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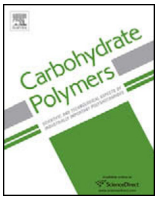

Influence of pH on mechanical relaxations in high solids LM-pectin preparations

K. Alba[a], S. Kasapis[b], V. Kontogiorgos[a,*]

[a] Department of Biological Sciences, University of Huddersfield, Huddersfield HD1 3DH, UK
[b] School of Applied Sciences, RMIT University, City Campus, Melbourne 3001, VIC, Australia

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ABSTRACT

The influence of pH on the mechanical relaxation of LM-pectin in the presence of co-solute has been investigated by means of differential scanning calorimetry, ζ-potential measurements and small deformation dynamic oscillation in shear. pH was found to affect the conformational properties of the polyelectrolyte altering its structural behavior. Cooling scans in the vicinity of the glass transition region revealed a remarkable change in the viscoelastic functions as the polyelectrolyte rearranges from extended (neutral pH) to compact conformations (acidic pH). This conformational rearrangement was experimentally observed to result in early vitrification at neutral pH values where dissociation of galacturonic acid residues takes place. Time–temperature superposition of the mechanical shift factors and theoretical modeling utilizing WLF kinetics confirmed the accelerated kinetics of glass transition in the extended pectin conformation at neutral pH. Determination of the relaxation spectra of the samples using spectral analysis of the master curves revealed that the relaxation of macromolecules occurs within ~0.1 s regardless of the solvent pH.

1. Introduction

Relaxation studies are frequently employed in biopolymer systems as a probe to shed light on macromolecular rearrangements. These motions in turn are pertinent to vitrification that is responsible for the mechanical performance and stability of biopolymer glasses. In the low moisture (or high solids) regime, including those found in dehydrated or frozen foods and low moisture pharmaceutical formulations (*e.g.*, tablets, powders), biopolymers are of particular importance in both academic and industrial fields. To that end, mechanical and thermal relaxations are commonly employed in the study of viscoelasticity for natural polymers and are now well understood so as to draw structure–function relationships (Kasapis, 2012).

Pectic polysaccharides traditionally play a central role as major components in the development of high-solid confectionery products (Al-Ruqaie, Kasapis, Richardson, & Mitchell, 1997; Almrhag et al., 2012; Kasapis, Al-Alawi, Guizani, Khan, & Mitchell, 2000a). Recently, their ability to create systems for controlled delivery of bioactives was also explored (Panyoyai, Bannikova, Small, & Kasapis, 2015). In previous investigations, high methoxylated (HM) pectin from citrus sources was utilized to explore the relaxation properties of the pectin–co-solute systems. The structural simplicity and usually low molecular weight of HM-pectin allows gelation under high solid (>~50%) and low pH conditions (Evageliou, Richardson, & Morris, 2000). Depending on the chemical nature of the sugar employed (monosaccharide *vs.* disaccharide *vs.* polydisperse glucose syrups) the morphology of networks and their viscoelasticity may change dramatically (Kasapis, Al-Marhoobi, Deszczynski, Mitchell, & Abeysekera, 2003a).

To build on previous findings, we increase the structural complexity of the polysaccharide as the fine structure of pectin influences to a great extend its physical behavior (Kim et al., 2013; Strom et al., 2007) and we introduced pectin extracted from okra pods with distinct physicochemical behavior. The major structural elements of pectin include homogalacturonan segments that are mainly composed of α-D-galacturonic acid residues and rhamnogalacturonan-I (RG-I) regions consisting of repeating units of α-D-galacturonic acid and α-L-rhamnose monomers with arabinan and/or galactan side chains (Vincken et al., 2003). In contrast, pectin extracted from okra is distinct as it mostly contains RG-I units and is highly acetylated with low degree of methoxyl substitution (LM-pectin) (Alba, Laws, & Kontogiorgos, 2015; Sengkhamparn et al., 2009a; Sengkhamparn, Verhoef, Schols, Sajjaanantakul, & Voragen, 2009b).

We have previously demonstrated that glassy pectin-matrix tablets slow the kinetics of drug release and furthermore create

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* Corresponding author. Tel.: +44 1484 472488.
E-mail address: v.kontogiorgos@hud.ac.uk (V. Kontogiorgos).

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stable emulsions indicating that they could be exploited in drug or nutrient encapsulation and delivery (Alba, Ritzoulis, Georgiadis, & Kontogiorgos, 2013; Ghori, Alba, Smith, Conway, & Kontogiorgos, 2014). In the present work, we aim to gather further evidence on the influence of macromolecular conformations on the mechanical properties of pectin, as impacted by the degree of ionization in the presence of small molecular weight co-solutes.

2. Materials and methods

2.1. Pectin extraction

Isolation and characterization of pectin samples were carried out according to earlier published protocols. Briefly, okra pods were subjected to an aqueous extraction at pH 6.0, precipitated with ethanol, dialyzed and freeze-dried to obtain dry pectin isolate. Total sugar analysis, protein content and D-GalA content, degree of methylation and acetylation, molecular weight and intrinsic viscosity were all determined, as described in our previous work (Alba et al., 2015). The major physical and chemical characteristics that are relevant to this work are reproduced in Table 1.

2.2. Glucose syrup

Glucose syrup Sipa-Wheat 69 (Sipal Partners, Herve, Belgium) with 80% total solids and dextrose equivalent of 69 was used for sample preparation. The total carbohydrates on dry matter were 97.5% w/w and carbohydrates with degree of polymerization greater than 2 constituted 13.5% w/w of the syrup. Glucose and maltose were 35 and 49% w/w, respectively.

2.3. Sample preparation

Pectin dispersions were prepared by dissolving the isolated polysaccharide at 1% (w/w) in either citric (pH 3.0) or phosphate (pH 7.0) buffers (100 mM) at room temperature under continuous stirring. Following dispersion of the polysaccharide, the temperature was raised to 70 °C and the appropriate amount of glucose syrup was added. The mixture was maintained at 70 °C until the required total solids content was obtained (80% w/w) by slowly evaporating water.

2.4. Rheological measurements

These were performed using a Bohlin Gemini 200HR-nano rotational rheometer (Malvern Instruments, Malvern, UK) equipped with plate-plate geometry (20 mm diameter and 1 mm gap). Temperature was controlled with a Peltier supported by a low temperature ethylene glycol bath (Julabo, F12, Germany) able to reach −30 °C. Experimental protocol of the present investigation included the following steps: Cooling scans were performed between 20 and −30 °C at a cooling rate of 2 °C/min, 0.01% strain and angular frequency of 1 rad/s. To investigate the viscoelastic behavior of the systems and create master curves of viscoelasticity, frequency sweeps were performed within the range of 0.628–62.8 rad/s at a strain of 0.01% with 4.4 °C temperature intervals. Modeling of rheological data was performed on Prism v.6 (Graphpad Software, San Diego, USA).

Table 1
Chemical and physicochemical properties of okra pectin isolate.

D-GalA[a]	56.9 ± 6.9
Degree of methylation (DM%)	24.6 ± 1.0
Degree of acetylation (DA%)	37.6 ± 3.0
Total sugars[a]	81.8 ± 6.4
Protein[a]	6.3 ± 0.1
$M_w \times 10^3$ (g/mol)	767
$[\eta]$ (dL/g)	4.4 (at pH 7.0) or 2.8 (at pH 3.0)

[a] All values are expressed as % on wet basis of the pectin powder.

2.5. Differential scanning calorimetry

Thermal analysis was performed using a Star System DSC1 (Mettler Toledo, Switzerland) with a Huber TC100 cooling system (Huber, Germany) to achieve temperatures down to −90 °C and a nitrogen DSC-cell purge at a flow rate of 50 ml/min. Samples with total solids of 80% (w/w) were weighed (about 15 mg) and hermetically sealed in aluminum pans, which were cooled from 10 to −90 °C at 2 °C/min. First derivative curve of heat flow and estimation of glass transition temperatures were determined with STARe Evaluations software supporting the instrument (v. 12.1, Mettler Toledo, Switzerland).

2.6. ζ-Potential titration

These measurements were performed using a ZetaSizer Nano Series ZEN2600 (Malvern Instruments, Malvern, UK) at 25 °C. Pectin solutions were dispersed at 0.625% w/v in citric buffer (100 mM) at pH 3.0 and titration was performed with 0.75 M sodium hydroxide to pH 7.0 or 0.75 M hydrochloric acid to pH 1.0. All measurements were performed in duplicates.

2.7. Numerical computation

This was performed in MATLAB (v7.0 R14 Service Pack 2, The Mathworks Inc., MA). The first step involves discretization of the viscoelastic functions of G' or G'' to create matrix A and was performed with the *discrG.m* script published elsewhere (Kontogiorgos, 2010). Following that step, algorithms *csvd.m* for calculation of the singular value decomposition of the matrix A and *l_curve.m* for computation of the optimum regularization parameter were used from Hansen's regularization tools package (Hansen, 1994). Finally, the algorithm *NLCSmoothReg.m* was used for the calculation of the relaxation spectra (Wendlandt, 2005).

3. Results and discussion

3.1. Sample characterization and viscoelasticity of pectin–co-solute mixtures

The pectin isolate used in the present investigation is of high purity (low protein concentration and high total carbohydrates) and high molecular weight (Table 1). Intrinsic viscosities were determined at two different pH values (7.0 and 3.0) matching those in sample preparation. These measurements were performed under the electrostatic screening of 0.1 M NaCl. This approach masks non-specific electrostatic interactions so that changes in coil dimensions are attributed to changes in the degree of ionization with variation of buffer pH (citric and phosphate). It is evident that pH plays a decisive role in coil dimensions resulting in an expanded conformation due to dissociation of D-GalA at high pH values (Table 1). It is expected that the high co-solute concentration (glucose syrup) in the samples will have an influence on the conformational properties of pectin as solvent quality changes. It has been reported for β-glucan (Grimm, Kruger, & Burchard, 1995), guar and locust bean gum (Richardson, Willmer, & Foster, 1998) and seed gums (Behrouzian, Razavi, & Karazhiyan, 2014; Mohammad Amini & Razavi, 2012) that intrinsic viscosity reaches a minimum before starting increasing again depending on the sugar type and concentration.

This has been attributed to changes in the solvent quality as sugar concentration varies up to 40% w/w solids, and to the degree