Control of the properties of xanthan/glucomannan mixed gels by varying xanthan fine structure

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A B S T R A C T

The interaction of native xanthan gum, deacetylated xanthan gum and depyruvated xanthan gum with konjac glucomannan has been studied using DSC and controlled stress rheometry. In the absence of electrolyte the DSC cooling curves for native xanthan and deacetylated xanthan showed a single peak and there was a corresponding sharp increase in the storage modulus indicating gel formation. It is apparent that on cooling, association of the konjac glucomannan with the native xanthan molecules is triggered by the xanthan coil–helix transition. In the presence of electrolyte, there were two DSC peaks observed. The higher temperature DSC peak was attributed to the xanthan coil–helix transition while the lower temperature DSC peak was attributed to konjac glucomannan–xanthan association as noted by an increase in the storage modulus. The gels formed were much weaker than those in the absence of electrolyte. The DSC cooling curves for depyruvated xanthan in the absence of electrolyte showed two peaks. The higher temperature peak was attributed to the coil–helix transition while the lower temperature peak corresponded to gelation as noted by an increase in the storage modulus. The gels were very much weaker than for native xanthan gum and deacetylated xanthan gum.

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1. Introduction

Xanthan gum is an exocellular polysaccharide produced by the bacterium *Xanthomonas campestris* and is nowadays very widely used in a broad range of products, including foods, pharmaceuticals, cosmetics, personal care, drilling muds, etc. because of its unique rheological properties (Sworn, 2009; Morris, 2006). Essentially, xanthan gum solutions exhibit a very high viscosity at low shear rates because of weak intermolecular association of the polysaccharide chains. However, the solutions are very shear thinning since the weak intermolecular associations are readily disrupted. The gum is, therefore, widely used to stabilise dispersions and emulsions because of its ability to inhibit particle sedimentation and droplet creaming (Velez, Fernandez, Munoz, Williams, & English, 2003). Xanthan molecules consist of a main chain of (1,4)-β-D-glucose residues with a trisaccharide side chain attached to every other glucose. The side chains are linked through the 3 position and consist of β-D-mannose, (1,4)-β-D-glucuronic acid, and (1,2)-α-D-mannose. The inner mannose may be acetylated and the terminal mannose may be pyruvated. Xanthan molecules have been shown by X-ray fibre diffraction studies to adopt a right-handed helical conformation with five-fold symmetry and a pitch of 4.7 nm (Moorhouse, Walkinshaw, & Arnott, 1977). It has been clearly demonstrated by a range of techniques including, optical rotation, viscosity, light scattering, electron microscopy, DSC, NMR and ESR, that the molecules undergo a conformational transition in solution to form a more flexible disordered state which is favoured at increasing temperature and low ionic strength (Foss, Stokke, & Smidsrød, 1987; Gamini, de Bleijser, & Leyte, 1991; Lui & Norisuye, 1988; Milas & Rinaudo, 1986; Norton, Goodall, Frangou, Morris, & Rees, 1984; Takigami, Shimada, Williams, & Phillips, 1993). There is much controversy reported in the literature as to whether the molecules adopt single helices, double helices or dimers through association of single helical chains in solution. For example, Norton et al. (1984) argued that the molecules adopt a single helical conformation with the side chains packed along the backbone and that ordered and disordered sequences may co-exist within the same molecule. Milas and Rinaudo (1986) also favoured the single helix model and suggested that the molecules can adopt three distinct molecular conformations. In the native state, the molecules are completely ordered and on heating become completely disordered. On further cooling the molecules order but are more expanded than the native state as a consequence of different side chain–backbone interactions. On the other hand Lui and Norisuye (1988) concluded that the ordered structure was a double helix and that on increasing

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the temperature the molecules unwind from both ends forming an expanded dimer joined by a short helical section. Foss et al. (1987) produced electron micrographs showing that the ordered xanthan consists of two associated molecules with sections of single strands at one or both ends. NMR studies by Gamini et al. (1991) provided support for the expanded dimer concept and also indicated that on heating considerable side chain mobility is established before any significant increase in the mobility of the backbone is observed. In the ordered form the side chains are associated with the cellulosic backbone thus stabilising the helical structure, while in the disordered form there is no association with the backbone and the side chains are free to rotate. Further confirmation of the involvement of the side chains in the ordering process has been provided through ESR studies by Takigami et al. (1993) using nitroxide spin labels attached to the carboxylate groups in the side chains. At high temperatures the side chains were found to be highly mobile giving rise to isotropic ESR spectra but on cooling to temperatures below the conformational transition, anisotropic spectra were obtained indicating a significant loss of mobility. The temperature of the conformational transition is dependent on the amount of acetyl and pyruvate groups present (Callet, Milas, & Rinaudo, 1987; Cheetham & Mashimba, 1992; Shatwell, Sutherland, Dea, & Ross-Murphy, 1990). It has been shown that it shifts to lower temperatures by removal of acetyl groups but increases to higher temperatures on removal of pyruvate groups. The acetyl groups on the side chains are able to interact with the cellulosic backbone through hydrogen or hydrophobic bonding thus promoting the ordered structure. The destabilising effect of the pyruvate groups has been attributed to the increase in electrostatic charge repulsion as a result of the side chains folding in on the backbone.

It is well known that xanthan gum will form thermoreversible gels in the presence of certain galactomannans, namely locust bean gum and tara gum and also with glucomannans (konjac mannan) (Abbaszadeh & Foster, 2012; Annable, Williams, & Nishinari, 1994; Callet et al., 1987; Cheetham & Mashimba, 1992; Dea et al., 1977; Dea & Morrison, 1975; Fitzsimons, Tobin, & Morris, 2008; Wielinga, 2009; Williams, Clegg, Day, Phillips, & Nishinari, 1991; Williams, Day, Langdon, Phillips, & Nishinari, 1991; Williams & Phillips, 1995). The galactomannans consist of linear chains of β-(1,4)-mannose residues with galactose residues linked at the 6 position (Takigami, 2009). The mannose to galactose ratio is 3:1 for tara gum and 4:1 for locust bean gum. Glucomannans consist of linear chains of β-(1,4)-glucose and mannose residues with branches consisting of up to 16 sugar units linked to the 3 position on the main chain (Nishinari, Williams, & Phillips, 1992). There is approximately one branch every 10 residues along the chain. The mannose to glucose ratio is 1.6:1. The molecules also contain acetyl groups (approximately 1 per 17 sugar residues). A common feature of galactomannans and glucomannans with respect to their interaction with xanthan gum is that they adopt an extended conformation in solution. The thermoreversible gels formed by mixing xanthan gum with tara gum, locust bean gum or konjac glucomannan are optically clear and highly elastic and have considerable commercial importance. The mechanism of the gelation process has been a matter of much controversy and a detailed review has been published previously (Williams & Phillips, 1995). It is clear that the main body of evidence indicates that gelation occurs as a consequence of the molecular association of xanthan molecules with galacto- and gluco-mannan chains. Brownsey, Cairns, Miles, & Morris (1988) studied the mixed systems using X-ray diffraction and the X-ray fibre pattern obtained provided direct evidence of intermolecular binding. Since gels were only formed when solutions were mixed at temperatures above the xanthan coil–helix transition, binding was assumed to involve the disordered xanthan chains. Dea and Morrison (1975), however, proposed that association involved the ordered xanthan helices. More recent work by Fitzsimons et al.

(2008) provides further evidence that the association does not require xanthan to be in the disordered form. These workers, however, demonstrated that stronger gels are formed after heating to 95 °C [i.e. above the temperature of the coil helix transition] and then cooling to 20 °C than those obtained on mixing at 20 °C. This is in agreement with our own previous work using a combination of DSC, rheology and ESR techniques which demonstrated that the molecular association between xanthan and galacto- and gluco-mannans only occurs at temperatures at or below the xanthan conformational transition (Annable et al., 1994; Williams & Phillips, 1995; Williams, Day, et al., 1991). A number of workers (Abbaszadeh & Foster, 2012; Shatwell, Sutherland, Ross-Murphy, & Dea, 1991; Tako, Asato, & Nakamura, 1984) have shown that the interaction of xanthan with galacto- and gluco-mannans is dependent of the degree of acetylation and pyruvation of the xanthan molecules. Deacetylated xanthan has been found to produce much stronger gels than native or depyruvated xanthan molecules. This study sets out to gain a further insight into the mechanism of gelation of konjac glucomannan/xanthan gum mixed systems using xanthan, deaceylated xanthan and depyruvated xanthan with a view to controlling the thermal and rheological properties of the mixed gels.

2. Materials and methods

2.1. Xanthan gum

A commercial sample of xanthan gum was dialysed against deionised water and the metal ion content determined using Atomic Absorption. It was found to contain 0.021% Na, 0.029% K and 0.002% Ca. The acetyl content was determined by alkaline hydrolysis as follows: Nitrogen gas was passed through 100 mL 0.5% (w/w) xanthan solution for 10 min and 3 mL of 1 M KOH added. The flask was stoppered and left for 2 days at room temperature with intermittent stirring. 30 mL 0.05 H_2SO_4 was added and the excess acid was titrated with 0.01 M KOH using phenolphthalein indicator. It was found that 93% of the side chains were acetylated. The pyruvate content was determined using the method reported by Sloneker and Orentas (1962) and it was found that 25% of the side chains contained a pyruvate group.

2.2. Deacetylated xanthan gum

The commercial sample reported above was deacetylated using the method described by Tako and Nakamura (1984).

2.3. Depyruvated xanthan gum

A sample of depyruvated xanthan was provided as a gift and it was found that 90% of the side chains were acetylated and 7% contained a pyruvate group.

2.4. Konjac glucomannan (KM)

A commercial sample of hydroprocessed konjac glucomannan was obtained and was used as received. It was found to contain negligible amounts of metal ions and had a molecular mass of 480 kDa and acetyl content of 1 per 17 sugar units.

2.5. Differential scanning calorimetry

Measurements were performed using a Seteram micro DSC fitted with 1 mL sample vessels. The polymer solution was accurately weighed into the sample cell and the reference cell was filled with the same weight of solvent. Instrumental baselines were determined with both the sample and reference cells filled with solvent