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Interaction mechanism in sol–gel transition of alginate solutions by addition of divalent cations

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Abstract

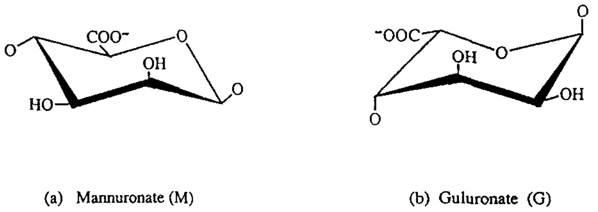
The interaction mode between divalent cations and alginate in aqueous solutions during sol–gel transition has been experimentally examined at different temperatures with the help of viscosity and density measurements. Comparison of temperature effects on the viscosity behaviour of alginate solution by addition of cupric and calcium cations confirmed different interaction modes existing between the Ca–alginate systems and the transition metal Cu–alginate systems. Different interaction mechanisms were proposed for the Cu–alginate systems at different temperature regions. Intra-molecular cross-linking clusters were found to pass through a conformational transformation with increasing temperature. This transformation of the interaction mode is not reversible when the solution is cooled. Visible and UV spectroscopic investigations suggest that the coordination of cupric ions mainly occurs to carboxyl groups, and the stoichiometric ratio *f* is proportional to the fraction of cross-links formed in the sol–gel transition process. © 1997 Elsevier Science Ltd.

Keywords: Sodium alginate; Sol–gel transition; Viscosity; Density

1. Introduction

Alginate is a linear polysaccharide of $(1 \rightarrow 4)$ linked α-L-guluronate (G) and β-D-mannuronate (M) arranged in a non-regular blockwise pattern (Fig. 1). A property of alginate of importance for both its biological and industrial applications is the formation of strong rigid gels with different divalent cations. However, Mg^{2+} does not promote gelation [1–3]. The physical and chemical properties in the sol–gel transition have been widely investigated [4–6]. Although explanations regarding the gelation phenomena have continually been put forward, the situation is less satisfactory with regard to the temperature effects during gel formation, which play an important role in the application of alginate.

In the present work, the viscosity and density behaviour of aqueous alginate solutions have been



(a) Mannuronate (M) (b) Guluronate (G)

Fig. 1. Constituent monomer units of alginate. (a) Mannuronate (M); (b) Guluronate (G).

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investigated at various concentrations of divalent cations and at different temperatures, with a view to obtaining an insight into the detailed reaction mechanism of metal–alginate complexes. Together with visible and UV spectra, the interaction modes between cupric ions and alginate monomers are discussed further.

2. Experimental

Sample preparation.—Sodium alginate was obtained from *Lessonia nigresens*. An average molecular weight of 2.1×10^5 was determined by means of gel permeation chromatography calibrated by pullulan standard samples. A value of 1.0 was obtained for M/G by the method described by Haug et al. [7]. The alginate samples were dialysed, centrifuged, and freeze-dried to obtain a purified sample.

Samples for sol–gel transition measurements were prepared at 10 °C by mixing 10 mL of 3 g/100 mL alginate aq soln with a certain concn and 1 mL of the chloride salts of Cu^{2+} and Ca^{2+} soln.

All samples were homogenous after gentle magnetic stirring for 10 h. Viscosity measurements were carried out after a further 24 h.

Viscosity measurements.—The viscosities of the samples were measured from 10 °C up to 50 °C for every 5 °C using an Ostwald-type capillary viscometer and then remeasured from 50 °C down to 10 °C. The temperature was controlled within ± 0.05 °C. The relative viscosity, η_{rel}, was calculated as a ratio between the solns with and without the salts at the same alginate concn, used as a measure for describing the extent of gel formation [5]. The viscometers were calibrated with water at each temperature concerned.

Density measurements.—The densities of the samples were measured with a vibrating tube digital density meter (model DMA60/602, Anton paar, Austria). The precision of this kind of density meter is $\pm 1.5 \times 10^{-6}$ $g \cdot cm^{-3}$. The temperature around the density meter was controlled with a CT1450 temperature controller and an CK-100 ultracryostat to maintain the bath temperature to within ± 0.005 K. The density meter was calibrated with water and dry air at each temperature concerned.

Spectroscopic measurements.—Visible and UV spectra were recorded at room temperature on a Lambda 17 UV/VIS spectrophotometer, using quartz 10- and 2-mm cells.

3. Results and discussion

Temperature effects on the viscosity behaviour of alginate solutions in sol–gel transition.—The stoichiometric ratio $f =$ [divalent cation]/[residues of alginate] was defined as a factor which controls the sol–gel transition, with the assumption that the ratio f is proportional to the cross-linking fraction formed. Fig. 2 shows the variation of the relative viscosity of cupric-added alginate solutions versus the cross-linking fraction f at different temperatures. It was shown that temperature affects sol–gel transition of alginate solution, especially near the gelation threshold. From this figure, we could conclude that high temperature improves gel formation of the Cu–alginate solution.

Fig. 3 shows the relationship between $\log \eta$ and $1/T$ for alginate solutions at different cupric concentrations obtained from both heating and cooling processes. It was shown that, for samples of alginate solutions with lower cupric concentrations ($f < 3 \times 10^{-2}$), a near linear relationship was confirmed between $\log \eta$ and $1/T$, showing a similar behaviour with that of other linear polysaccharide solutions without added salt. This result concurs with the suggestion that for alginate solutions with very low density of cross-linkers, the chain behaves as if it were alone [8,9]. With increasing f, the linear relationship between $\log \eta$ and $1/T$ is destroyed. The decrease of viscosity by heating becomes slow, especially when the temperature is above 25 °C. However, a quite good linearity is reconfirmed in the

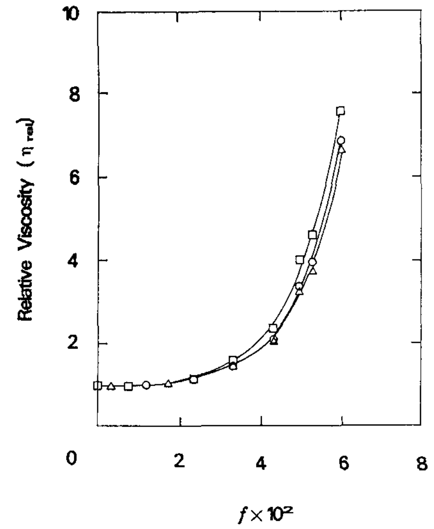

Fig. 2. Variations of relative viscosity for 3%wt alginate solutions vs. fraction of cupric cations at different temperatures (○: 10 °C; △: 30 °C; □: 50 °C).

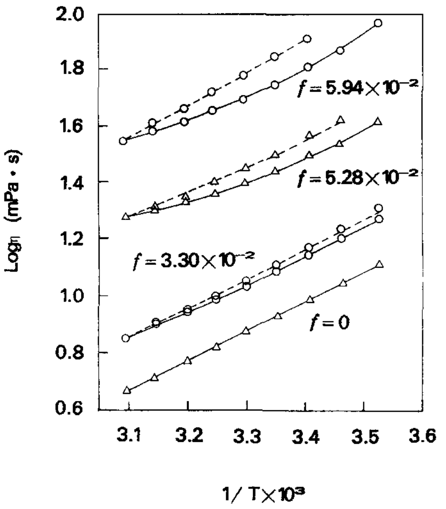

Fig. 3. Log η plots vs. $1/T$ at different fractions of cupric cations for Cu–alginate system. (Solid lines refer to the heating while dashed lines refer to the cooling process which follows.)

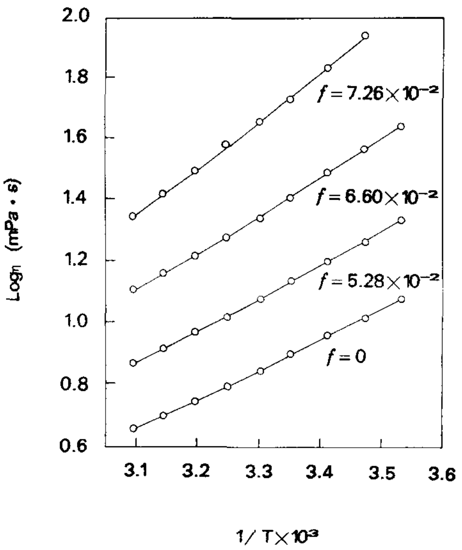

Fig. 4. Log η plots vs. $1/T$ at different fractions of calcium cations for Ca–alginate system.

cooling process which shows an irreversible process of viscosity variation with temperature.

Considerable overlap exists in semi-concentrated polyelectrolyte solutions leading to many transient networks which become more stable when a simple salt is introduced. Thus an interaction mode between monomers of intra- and inter-molecular cross-linking was suggested to occur simultaneously when divalent cations were added to a solution of alginate [10]. With increasing cupric concentration, the counterions along the chain increase, which contributes to a flexible polychain. Intra-molecular cross-linkings have a contractive effect on chain conformation while the formation of inter-molecular cross-linkings is the main factor responsible for viscosity increase and gel formation [11]. Both the two kinds of cross-linkings differ in their thermostability [10]. At lower temperature, the mobility of chain segments is weak, and the networks formed by entanglements of polychains are relatively stable. The formation of intra-molecular cross-linking is possible, but could be destroyed due to the expansive effects of the violent mobility of chain segments and the charge repulsion along the alginate chain. A transformation of interaction mode from intra- to inter-chain cross-linking occurs when the solution is being heated and results in more and larger clusters in the system. The decrease of viscosity with increasing temperature is then slowed down. For the cooling process which follows, the linearity between log η and $1/T$ suggests that the transformation from intra- to inter-molecular cross-linkings when heated is not reversible. This result reconfirmed that inter-molecular cross-linking is thermodynamically stable whereas the intra-molecular cross-linking is sensitive to temperature.

For Ca–alginate systems, however, the effects of temperature on viscosity behaviour were not the same as that of Cu–alginate systems. As shown in Fig. 4, the log η of alginate solutions exhibits linearity with varying $1/T$ which qualitatively suggested that the interaction mode between Ca^{2+} and alginate monomers is relatively stable and not destroyed by heating. This result is consistent with ^{13}C-NMR [12,13] and CD [14] spectroscopic investigations which have indicated that the structure is not destroyed by heating up to 100 °C. The difference in viscosity behaviour for the two systems results from different cross-linking mechanisms [15]. In the Ca–alginate systems, there exists a specific interaction in which all the functional groups in the guluronate residues are considered to involve the interaction with calcium ions in the egg-box junction. On the other hand, in Cu–alginate systems, interaction between cupric ions and alginate monomers is characterised by a simple complex formation in which only carboxyl groups are considered to coordinate to cupric ions.

Temperature effects on the density behaviour of alginate solutions in sol – gel transition.—Fig. 5 shows the variations of the densities of alginate solutions vs. f at different temperatures. At lower temperature, it was shown that for the region of $f < 3.5$

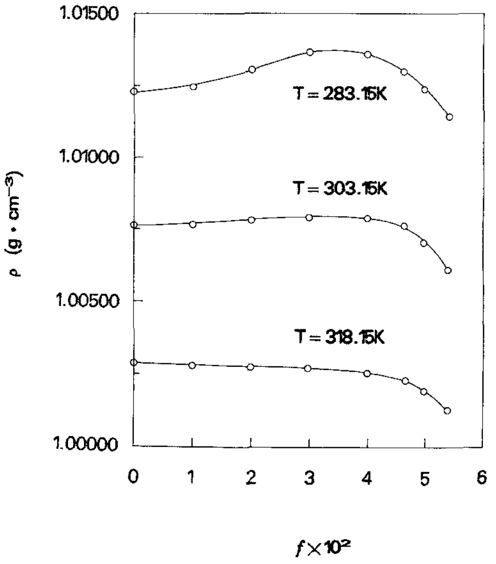

Fig. 5. Plots of the densities of alginate solutions vs. cupric fractions at different temperatures, 10, 30, and 45 °C, respectively.

× 10^{-2}, there is an increase of density value with increasing f. Considering that the increase of viscosity of the samples in this f region is not obvious (see Fig. 2), the increase of densities can be due to the formation of intra-molecular cross-linking clusters which makes molecular conformation compact. The increasing concentration of the added salt, cupric chloride, is the other factor which contributes to the density increase. In the higher f region, however, although with the increasing concentration of cupric chloride in the system, a decreasing tendency of density with increasing f was obtained. Together with the rapidly increasing viscosity of the system (see Fig. 2), this result reveals that the effects of cluster formation by inter-molecular cross-linking is the dominant factor which generates a highly ordered structure by mutipolychain association in the system [13] responsible for decrease of density value.

By contrast, at higher temperature, an almost monotonous decrease of density of alginate solutions in the whole f region was obtained, suggesting that the alginate cluster on a single chain is not as large as it is at lower temperature. The inter-molecular cross-links can be considered as the dominant interaction mode between alginate chain and cupric ions.

Spectroscopic investigations.—Visible and UV spectra of aqueous Cu–alginate solutions at room temperature show that there are two absorption bands at about 750 and 240 nm. The absorption at 750 nm is responsible for the blue colouration of aqueous Cu–alginate solutions. It has been reported [16] that the absorption at 240 nm may be due to the pπ(O) → $d_{x^2-y^2}$(Cu) transfer transitions, where pπ(O) denotes the pπ orbital of ligand. Compared with the visible and UV spectra of aqueous Cu^{2+} solutions without alginate, almost no shift of the peaks was observed for the two kinds of systems, although the absorbance was different.

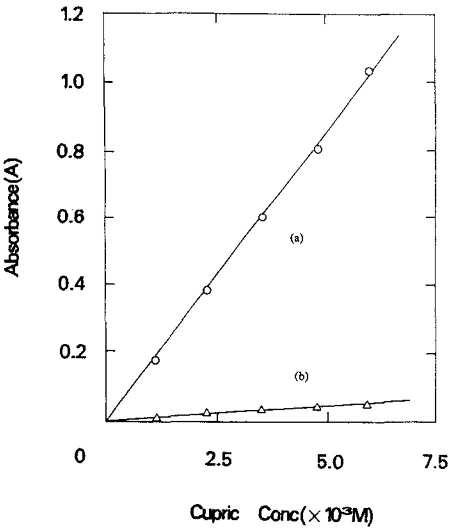

Fig. 6. Variations of absorbance for (a) alginate solutions and (b) aqueous solutions vs. the cupric concentrations in the systems at which $\lambda_{max} \approx 240$ nm.

Fig. 6 shows the variation of absorbance of the two systems at 240 nm vs. cupric concentrations. It was shown that the absorbance increases linearly with increase of cupric concentration for both of the two systems. Very different slopes of the two graphs suggest that naturally different ligands exist in the two systems investigated, indicating that carboxyl groups are coordinated to cupric ions in the aqueous Cu–alginate system. The linearity relationship between absorbance and cupric concentration may also suggest that there is no great difference in the interaction modes between cupric ions and alginate monomers during the sol–gel transition and is consistent with our assumption that f is proportional to the fraction of cross-links.

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