International Journal of Biological Macromolecules xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Influence of aggregation on characterization of dilute xanthan solutions

Arturo Merino-González, Anna Kozina*

Instituto de Química, Universidad Nacional Autónoma de México, P.O. Box 70-213, 04510 Mexico City, Mexico

ARTICLE INFO

Article history:
Received 9 May 2017
Received in revised form 10 July 2017
Accepted 17 July 2017
Available online xxx

Keywords:
Xanthan
Dilute solutions
Aggregation
Viscosity
Light scattering

ABSTRACT

Xanthan is an extracellular polysaccharide of polyanionic nature widely used in industrial processes as flow modifier. Its characterization in dilute solutions is complicated by the strong tendency to aggregation. We explore the possibility to obtain dilute xanthan solutions without aggregates. We applied some steps of the sample preparation procedures from previous works on xanthan, such as ultrasonication, heating and micro filtration. The influence of this type of treatment on the observed properties of xanthan 0.1 M NaCl aqueous solutions is studied. Renaturalization of xanthan solutions above the overlap concentration does not break the aggregates but, on the opposite, produces the ones that are more resistant to ultrasound. Ultrasonication breaks only large aggregates and at long sonication times brings the risk of the single chain degradation. The best results are provided by a procedure that combines a short ultrasonication time followed by micro filtration but it is impossible to obtain a solution completely free of small aggregates by conventional sample preparation methods. Nevertheless, a significant reduction of large aggregates results in a linear concentration dependence of xanthan reduced viscosity, which allows more confident determination of the intrinsic viscosity. Another advantage of large aggregates removal is a possibility of physical interpretation of xanthan molecular parameters by static light scattering, taking into account its association tendency.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction

Xanthan is a microbial polysaccharide widely used in food, pharmaceutical and cosmetic industry to control flow properties of products. Its primary structure contains a cellulose backbone composed of β-(1→4)-D-glucose units with trisaccharide side chains made of (3→1)-α-D-mannopyranose-(2→)-β-D-glucuronic acid-(4→)-β-D-mannopyranose [1]. Up to two carboxyl groups and an acetate group can be present on each side chain making xanthan an anionic polyelectrolyte. The substitution degree (a fraction of substituted sites per side chain) may vary with the conditions of bacterial production as well as with the posterior treatment [2]. The secondary structure of xanthan is mainly caused by the presence of a large number of hydroxyl groups that are able to form hydrogen bonds and depends on solution temperature and ionic strength [3,4]. At room temperature and at high ionic strength the secondary structure of native xanthan is an ordered helix. More exact helical structure is controversial, some of the reports suggest a single stranded helix [4,5], while the majority of others suggest a double helix [6–12], the latter is taken into account in the present work. On heating, order–disorder transition occurs with partial or complete dissociation of double helices [13]. This disordered conformation corresponds to denatured xanthan. On cooling, the double helices are paired but the process is not completely reversible and the native conformation is not recovered but rather altered resulting in renatured xanthan. The substitution degree affects order-disorder transition: acetate groups favor ordered while pyruvate groups favor disordered conformation [14–17].

Characterization of dilute xanthan solutions pursues the study of macromolecule conformational changes on variation of external conditions, such as temperature, ionic strength, pH, solvent, etc. However, as the majority of other polysaccharides, xanthan is very prone to association. High molecular weight samples ($M > 10^6$ g/mol) contain a fraction of insoluble polymer or microgel. These aggregates contribute to the viscosity of dilute solutions and thus, the measured intrinsic viscosity does not reflect the size and conformation of a single molecule. Light scattering technique is even more affected, since it is extremely sensitive to any minor presence of large objects. Thus, the aggregates may call into question the parameters determined for single molecules by light scattering or their reproducibility.

* Corresponding author.
E-mail address: akozina@unam.mx (A. Kozina).

http://dx.doi.org/10.1016/j.ijbiomac.2017.07.110
0141-8130/© 2017 Elsevier B.V. All rights reserved.

To tackle this difficulty, a special emphasis on solution preparation must be put. There is no accepted standard procedure for sample preparation and every research group chooses the variant that works best for the particular purpose. This is one of the reasons why sometimes the reports are difficult to compare or reproduce. Additionally, the substitution degree is not always reported, which makes the comparison even more complicated. There is a variety of ways of sample preparation. The following steps are mentioned in the literature. The polymer may be only dissolved by stirring for a certain time without further treatment [10,11,18–20], the solutions may be filtered [5,9,21,22], ultrasonicated and fractionated [23], ultrasonicated and filtered [8,16], ultracentrifuged [24] or heated under high pressure and filtered [25]. The list is not exhaustive. Nevertheless, it is a difficult task to choose the 'correct' sample preparation procedure for someone new to the field or for routine characterization in a lab.

Since xanthan associates with time, even after careful removal of large aggregates, the self-assembly process continues. With time it results in the formation of new aggregates. It is not known to what degree these aggregates contribute to the observed physical properties of dilute xanthan solutions. Therefore, it is necessary to obtain more information on how the presence of aggregates alters the observed polymer viscosity and light scattering. Therefore, in the present work we explore the problem of aggregates involved in characterization of dilute xanthan solutions and how its partial removal is reflected in the dilute solution properties. We also explore to what extend of aggregate presence the characterization techniques provide physically meaningful results. Thus, we adopted some of sample preparation steps typically used to remove aggregates from commercially available samples. We investigated how these steps affect the observed dilute xanthan solution properties. We analyzed the efficiency and the limitations of each step for elimination of aggregates. We suggest a protocol of sample purification which combines the known steps in a correct order to remove the majority of large aggregates. This allows sample characterization by both rheometry and light scattering in a reproducible way. The heat treatment and its effect on polymer association is also discussed.

2. Materials and methods

2.1. Materials

Commercial xanthan gum was supplied by Sigma-Aldrich (G1253, USA). According to the manufacturer, the polysaccharide is produced from a fermentation broth that is then pasteurized and the polysaccharide is precipitated in alcohol, recovered and milled. The moisture content was determined according to the standard procedure [26] and is 10.78%. Sodium chloride and absolute ethanol were purchased with Sigma-Aldrich and J.T. Backer (USA), respectively. Deionized water (Milli-Q, resistivity $\rho = 18.1\ M\Omega{\cdot}cm$) was used.

2.2. Sample preparation

The two types of preparation procedure were studied as described further, Fig. 1.

2.2.1. Preparation 1

In the first preparation process initial 'native' xanthan solution with concentration of 0.001 g/mL was prepared by dissolution of the polymer as received from the supplier in water at 25 °C for 1 h at vigorous stirring. Then, the dry sodium chloride was added to the polymer solution in such amount that its concentration in the solution was 0.1 M, step (1). These samples are named for convenience 'native' (NT) xanthan, although the heat treatment by manufacturer should be borne in mind. After that, 'renatured' xanthan samples (RN) were prepared by heating of NT xanthan stock solution at 90 °C for 40 min followed by slow cooling to the room temperature, step (2). Later on, both NT and RN xanthan samples were subjected to an ultrasonic treatment for 30 min, 1, 2 or 3 h, steps (3) and (4). The sonication process was performed in the following way. First, the stock solutions with xanthan concentration $C = 0.001$ g/mL were centrifuged at 2000 rpm for 10 min to eliminate air bubbles. Then, the samples were placed in an ultrasonic bath (Elma-Sonic S30H, Germany) operating at a fixed ultrasonic frequency of 37 kHz and sonicated for a given time at 25 °C maintaining the constant bath temperature with addition of ice. After that, the dilutions from the stock solution were made in the range of xanthan concentrations between 0.0001 and 0.0005 g/mL.

2.2.2. Preparation 2

In the second preparation procedure, NT xanthan sample with the polymer concentration of 0.001 g/mL was prepared in 0.1 M NaCl by dilution of the solid polymer in water and then addition of the salt in the same way as described above (1 h of stirring), step (1). Then, 30 min or 1 h of ultrasonication was applied, step (2). Then, the sonicated solution was filtered with a nylon syringe filter with pore diameter of 0.45 μm, step (3). The polymer in the filtered solution was precipitated in absolute ethanol, step (4) and the solid content was separated and dried in vacuum at 30 °C for 48 h, step (5). At this stage the polysaccharide is already purified from the aggregates, so it is named 'native' purified xanthan (NTP). To prepare the samples for measurements, the stock solution of NTP xanthan in 0.1 M NaCl with concentration of 0.001 g/mL was made by direct dilution in water followed by the salt addition (1 h stirring), step (6), and then xanthan was renatured in already mentioned way, step (7), resulting in purified renatured xanthan PRN.

2.3. NMR structure characterization

Solution-state ^1H NMR spectra of xanthan without any treatment (NT) were recorded with an Avance III HD spectrometer (Bruker, Germany) at 500 MHz in D_2O as a solvent at 25, 50 and 80 °C. Xanthan was directly dissolved in deuterium oxide at concentration of 0.005 g/mL by stirring overnight. After that, sodium acetate (AcNa) was added as an external standard to the sample so that its concentration was 3×10^{-3} mol/L. All the chemical shifts were referred to the standard with $\delta = 1.9$ ppm. Xanthan acetate and pyruvate substitution degree (SD) was calculated as follows [27]:

$$SD_{ac} = \frac{829N_{ac}}{1 - 92N_{pyr} - 42N_{ac}} \quad (1)$$

and

$$SD_{pyr} = \frac{829N_{pyr}}{1 - 92N_{pyr} - 42N_{ac}}, \quad (2)$$

where N_{ac} and N_{pyr} are the numbers of the corresponding groups (acetate or pyruvate) per gram of polysaccharide in mol/g, 829 is the molar mass of one xanthan repeating unit without acetate or pyruvate, 42 is the molar mass of an acetate group and 92 is the molar mass of a pyruvate group. N_{ac} and N_{pyr} can be determined as:

$$N_{ac} = \frac{A_{ac}}{A_{st}} \cdot \frac{C_{st}}{C_{xt}} \quad (3)$$

and

$$N_{pyr} = \frac{A_{pyr}}{A_{st}} \cdot \frac{C_{st}}{C_{xt}}, \quad (4)$$

where A_{ac}, A_{pyr} and A_{st} are the areas below the signals corresponding to protons of xanthan acetate, pyruvate and the standard AcNa,

Please cite this article in press as: A. Merino-González, A. Kozina, Int. J. Biol. Macromol. (2017), http://dx.doi.org/10.1016/j.ijbiomac.2017.07.110

Download English Version:

https://daneshyari.com/en/article/8329637

Download Persian Version:

https://daneshyari.com/article/8329637

Daneshyari.com