact three fold (3_1) form due to a reduction in charge density upon pH reduction and stabilisation via intramolecular hydrogen bonding[10,11]. Furthermore, intermolecular hydrogen bond are formed between protonised and unprotonised carboxyl groups and between carboxyl groups and O(3) on a neighbouring anti-parallell pectin helix. It has been suggested that these bonds are favored by a blocky distribution of the carboxyl groups allowing for longer stretches of hydrogen bonds to be formed and a certain degree of co-operativity[9]. Similar suggestion has been made for acid alginate gels[12].

While it has been shown that the acid pectin gels obtained using a random pectin can be described as a strong gel, we intend in this study to investigate the rheological properties of blocky pectin samples at pH close to pKa, in absence and presence of monovalent ions.

MATERIAL & METHODS

Materials. The pectin used in this study was kindly provided by CP Kelco with the following analytical information: DM of ~37% and a galacturonic acid content of ~ 89%. The pectin had been de-esterified at 45°C using a PME extracted from papaya, at a pH of 4.5 and a NaCl concentration of 0.4M. A conventional pectin of DM 35% was further used.

Methods: The determination of DB and DB_{abs} were performed by incubating the pectin with endopolygalacturonase [EC3.1.2.15][13]. The oligomers were analysed using capillary electrophoresis according to a method previously described[9] giving a DB of ~ 57% and DB_{abs} of ~ 36%. The DB and DB_{abs} of the conventional pectin were 40 and 26% respectively.

The starting pectin solutions were prepared at concentrations of 2-3 wt%. This solution was diluted to the desired end concentrations using either deionised water or salt (NaCl) solutions. Both pectin and dilution solution was heated to 45°C prior being mixed together. Acidification of the gels was also performed at T= 45°C using hydrochloric acid (0.1N or 1N). Dilution and addition of acid or base was done at elevated temperature (45°C) as most samples behaved as solutions at this temperature. This minimise effect from shear or inhomogeneities formed upon acidification or salt addition. The solutions were then acidified using hydrochloric acid (0.1N or 1N) at 45°C. To achieve higher pH values, 1M sodium hydroxide (NaOH) was used.

The rheological measurements were performed using a cone-plate geometry on a TA-AR1000 rheometer. The cone had a diameter of 40 mm an angle of 1.59° and gap of 7 μm. The cone and the plate were pre-heated to 50°C prior loading of the pectin sample. The pectin sample itself was heated to 45°C prior being loaded. Once loaded, the sample was quickly quenched to 5°C and equilibrated at 5°C for 30 mins before measurement started. Oscillatory measurements were conducted at 5°C to measure the gel moduli, G' and G'' and temperature (5-40°C and 40-5°C). Gelation and melting temperatures were established from the temperature sweep. The temperature was varied with 2°C per minute. In these measurements, a constant strain of 0.5% and a constant frequency of 1Hz were used. Samples were measured in triplicate and variations of moduli during time sweep and melting and setting temperature were within 10%. A further frequency sweep at T = 5°C was conducted on selected samples. The pH meter used was a Schott CG-825 and it was calibrated between pH=7 and 4 before each experiment.

RESULTS

Rheological characterization of pectin samples in absence of monovalent ions:

The slope of the viscosity increase as a function of polymer concentration at two different pH values differed for the random and the blocky pectin studied (Figure 1). Similar increases as a function of polymer concentration is shown for both pectin at pH 5 while at pH 3, the viscosity of the blocky pectin increases more rapidly compared to the non blocky pectin. The estimated c* obtained for the blocky pectin at pH 5 is 0.4%, close to the values obtained for the random pectin at both pH 5 and 3. At pH 3, the blocky pectin obtain a c* of 0.25%.

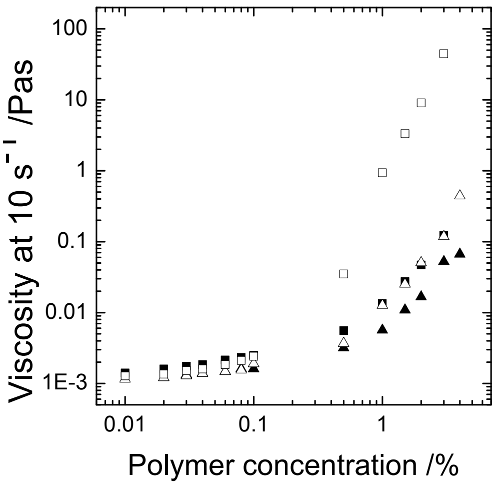

Figure 1. Viscosity at a shear rate of $10s^{-1}$ as a function of pectin concentration at pH 5 (triangles) and pH 3 (squares) for pectin with similar degree of methylation but with random (solid symbols) and blocky (open symbols) charge distribution.

Oscillatory measurements show that the moduli of the sample prepared using the blocky pectin increases as the pectin concentration increased from 1.0% to 3.0% at a fixed pH value of 3.0 (Figure 2). Tan δ (defined as G''/G') decreases from 0.63 to 0.35 as pectin concentration increases from 1 to 3% showing an increased importance of the elastic component with increasing polymer concentration. Loss moduli was higher than storage moduli for the non – blocky pectin at the pH and temperature tested.

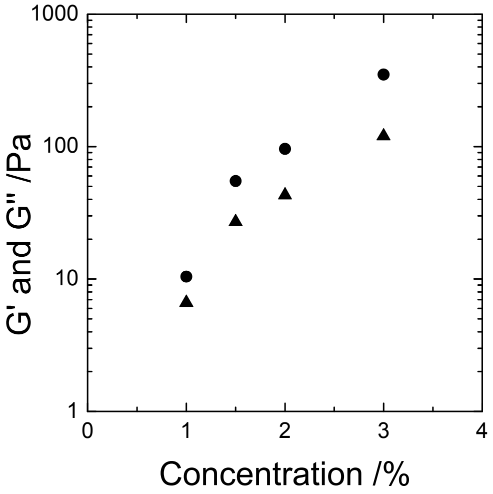

Figure 2. G' (circles) and G'' (triangles) as a function of pectin concentration and at pH = 3.0, T = 5°C, f=6.28rad/s and strain = 0.5%.

The mechanical spectra at the lowest pectin concentration tested (1%) is typical of a gelling sample where the degree of crosslinks is just sufficient to form a continuous network. Log G' and log G'' vary linearly with log ν over the frequency range studied and with the same slope (Figure 3). Upon increase in pectin concentration, the absolute value of G' is higher than G'' at frequencies between 0.02 and 80 $rads^{-1}$. It appears as though the slopes of log G' is slightly higher than the slope of log G''. The mechanical spectra further suggest an overlap of G''>G' at low frequencies.

Pre-shearing the samples at different shear rates prior oscillatory measurements show a fast (within a minute) recovery independent of pre-shear speed (1, 10, $100s^{-1}$) and the moduli obtained the same absolute values independent of pre-shear rate (results not shown).

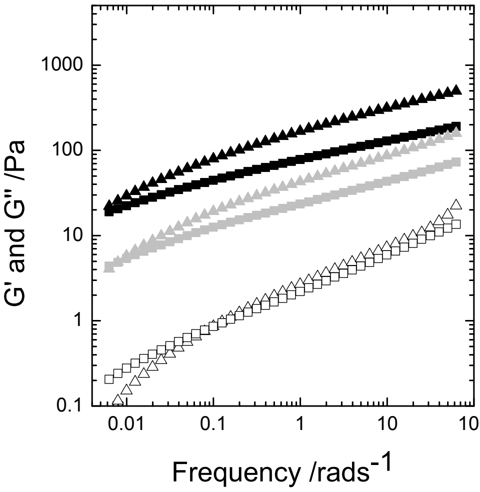

Figure 3. G’ (triangles) and G’’ (squares) as a function of frequency at a strain of 0.5%, T = 5°C and pH = 3.0. Black symbols represent 3%, grey 2% and open 1% pectin.

The temperature sweep of the pectin samples are presented in Figure 4a and b. Reducing the temperature increases the gel strength of all samples and increasing pectin concentration increases the setting and melting temperature of the pectin gels. There is little hysteresis between the melting and the setting temperatures of the gel.

<u>Rheological characterization of acid pectin samples in presence of monovalent ions:</u>

The addition of NaCl (0 to 0.2M) to the acid pectin gels increased G’ while tan delta (G’’/G’) was reduced (Figure 5a). The mechanical spectra of the pectin sample with increasing amount of salt (Figure 5b) show high absolute value of moduli but more importantly a larger difference between G’ and G’’ and less frequency dependence. In addition to the increase in G’ and G’’, the gelation and melting temperatures also increased (Figure 5c) as did the thermal hysteresis of the where NaCl was present.

In some cases, such as that corresponding to pH 2.45 with 0.05M NaCl, the melting temperature of the gel was above the preparation and testing temperature of 45°C. These gels could be visually observed to be synergetic and inhomogeneous. Rheological tests on these conditions were not done since the high temperature required to melt the gel could cause evaporation (cone and plate geometry used) and lead to unreliable data. Change of geometry to bob and cylinder would allow

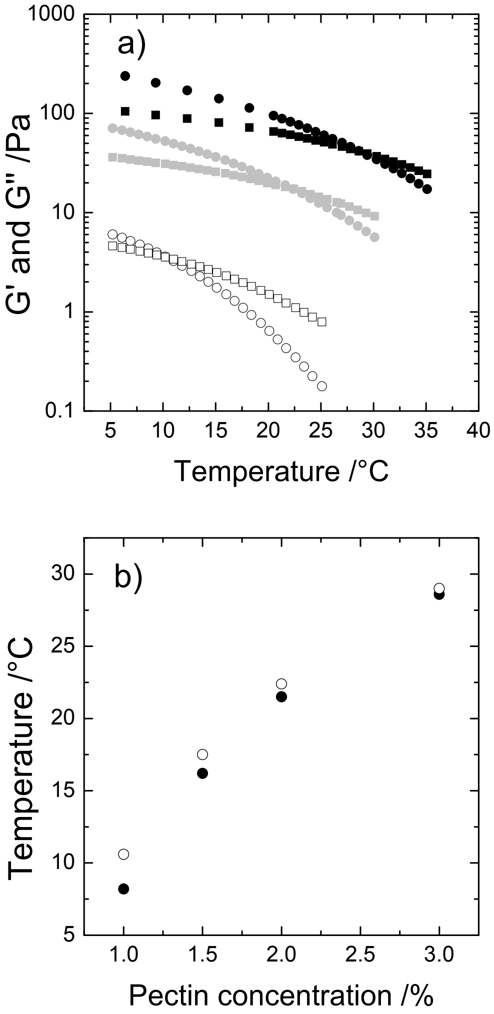

Figure 4. a) G’ (circles) and G’’ (squares) as a function of temperature at a f=6.28rad/s, a strain of 0.5%, T = 5°C and pH = 3.0 where black symbols represent 3%, grey 2% and open 1% pectin. b) melting (filled symbols) and setting (open symbols) temperatures as a function of pectin concentration.

better protection from evaporation by the addition of oil on the surface of the sample, however, this would have required too large quantities of pectin sample than we had access to.

DISCUSSION

Acid pectin gels formed upon slow acidification using glucono-δ-lactone and a pectin of DM 31% form strong gels at 3% pectin and pH <3[4]. A strong gel is here defined as G' > G'' coupled with no frequency dependence of the moduli[14].

The present investigation of the rheological properties of a sample containing a non random pectin with a DM~37% differ from the expected behaviour of a strong gel at pH 3 or lower. The mechanical spectra of the pectin samples containing 2 and 3% pectin suggest that the samples can be characterised as weak gels between the frequency range of 0.02-80rad/s, where a weak gel is defined as G' > G'' but where the slope of log G' and log G'' vary with the frequency[14]. However, the results at lower frequencies suggest that an overlap of G'' > G' occur, which indicates that the samples should be described as entangled solutions, that is, topological interaction of polymer chains show a pseudo gel behaviour[14].

The pre-shear results coupled with the temperature dependence of the system suggest fast aggregations or no aggregations occurring at all, as no pre-shear influence is observed and no or little hysteresis upon temperature sweep (Figure 4). One could hypothesis that a blocky pectin would change its conformation from 2_1 to 3_1 more easily due to larger stretches of stretches of hydrogen bonds within a single polymer chain. This could be supported by the reduction in c* as shown in Figure 1 showing a reduction in c* upon a pH from 5 to 3 for a blocky pectin, which is not observed in the case of a random pectin.

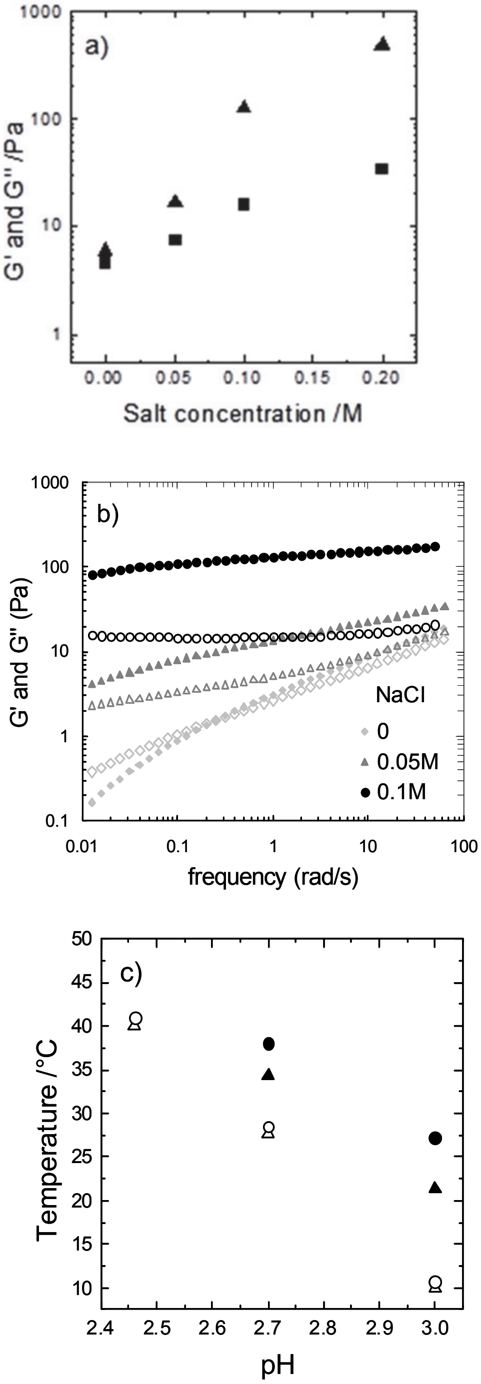

Figure 5. Influence of NaCl concentration on acid pectin samples at pH 3.0 (a) G' (triangles) and G'' (squares) (b) frequency dependency where closed symbols represent G' and open G'' (c) gelation (circle) and melting (triangle) temperatures in the absence of NaCl (open symbols) and in the presence of 0.05M NaCl (solid symbols).

The presence of monovalent ions was found to change the rheological properties of the acid pectin samples considerably. The results clearly show that the samples become closer to strong gels where little frequency dependence of G' and G'' is obtained and where the temperature hysteresis become larger (Figure 5). Furthermore, visual observations showed a sort of lumping of the sample at higher salt concentrations, all signs of aggregations. It is important to note that the presence of the ions alone did not result in any gelation, since tests repeated at higher pH values of 4.0 and 5.0 did not lead to any gel formed, i.e. G''>G' for these systems. Further, addition of urea was found to inhibit gelation of the pectin solutions with and without salt suggesting that hydrogen bonds were the main driving force for the pectin gels both in the absence and presence of the monovalent ions. However, one could argue that the presence of monovalent ions will screen the negative charge and allow the chains to come closer and possibly facilitate aggregations between single chains. This would be in agreement with the relatively little influence on G' that a pH reduction (from pH 3.0 to 2.5) has in the systems where salt is present (and thus where repulsive charges are reduced due to protonisation) compared to the absence of salt.

Further studies should elude whether a strong gel is obtained at pH between 3 and 2 as well as better understanding of potential time influence on the setting of gel and if this could explain the discrepancy found between this study and the one of Gilsenan and co-workers.

CONCLUSIONS

The rheological behaviour of a blocky low-methoxy pectin was studied at low pH (pH 4-2) and in the absence and presence of monovalent ions. This study suggests that pectin samples at low pH (<3.5) do not form strong gels in the sense that the gel consist of disordered flexible polymer chains linking in ordered junction zones. Instead the rheological characterisation of the acid pectin samples shows the characteristics of an entangled polymer solution.

Upon addition of monovalent ions the pectin sample turned into a typical strong gel. Little influence of frequency on the moduli, hysteresis upon temperature sweeps suggesting larger aggregates being formed (also observed visually by the sample turning turbid instead of clear) and occurrence of syneresis at increased salt concentration. It is suggested that the presence of monovalent ions reduce the repulsive charges between pectin samples but that they do not induce salt bridges as the presence of urea reduce the gel strength, suggesting that hydrogen bonds are the driving force for gelation to occur.

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