Food Hydrocolloids xxx (2014) 1–7

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Comparative heterogeneity, molecular weights and viscosities of xanthans of different pyruvate and acetate content

Tayyibe Erten [a], Gary G. Adams [a,b], Timothy J. Foster [c], Stephen E. Harding [a,*]

[a] University of Nottingham, National Centre for Molecular Hydrodynamics, Sutton Bonington, LE12 5RD England, UK
[b] University of Nottingham, School of Health Sciences, Queen's Medical Centre, Nottingham, NG7 2HA England, UK
[c] University of Nottingham, Division of Food Sciences, Sutton Bonington, LE12 5RD England, UK

ARTICLE INFO

Article history:
Received 3 February 2014
Accepted 2 April 2014
Available online xxx

Keywords:
Xanthan
Pyruvate
Molecular weight
Ultracentrifugation

ABSTRACT

Introduction: The bacterial exopolysaccharide xanthan is a well-known hydrocolloid, with a high viscosity deriving from its large molecular weight and volume.
Materials and methods: Four different xanthans deriving from *Xanthomanas campestris* – two with similar pyruvate and acetate contents and two with different contents – were characterized in dilute aqueous buffered solution (pH 7.0, ionic strength 0.3 M) using sedimentation velocity and sedimentation equilibrium in the analytical ultracentrifuge, supplemented by dynamic light scattering.
Results: This facilitated a comparison with regards heterogeneity (sedimentation coefficient distribution) and molecular weight, despite their large size and low critical overlap concentration. The xanthans were also compared with regard to reduced specific and intrinsic viscosity behaviour. The xanthans generally show strong similarity in properties with the exception of the lowest pyruvate xanthan, a finding which should be useful for future applications of these materials.

© 2014 Published by Elsevier Ltd.

1. Introduction

Xanthan is an anionic, extracellular microbial polysaccharide produced by *Xanthomanas campestris* (Berth et al., 1996; Khouryieh, Herald, Aramouni, & Alavi, 2007; Rodd, Dunstan, & Boger, 2000). The primary structure of xanthan consists of a linear β-(1 → 4) D-glucan backbone with branched trisaccharide chains containing D-glucuronic acid unit between two D-mannoses. These trisaccharide chains are linked to a backbone at C-3 on every second unit. Approximately half of the terminal D-mannose units contain a pyruvic acid residue linked via a keto group to the 4 and 6 positions, whose distribution patterns are beginning to be described (Kool, Gruppen, Sworn, & Schols, 2014).

The D-mannose linked to the main chain can also be acetylated group at position C-6 as shown in Fig. 1 (see, e.g. (Tombs & Harding, 1997)). The effect of pyruvate and acetyl substituents on rheological properties both in the presence of supporting electrolyte and also without, has been the subject of considerable previous studies, and with somewhat contradictory conclusions. Some researchers have claimed that the acetyl and pyruvate groups may have a significant effect on viscosity (see, for example, (Cheetham & Nik Norma, 1989; Sandford et al., 1977; Smith, Symes, Lawson, & Morris, 1981; Tako & Nakamura, 1984)), whereas other groups claim the opposite (Bradshaw, Nisbet, Kerr, & Sutherland, 1983; Callet, Milas, & Rinaudo, 1987; Shatwell, Sutherland, & Ross-Murphy, 1990). Khouryieh et al. (2007) concluded that acetate and pyruvate substituents can affect viscosity depending on temperature, salt concentration and sample concentration and that they play an important role on the conformation of xanthan.

In this study, we seek to throw some light on this rather uncertain state of affairs from a hydrodynamics perspective, focussing on the effect of molecular weight and pyruvate content under properly controlled solution conditions. Four commercial xanthan samples, were studied, two with identical degrees of pyruvalation ("Standard pyruvate" –3.9%) but different molecular weights, and two further ones of different degrees of pyruvalation :"High" (6.5%) and "Low" (2%) were characterized in dilute aqueous buffered solution (pH 7.0, ionic strength 0.3 M) using high-precision viscometry, sedimentation velocity, sedimentation equilibrium and dynamic light scattering (DLS), facilitating a rigorous comparison with regards heterogeneity (sedimentation coefficient distribution), molecular weight and conformation (translational frictional ratio). Resolution of the controversy is deemed important because of the huge commercial significance of xanthan in the food and biopharmaceutical industries.

* Corresponding author.
E-mail address: steve.harding@nottingham.ac.uk (S.E. Harding).

http://dx.doi.org/10.1016/j.foodhyd.2014.04.012
0268-005X/© 2014 Published by Elsevier Ltd.

Please cite this article in press as: Erten, T., et al., Comparative heterogeneity, molecular weights and viscosities of xanthans of different pyruvate and acetate content, *Food Hydrocolloids* (2014), http://dx.doi.org/10.1016/j.foodhyd.2014.04.012

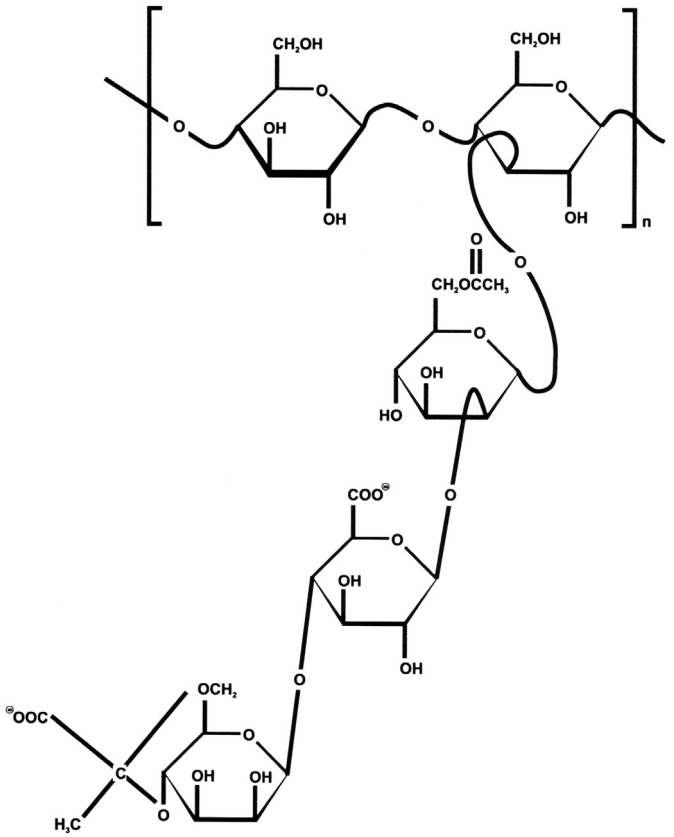

Fig. 1. Chemical structure of part of a xanthan molecule showing the beta glucan backbone and side chain with an acetate on the first branched sugar residue (mannose) the terminal mannose residue pyruvalated.
Adapted from Tombs & Harding 1997.

A major problem however in the characterization of xanthans is their very large size compromising the use of size exclusion chromatography based procedures, the low value of the "critical overlap concentration" c^* and large thermodynamic non-ideality of these substances. However we can now take advantage of some recent and relevant advances in the analysis of polymeric materials by analytical ultracentrifugation (Abdelhameed et al., 2010; Dam & Schuck, 2004; Schuck et al., 2014).

2. Materials and methods

2.1. Xanthans

Xanthan samples with standard (low and high molecular weight – "*Standard LMW*", and "*Standard HMW*" respectively), low and high pyruvate contents "*Low*" and "*High*" respectively were supplied by Dupont (Paris). The two standard samples had been shown to have the same pyruvate content (3.9%), as determined by chemical analysis, with the high and low pyruvate samples having 6.5% and 2% respectively. The proportions of acetate as determined had also been evaluated and given in Table 1.

Stock solutions of xanthan were prepared by dissolving a known amount in distilled deionised water, at room temperature with gentle stirring at 3 h followed by overnight dialysis against phosphate-chloride buffer supplemented with NaCl according to Green (1933) at pH 7.0 and an ionic strength I of 0.3 M. The concentrations, c (g/ml) of the stock xanthan solutions were measured after dialysis using an Atago (Japan) DD-7 refractometer calibrated with glucose standards, and a refractive increment dn/dc of 0.15 ml/g (Berth et al., 1996).

Table 1
Pyruvate and acetate content of the xanthans.

Xanthan	Pyruvate (%)	Acetate (%)
Standard LMW	3.9	6.2
Standard HMW	3.9	6.2
High	6.5	5.1
Low	2.2	6.0

2.2. Sedimentation velocity analytical ultracentrifugation

An Optima XL-I (Beckman Instruments, Palo Alto, USA) equipped with Rayleigh interference optics was used to determine the sedimentation behaviour and molecular homogeneity of xanthan samples. Solution concentrations ranging between 0.05 and 0.225 mg/ml for each xanthan were studied at a temperature of (20.0 ± 0.1) °C. 400 μl of solution and an equal volume of the pH 7.0, I = 0.3 M reference buffer (400 μl) were injected into the solution and solvent channels of 12 mm optical path length double sector cells. The Rayleigh interference optical system was used to record changes in the concentration versus radial displacement profiles with time. Scans were taken at 2 min intervals for a run time of approximately 18 h at 30,000 rpm. A partial specific volume, $\bar{\upsilon}$ of (0.602 ± 0.010) ml/g was used for the xanthans (Anderson, 1991; Dhami et al., 1995). For comparison purposes, sedimentation coefficients were normalised to standard solvent conditions, namely the density and viscosity of water at 20.0 °C (Abdelhameed, 2010; Laue and Stafford III, 1999):

$$s_{20,w} = \{(1-\bar{\upsilon}\rho_{20,w})/(1-\bar{\upsilon}\rho_0)\}\cdot\{\eta_0/\eta_{20,w}\}s_{T,b} \quad (1)$$

where ρ_o and η_o are respectively the density and viscosity of the solvent or buffer, b, at temperature T and $\rho_{20,w}$ and $\eta_{20,w}$ the corresponding values of water at 20.0 °C. The data was analysed using the "least squares ls-g(s) model" SEDFIT algorithm of P. Schuck and colleagues (Dam & Schuck, 2004) in terms of distributions of sedimentation coefficient distribution (see also (Harding, 2005c)). The ls-g(s) *vs.* s profiles were used to provide an assessment of sample polydispersity. The s and $s_{20,w}$ values are 'apparent' values because of non-ideality. To remove this effect, $s_{20,w}$ is extrapolated to zero concentration and $s^\circ_{20,w}$ is obtained from reciprocal plot of $s_{20,w}$ ($1/s_{20,w}$) *vs.* concentration (see for example, (Harding, 2005c; Laue & Stafford III, 1999; Tanford, 1961)):

$$1/s_{20,w} = (1/s^\circ_{20,w})\cdot(1+k_s c) \quad (2)$$

where $s^\circ_{20,w}$ is sedimentation coefficient at zero concentration and standard condition, k_s is the Gralen parameter (Gralen, 1944); see also (Harding, 2005a, 2005b; Morris et al., 2001).

2.3. Sedimentation equilibrium analytical ultracentrifugation

Sedimentation equilibrium experiments were also performed using the Beckman (Palo Alto, California, U.S.A.) Optima XL-I analytical ultracentrifuge and employing Rayleigh interference optics and an automatic on-line data capture system. The modified long (20.0 mm) optical path length double-sector titanium cells with sapphire windows were loaded with (~120 μl) of dialysed sample and a matching amount of reference buffer dialysate in appropriate channels. The balanced cells were then loaded into an analytical 8-hole titanium rotor An50-Ti and placed in the AUC. After time was allowed for vacuum formation (≤50 microns) and for temperature equilibration (20.0 °C), the rotor was then accelerated to 2000 rpm; rotor speeds were selected to be sufficiently apart to give good molecular weight distributions (Scott & Schuck,

Please cite this article in press as: Erten, T., et al., Comparative heterogeneity, molecular weights and viscosities of xanthans of different pyruvate and acetate content, *Food Hydrocolloids* (2014), http://dx.doi.org/10.1016/j.foodhyd.2014.04.012

Download English Version:

https://daneshyari.com/en/article/10375833

Download Persian Version:

https://daneshyari.com/article/10375833

Daneshyari.com