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On the percolation of alginate/calcium systems at low concentrations

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ABSTRACT

The sol-to-gel transition of an alginate rich in β-D-mannuronic acid residues and at a concentration of 0.1% w/v in 15 mM NaCl in the presence of calcium ions of 0 to 3.5 mM was studied with dynamic light scattering. The dynamics of the different systems added further insight into the alginate gel forming mechanisms. Below a Ca^{2+} concentration of 0.7 mM, the build-up of small aggregates could be verified. Moreover, at a critical concentration, close to 0.9 mM Ca^{2+}, a percolated, non-ergodic network started to form from some of these aggregates, with smaller aggregates still diffusing in the network. The system displayed strong non-ergodicy at high Ca^{2+} concentrations with a non-ergodicity parameter that appeared to form discontinuously from near zero to a clearly non-zero value at the critical Ca^{2+} concentration.

1. Introduction

Gel forming materials based on polysaccharides are used as passive ingredients to provide, e.g. a desired texture and tactile perception of foods, and as functional materials reacting to external stimuli, e.g. in pharmaceutical formulations (Whistler & BeMiller, 2003; Augst, Kong, & Mooney, 2006). However, for rational product development it is necessary to understand the mechanisms behind the sol-to-gel transition and how a network with controlled properties can be formed under specific conditions. The gel forming mechanisms for polysaccharides depend on the type of polymer and they range from *entangled systems* in e.g. hydroxypropyl methylcellulose and *reversible physically cross-linked systems*, e.g. alginates and carrageenans to *irreversible chemically cross-linked systems*. It is well known that these systems are very different on a microstructural scale and that they are structurally quite heterogeneous (McClements, 2007).

Alginates are linear ionic polysaccharides derived from brown seaweeds and consist of residues of (1,4)-linked β-D-mannuronic (M) and α-L-guluronic (G) acid in blocks and/or as MG-constituents. The polymer and its gel-forming mechanisms under different conditions have been well documented in the literature (Smidsrod & Haug, 1965). Based on circular dichroism studies (Grant, Morris, Rees, Smith, & Thom, 1973) it has long been generally accepted that ionotropic gelation of alginate takes place by a mechanism first described by the so-called egg-box model. According to this model, paired G-sequences form cavities to accommodate cations, e.g. Ca^{2+}, forming junction zones of two or more laterally associated chains. The G-sequences are more susceptible to calcium binding compared to M-sequences.

Later studies have led to further refinement of this model, providing more insight into the association steps involved in the sol-to-gel transition. For instance, also the M-blocks have been found to exhibit an affinity towards binding calcium, especially when their neighboring groups are G-blocks as in MGM-sequences (Wang, Zhang, Konno, & Saito, 1993, and Donati et al., 2005). Also, alginates with high mannuronic content in the presence of monovalent cations (Karakasyan et al., 2010) form thermotropic gels. The most stable junction zones appear to be formed by dimerization (Morris, Rees, Thom, & Boyd, 1978; Wang et al., 1993; Wang, White, Konno, Saito & Nozawa, 1995), although lateral association of G-blocks seems to be dominating at high calcium concentration and degree of polymerization of the G-blocks (Stokke et al., 2000). A multiple step mechanism for the binding of calcium was proposed by Fang et al. (2007). The process was suggested to take place in three steps viz. the formation of monocomplexes, dimerization, and finally lateral association. A similar suggestion was made by Zhao, Hu, Evans, and Harris (2011) who presented a theoretical phase diagram in very good agreement with experiments. The phase diagram predicts that gels are formed also at very low concentrations of alginate and calcium. In a ^{13}C NMR it was shown that the polymer dynamics slows down upon binding of calcium leading eventually to a gel network (Wang et al., 1993). Rheology and atomic force microscopy (AFM) reveal that both individual polymers and

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aggregates of polymer co-exist in the gel state (Jorgensen, Sletmoen, Draget, & Stokke, 2007; Funami et al., 2009). At low calcium concentrations, associations between G- and MG-blocks are largely responsible for the gelation while at high calcium concentration G-blocks are more common (egg-box dimers). The two different association modes lead to the formation of microgels in solution at low calcium concentrations and an ordered, percolating fiber-like structure at high calcium concentrations. The importance of the size of species in solution prior to gelation was discussed by Lu, Liu, Tong, and Gao (2006), who showed how different molecular weights of the alginate dictate whether scaling of rheological parameters according to a percolation model is obtained or not. A comprehensive review of alginate production, properties and use has been given by Draget, Smidsrod, and Skjak-Braek (2005).

Here we study the dynamics of small species in different polysaccharide systems and more specifically how it is influenced by the microstructure and dynamics of the polymers. We use dynamic light scattering (DLS) to follow changes in the dynamics at low concentrations (0.1% w/v alginate, 0–3.5 mM Ca^{2+}). By DLS we study non-ergodic properties of the network and we distinguish between different types of relaxation of both free and aggregated species.

2. Experimental

2.1. Materials and sample preparation

An ultrapure sodium alginate sample with stated average molecular weight of 250 kD and ratio M/G > 1 was purchased from FMC BioPolymer, Norway (Pronova UP MVM, 4200301). D-Glucono-δ-lactone (GDL) and calcium carbonate (99.999%) was purchased from Sigma-Aldrich Co. An alginate stock solution was prepared with a polymer concentration of 4 mg/mL by dissolving the alginate powder in 15 mM NaCl(aq) while gently stirring. The water was of Milli-Q quality (Millipore). The solution was then heated to 70 °C for 1 h while stirring and then slowly cooled to room temperature and continuously stirred overnight. The NaCl solution was degassed and filtered through a 0.1 μm Sartorius Minisart filter prior to use. Homogeneous calcium alginate systems were prepared by filtering through a 0.45 μm Life Science filter followed by mixing the alginate stock solution (with a calcium carbonate suspension of appropriate concentration in 15 mM NaCl(aq), followed by the addition of slowly hydrolyzing GDL while vigorously shaking the sample. The concentration of GDL was always kept twice that of the calcium carbonate in accordance with the procedure described in literature (Draget, Ostgaard, & Smidsrod, 1990). It was verified that the filter sizes used did not influence the concentrations of the different chemicals by monitoring of the refractive index increment using a differential refractometer (PN3120 dndc, Postnova, Germany). The final alginate concentration was 1 mg/mL for all studies and the calcium concentration was varied from 0.5 mM to 3.5 mM. Solutions of alginate without GDL and calcium were prepared from the stock solution and diluted with the degassed and filtered NaCl(aq) to give a final concentration of 1 mg/mL alginate in 15 or 50 mM NaCl(aq) and finally treated the same way as the calcium systems. Importantly, care has been taken to allow for a slow introduction of Ca^{2+} ions and samples have been left to equilibrate for a sufficient period of time to minimize aging effects. This leads to a more reliable analysis of the dynamics in that any parameters extracted in the analysis can be regarded as essentially independent of time. In particular, this enables an unambiguous determination of the non-ergodicity parameter for this gelation scenario.

All samples used were freshly prepared and transferred to 10 mm light scattering cells (Hellma GmbH, Germany) or NMR-tubes (Armar AG, Switzerland) and kept in an incubator at 23 ± 0.5 °C for five days before the measurements. A 2% w/v alginate solution was prepared for NMR experiments following the procedure described by Grasdalen, Larsen, and Smidsrod (1979).

2.2. Light scattering instruments

Dynamic light scattering (DLS) experiments were carried out with an ALV-goniometer system (ALV-CGS3, ALV-GmbH, Langen/Germany) with an ALV-multiple tau correlator (shortest sampling time = 0.125 ns, maximum number of channels = 288) and an ALV rotation unit. The instrument is equipped with a HeNe laser (22 mW output power) operating at a wavelength of 632.8 nm. A Glan-Thompson prism in vertical position was used before the ALV avalanche photo diode with respect to the scattering plane to give a vertical/vertical configuration. All experiments were carried out at 23 ± 0.05 °C and at a detection angle of 90° after one hour of thermally equilibrating the sample in the sample holder. The value of the q-vector, defined as

$$q = \frac{4\pi n}{\lambda} \sin\left(\frac{\theta}{2}\right) \quad (1)$$

where n is the refractive index of the solvent, λ is the wavelength in vacuum of incident light, and θ is the angle of detection, was determined by the ALV software and corresponded to the detection angle of 90°.

2.3. Light scattering methodology and data analysis

Several different methods to sample DLS data from non-ergodic systems have been described in literature (Pusey & van Megen, 1989; Joosten, Gelade, & Pusey, 1990; Joosten, McCarthy, & Pusey, 1991; Geissler, Hecht, Horkay, & Zrinyi, 1988; Xue, Pine, Milner, Wu, & Chaikin, 1992; Sellen, 1987, 1993), some of which were compared by Schätzel with respect to accuracy (Schätzel, 1993). In this work we have used three of these methods to obtain different correlation functions as function of time, t. To avoid too much light exposure to the detector, an acceptable attenuator aperture regulating the amount of light was first found by manually rotating the cuvette step-wise through many positions. The same attenuator setting was then used in all three types of experiments.

(I) The method by Xue et al. (1992), in which the cuvette is continuously rotated while sampling the data, was used. In this way the obtained time-averaged correlation function $g_T^{(2)}(q,t)$ is equal to the ensemble-averaged function $g_E^{(2)}(q,t)$ except for an extra decay introduced in the correlation function due to the rotation. In our experiments data were collected during typically 100 s (solutions) and 600 s (non-ergodic samples) while rotating the cuvette at the lowest possible speed (0.03 rps). The data were used to estimate the ensemble-averaged total scattered intensity $\langle I \rangle_E$ and the intercept $g_E^{(2)}(q,0)$ which is denoted β^2 below. The intercept was estimated by a third-order polynomial fit to data below 0.1 ms, and by a linear fit for times <0.01 ms. These (cumulant) fits also gave the initial relaxation time for a correlation function. The averages of these two types of fits were used for the intercept and the initial relaxation time.

(II) The modified heterodyne method as presented by Sellen (1987, 1993) was subsequently used with sampling times typically between 30 and 100 s. In this method a position of the cuvette is chosen so that the time-averaged scattered intensity $\langle I \rangle_T$ at that position is equal to the ensemble averaged intensity $\langle I \rangle_E$. The latter was taken from the rotation data. Deviations between the two averages of less than 5% were found to influence the results from the analysis only marginally. In the