ELSEVIER

0144-8617(95)00102-6

Carbohydrate Polymers 28 (1995) 101–105

0144-8617/95/$09.50+.00

A light scattering study of carrageenan/galactomannan interactions

Christer Viebke

Physical Chemistry 1, Lund University Chemical Center, Box 124, S-221 00 Lund, Sweden

(Received 27 March 1995; revised version received 19 August 1995; accepted 22 August 1995)

New experimental results that support the formation of mixed aggregates in the carrageenan/galactomannan system are presented. Kappa- and iota-carrageenan were degraded by ultrasonication to obtain low molecular weight samples. These samples were mixed with galactomannan in various proportions and studied in dilute solutions by multi-angle laser light scattering. Two different galactomannans were used, locust bean gum and guar gum. The ion specificity of the carrageenans was used to control their conformation and association. This made it possible to separately study how double helices and aggregates interact with the galactomannans. Under self-aggregated conditions, kappa-carrageenan was seen to associate with locust bean gum, but not with guar gum. Iota-carrageenan did not show any signs of association under any conditions.

INTRODUCTION

It is well established that the solution properties of certain biopolymer mixtures could be quite different from those of the pure polymer systems (Dea, 1979; Dea & Morrison, 1975; Morris, 1990). A large enhancement in viscosity or even a gelation, below the concentration at which this takes place for either of the pure polymer systems, may be found. Such synergistic effects may be caused either by an effective attraction or a repulsion between the two polymers, resulting in mixed aggregate formation or phase separation. Most often, a phase separation in aqueous polymer mixtures is segregative, but a combination of association and phase separation is also possible, where the polymer association is followed by a phase separation (Morris, 1990; Piculell *et al.*, 1994*a*). In gelling mixtures, an infinite three-dimensional network has to be formed, including junction zones and branching points. Different types of gel structure are possible in mixed systems. Cairns *et al.* (1987) distinguish between separate polymer networks, which interlace and form an interpenetrating network, a phase separated network, and a network where one of the polysaccharides binds to the other and forms a coupled network. In these three structures both polymers take part in the network(s). A fourth structure involves a network of one of the polymers, in which the other polymer is dissolved.

One type of mixture where synergistic effects are well known to occur is that involving certain galactomannans and certain helix-forming galactans from the carrageenan family. Galactomannans consist of linear chains of β-(1→4)-linked D-mannopyranosyl residues, to which side chains of (α-(1→6)-linked galactopyranosyl are attached (Fig. 1a). They are normally non-gelling polysaccharides used as food additives. Various types of galactomannans differ in the degree and pattern of side chain substitution (Morris, 1990). The helix-forming carrageenans (Fig. 1b) differ in the degree of sulphation per repeating disaccharide unit in the order furcellaran (0.6) < kappa-carrageenan (1) < iota-carrageenan (2). It is widely accepted that the gelling of carrageenan involves a coil-to-double helix transition of the molecule.

The extent of synergism observed in the galactan/galactomannan mixtures varies from none, in certain cases, to very large in others. Generally, conditions enhancing the tendency of galactan self-association (lower sulfate content, presence of certain salts) also enhance the synergistic effects (Morris, 1990). As regards the galactomannans, the synergistic effects depend on both the degree and the pattern of side chain substitution. A large synergistic effect is seen for locust bean gum (lbg), which typically has a mannose:galactose ratio of 3.5, whereas little or no effect is seen for guar gum (gg), where the mannose:galactose ratio is 1.55 (Morris, 1990).

The first mechanism of synergistic gelation that was suggested for galactomannan/galactan mixtures was a binding of the bare mannan backbone of the galactomannan to the double helix of the galactan (Rees, 1972*a*, *b*; Dea *et al.*, 1972). This model was based

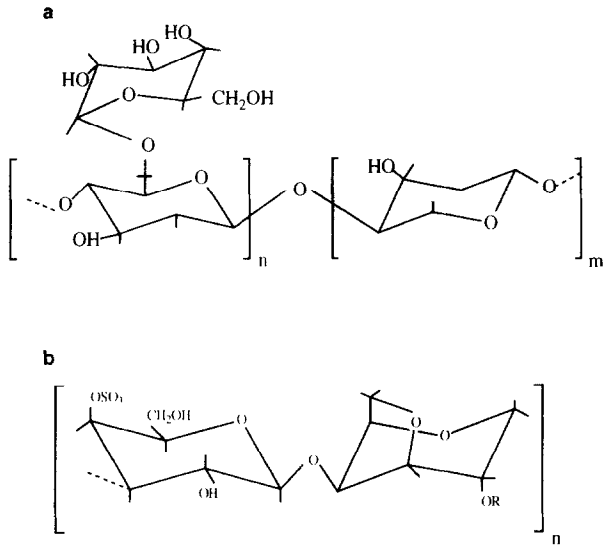


Fig. 1. Repeating units of: (a) galactomannan; and (b) carrageenan. For galactomannans, the lengths of the n and m blocks vary within and between samples. For carrageenans, R = H (kappa-carrageenan), R = SO_3^- (iota-carrageenan).

on experiments with agarose and kappa-carrageenan mixed with various galactomannans. It was shown that, by adding galactomannan, a gelation could be induced below the normal gelling concentration for the galactans. Furthermore, it was seen that low molecular weight galactans that normally did not form a gel, gelled or precipitated when galactomannan was added to the solution. The synergistic interaction was highest for the least substituted galactomannan polymers, which was taken as evidence that the mannan backbone took part in the gelation. The intermolecular binding between the mannan backbone and the galactan double helix was later challenged by Cairns *et al.* (1987). They studied the X-ray diffraction patterns for kappa-carrageenan and furcellaran, alone and in mixtures with galactomannans, and found no differences between the pure and the mixed systems. On the other hand, the authors were equally unable to find evidence of a segregative phase separation, since sulphur and potassium mapping showed the mixed gels to be homogeneous at a resolution of 1 μm. In the absence of evidence of either a coupled or a phase-separated network, the authors favoured a gel structure where the galactomannan chains were simply contained in the galactan network. Since then, however, experimental evidence by nuclear magnetic resonance (NMR) (Rochas *et al.*, 1990) and electron spin resonance (ESR) (Williams *et al.*, 1993) has been presented, showing that both galactomannan and glucomannan chains are involved in some association in the corresponding mixed gels. Very recent evidence has shown that the association of the galactan helices is also affected in the mixed gels (Piculell *et al.*, 1994*a*, *b*). The latest model for the galactan/galactomannan mixtures that has been suggested (Williams *et al.*, 1993; Piculell *et al.*, 1994*a*) is a mixed aggregate, where the flexible galactomannan molecules adsorb onto the surface of aggregates of galactans.

In this study, we have used multi-angle laser light scattering (MALLS) to study the interaction between carrageenan and galactomannan in the dilute solution regime. The conformation of the carrageenan molecules has been controlled by the added electrolyte. This makes it possible to separately study interaction of the double helix and the aggregates with galactomannans.

MATERIALS AND METHODS

Locust bean gum, guar gum, kappa- and iota-carrageenan were gifts from Sanofi Bio-industries. The carrageenans were ion-exchanged to suitable pure ion forms (sodium and potassium) by pouring a hot carrageenan solution through an ion-exchange column at elevated temperature. The galactomannans were used without further purification.

Light scattering was measured at 632.8 nm on a Dawn F MALLS photometer (Wyatt Technology, Santa Barbara, CA) equipped with a 5 mW He–Ne linearly polarised laser. Pure toulene with a known Rayleigh ratio was used to absolutely calibrate the MALLS. The instrument can either be coupled to a GPC system for on-line analysis or used in batchwise mode, to obtain classical Zimm plots. The intensity of scattered light was measured at 15 different angles, from 30 to 160°. The software used for analysis was AURORA, supplied by Wyatt Technology.

The depolymerisation of the carrageenans was carried out by ultrasonication, using a 600 W, 20 kHz, high intensity ultrasonic processor (model VC50, Sonics and Materials Inc., Danbury, USA). The coil-to-helix transition was followed by optical rotation at 435 nm on a Jasco DIP-360 polarimeter in a jacketed cell with a 5 cm path length.

Sample preparation

Kappa- and iota-carrageenan were dissolved (0.1% w/w) in the appropriate electrolyte solution at 70°C. The samples were depolymerised by sonication for 120 min, which gave a weight average molecular weight less than 1.10^5 (Viebke, to be published) for the coil form. The galactomannans (0.1% w/w) were dissolved in the appropriate electrolyte solution by stirring overnight at room temperature, followed by heating in a water bath for at least 30 min at 70°C to obtain a true solution. All mixtures were prepared from these stock solutions. The mixtures were heated in a water bath to 80°C before analysis at 25°C. The samples were filtered through a 0.45 μm pore size filter into the cell to avoid contamination of dust.

Evaluation of light scattering data

The scattering of light from a polydisperse macromolecular solution can be used to measure the weight-average molecular weight. This is usually obtained by the Zimm method, i.e. the scattering intensity is measured at different angles and concentrations, followed by an extrapolation to zero angle and zero concentration (Kratochvil, 1987). This procedure will give the absolute value of the weight-average molecular weight. It is also possible to calculate an apparent weight-average molecular weight by doing only the extrapolation to zero angle at a finite concentration. In light scattering, the apparent weight-average molecular weight is defined as (Huglin, 1972)

$$< M_w >_{app} = R_0/(Kc) \quad (1)$$

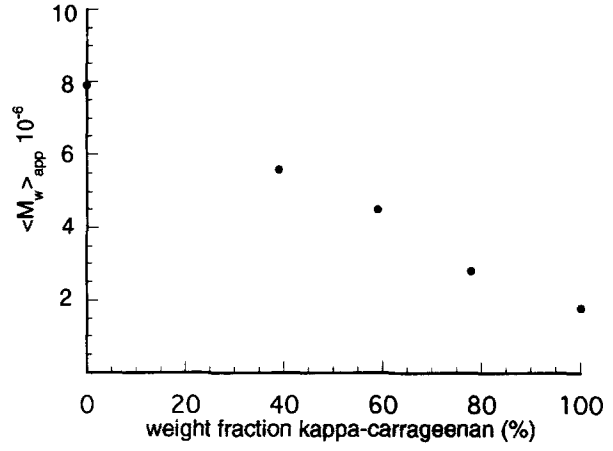
where K is a optical constant, c is the concentration and R_0 is the excess Rayleigh scattering at zero angle. This expression was used to evaluate the data obtained from the measurements. The Rayleigh scattering was obtained at different angles and an extrapolation to zero angle was performed. The total polymer concentration was 0.05% (w/w), but the composition was varied between carrageenan and galactomannan. The optical constant was assumed to be constant for the different compositions. The dn/dc values used in the analysis of light scattering data for carrageenans were 0.127 and 0.140 ml/g for iota- and kappa-carrageenan, respectively. These values were measured in pure water.

RESULTS

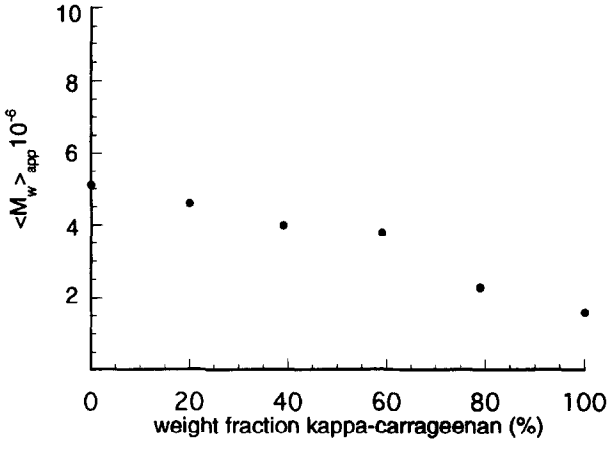
The accuracy and reproducibility of light scattering measurements depend, to a large extent, on the preparation of the samples and the way they are injected into the sample cell. The scattering intensity depends on the size of the scattering particle. This makes it necessary to filter the samples into the cell, to avoid contamination of dust. The procedure which involves filtration of the samples at elevated temperature (coil conformation of the galactans) through a 0.22–0.45 μm filter into the sample cell has been used (Viebke, to be published) to obtain Zimm plots for pure kappa- and iota-carrageenan samples under different electrolyte and temperature conditions. This procedure has shown a good reproducibility and the results obtained have agreed well with results obtained from MALLS/GPC measurements, both for the coil and the helix conformation. The carrageenan/galactomannan mixtures have been treated according to the same procedure as the pure carrageenan samples.

It is well known that iodide ions stabilise the double helix and prevent further association of kappa-carrageenan (Grasdalen & Smidsrod, 1981; Slootmaekers *et al.*, 1988). Accordingly, we chose 0.1 M sodium iodide at 25°C to study the interaction of the double helix with the galactomannan. Under these conditions the helical content of kappa-carrageenan is at least 80% according to optical rotation measurements. The onset of the helix formation, T_0, occurred at 38°C for the pure kappa-carrageenan sample. In Fig. 2a, the $< M_w >_{app}$ variation with the kappa-carrageenan/gg composition is displayed. It is clearly seen that the $< M_w >_{app}$ is a linear combination of the composition and no signs of association can be observed. The same is observed, under similiar conditions, for kappa-carrageenan/lbg mixture as displayed in Fig. 2b.

It is known that potassium ions promote the self-association of kappa-carrageenan (Rochas & Rinaudo, 1980). Therefore, we chose the condition of 50 mM potassium chloride at 25°C to study the interaction between self-aggregated kappa-carrageenan and galactomannan. The helical content is at least 80%, and

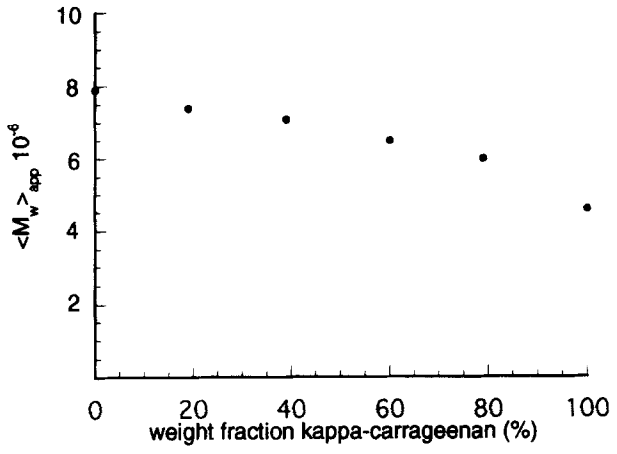


(a)

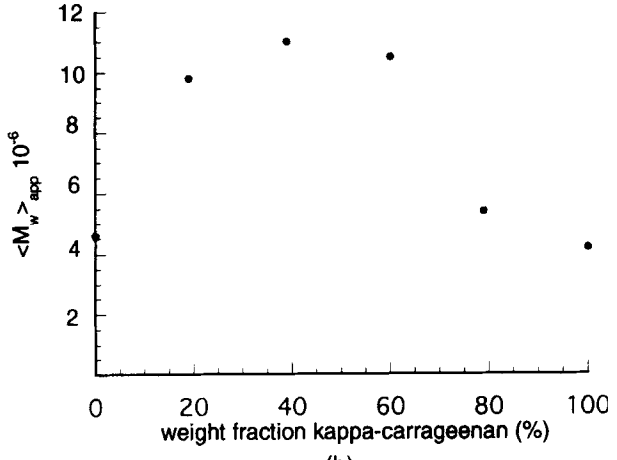


(b)

Fig. 2. $< M_w >_{app}$ for: (a) kappa-carrageenan/gg; and (b) kappa-carrageenan/lbg mixtures in 0.1 M sodium iodide, 25°C. Total polymer concentration 0.05 (w%w).



(a)



(b)

Fig. 3. $<M_w>_{app}$ for: (a) kappa-carrageenan/gg; and (b) kappa-carrageenan/lbg mixtures in 50 mM potassium chloride, 25°C. Total polymer concentration 0.05 (w%w).

T_0 was found at 43°C for the pure kappa-carrageenan sample. Figure 3a shows the $<M_w>_{app}$ variation with the kappa-carrageenan/gg composition. There is no evidence of any association in this system under these conditions, although synergism is known to occur in this system (Morris, 1990). In Fig. 3b, the $<M_w>_{app}$ vs kappa-carrageenan/lbg composition is displayed. This system shows an enhancement of $<M_w>_{app}$ in the mixtures indicating that some association process other than the self-association of the galactans is occurring. It is well known that no synergism is observed for iota-carrageenan/galactomannan mixtures (Morris, 1990). In Fig. 4a, a plot of the $<M_w>_{app}$ vs the composition of iota-carrageenan/lbg is displayed. The condition is 0.1 M KCl 25°C which should correspond to all-helical solution for the iota-carrageenan, $T_0 = 47$°C (Piculell *et al.*, 1987). No sign of association is observed in this system, thus, confirming that no mixed association is occurring in the iota-carrageenan/galactomannan system.

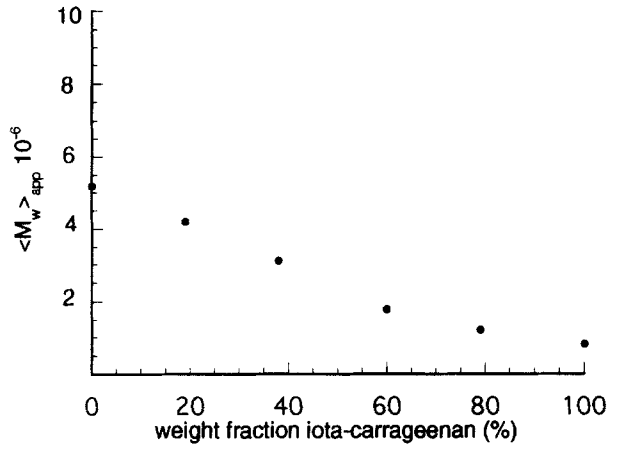


Fig. 4. $<M_w>_{app}$ for iota-carrageenan/lbg mixture in 0.1 M potassium chloride, 25°C. Total polymer concentration 0.05 (w%w).

DISCUSSION

In the analysis of the experiments some approximations have been made. No adjustments for changes in refractive index increment with the degree of association have been performed. Further, an apparent molecular weight has been calculated, i.e. no extrapolation to zero concentration has been performed. Still, the above experiments in the dilute concentration regime show the same trend as is found under more concentrated conditions (Viebke, unpublished data) using viscosity measurements. The association is seen to depend on the self-association of the carrageenan; no association under double helix conformation or with iota-carrageenan was observed. The interaction was also dependent on the nature of added galactomannan, i.e. the mannose:galactose ratio. The result could be explained in the framework of a recent suggested model (Williams *et al.*, 1993; Piculell *et al.*, 1994*a*), where the flexible galactomannan molecule adsorbs onto the surface of the self-aggregated galactans. Support for the existence of an association in galactan/galactomannan systems has recently been presented in two studies (Viebke & Piculell, 1995; Parker *et al.*, 1995). The nature and the driving force of this association are still poorly understood. The observation that the interaction depends on the size of the galactan aggregates may suggest that this is important. However, the effect of specifically adsorbing ions that compete with the galactomannan cannot be ignored. The association of lbg and gg onto agarose has been shown to decrease with the addition of iodide compared with nitrate ions (Viebke & Piculell, 1995). Another effect that may occur is the counterions that compete with the neutral adsorbing polymer segments. The counterions could displace the adsorbing polymer if the charge density is sufficiently high (Piculell *et al.*, 1994*a*). This could explain why no association is seen for iota-carrageenan

under any type of salt addition and explain the observed trend that the synergism (association) increases with a decrease in charge density.

ACKNOWLEDGEMENTS

This work was supported by grants from the Swedish Natural Science Council (NFR). The MALLS instrument was funded by a grant from the Swedish Council for Planning and Coordination of Research (FRN).

REFERENCES

Cairns, R., Miles, M.J., Morris, V.J. & Brownsey, G.J. (1987). X-ray fibre-diffraction studies of synergistic, binary polysaccharide gels. *Carbohydr. Res.*, **160,** 411–423.

Dea, I.C.M. (1979). In *Polysaccharides in Food*, eds J.M.V. Blanshard & J.R. Mitchell. Butterworths, London, pp. 229–247.

Dea, I.C.M., MacKinnon, A.A. & Rees, D.A. (1972). Tertiary and quaternary structure in aqueous polysaccharide systems which model cell wall cohesion: reversible changes in conformation and association of agarose, carrageenan and galactomannan. *J. Mol. Biol.*, **68,** 153–172.

Dea, I.C.M. & Morrison, A. (1975). Chemistry and interactions of seed galactomannans. *Adv. Carbohydr. Chem. Biochem.*, **31,** 241–312.

Grasdalen, H. & Smidsrod, O. (1981). Iodide-specific formation of kappa-carrageenan single helices. *Macromolecules*, **14,** 1842–1845.

Huglin, M.B. (1972). *Light Scattering from Polymer Solutions.* Academic Press, London.

Kratochvil, P. (1987). *Classical Light Scattering from Polymer Solutions.* Elsevier, Amsterdam.

Morris, E.R. (1990). Mixed polymer gels. In *Food Gels*, ed. P. Harris. Elsevier Applied Science, London, pp. 291–359.

Parker, A., Lelimousin, D., Miniou, C. & Boulenguer, P. (1995). Binding of galactomannans to kappa-carrageenan after cold mixing. *Carbohydr. Res.*, submitted.

Piculell, L., Håkansson, C. & Nilsson, S. (1987). Cation specificity of the order–disorder transition in iota carrageenan: effects of kappa carrageenan impurities. *Int. J. Biol. Macromol.*, **9,** 297–301.

Piculell, L., Iliopoulos, I., Linse, R., Nilsson, S., Turquois, I., Viebke, C. & Zhang, W. (1994*a*). Association and segregation in ternary polymer solutions and gels. In *Gums and Stabilisers for the Food Industry 7*, eds G.O. Phillips, P. Williams & D.J. Wedlock. IRL Press, Oxford, pp. 309–322.

Piculell, L., Zhang, W., Turquois, T., Rochas, C., Taravel, F.-R. & Williams, P.A. (1994*b*). Effects of added galacto- and glucomannans on the NMR spectra of ^{133}Cs ions in kappa-carrageenan gels. *Carbohydr. Res.*, in press.

Rees, D.A. (1972*a*). Shapely polysaccharides. *Biochem. J.*, **126,** 257–273.

Rees, D.A. (1972*b*). Polysaccharide gels. A molecular view. *Chem. Ind.*, 630–636.

Rochas, C. & Rinaudo, M. (1980). Activity coefficients of counterions and conformation in kappa-carrageenan systems. *Biopolymers*, **19,** 1675–1687.

Rochas, C., Taravel, F.-R. & Turquois, I. (1990). N.M.R. studies of synergistic kappa carrageenan-carob galactomannan gels. *Int. J. Biol. Macromol.*, **12,** 353–358.

Slootmaekers, D., De Jonghe, C., Reynaers, H., Varkevisser, F.A. & Bloys van Treslong, C.J. (1988). Static light scattering from kappa-carrageenan solutions. *Int. J. Biol. Macromol.*, **10,** 160–168.

Viebke, C. & Piculell, L. (1995). Adsorption of galactomannans onto agarose. *Carbohydr. Polym.*, submitted.

Williams, P.A., Clegg, S.M., Langdon, M.J., Nishinari, K. & Piculell, L. (1993). Investigation of the gelation mechanism in κ-carrageenan/konjac mannan mixtures using differential scanning calorimetry and electron spin resonance spectroscopy. *Macromolecules*, **26,** 5441–5446.