# Effect of ι-Carrageenan on Flocculation, Creaming, and Rheology of a Protein-Stabilized Emulsion

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The influence of ι-carrageenan on the surface activity of bovine serum albumin (BSA) and on the properties of BSA-stabilized oil-in-water emulsions is reported. Surface tension data at low ionic strength indicate a weak electrostatic protein−polysaccharide interaction at neutral pH, which becomes much stronger at pH 6 but disappears in 0.1 M NaCl. The effect of the attractive BSA−ι-carrageenan interaction on the droplet-size distribution and creaming stability of protein-stabilized emulsions (20 vol % *n*-tetradecane, 1.7 wt % BSA, 5 mM) has been investigated over a range of pH values. At pH 6 the system behavior is interpreted in terms of bridging flocculation leading to a gel-like emulsion network over a certain limited polysaccharide concentration range. This was confirmed by small-deformation oscillatory rheological measurements on equivalent concentrated emulsions (40 vol %). There is a good correlation with the rheology of the dextran sulfate plus BSA emulsions studied previously, suggesting that the flocculation mechanism is similar for the two sets of systems.

**Keywords:** *Protein−polysaccharide interaction; ι-carrageenan; bovine serum albumin; emulsion stability; creaming; bridging flocculation; rheology*

## INTRODUCTION

Proteins and polysaccharides are present together in many food emulsion products. Depending on polysaccharide concentration, and the nature and strength of the biopolymer interactions, the presence of polysaccharides in protein-stabilized emulsions can in principle have a widely variable effect on stability and rheological properties. For a protein−polysaccharide interaction that is net attractive and a polysaccharide concentration that is very low, the system may become flocculated due to the formation of polymer bridges between emulsion droplets. On the other hand, if there is more than enough of the interacting polysaccharide to cover completely the surface of the protein-coated droplets, the emulsion may remain unflocculated due to restabilization by the secondary steric stabilizing polysaccharide layer (Dickinson and Euston, 1991; Dickinson and McClements, 1995). We demonstrated recently using small-deformation rheological measurements (Dickinson and Pawlowsky, 1996a) that a highly anionic polysaccharide, dextran sulfate, can induce bridging flocculation at neutral pH and low ionic strength in a concentrated emulsion stabilized by the protein bovine serum albumin (BSA). Now we consider how stability and rheology of the same BSA-stabilized emulsion are affected by another anionic polysaccharide, ι-carrageenan.

Carrageenans are commonly used as stabilizers, thickeners, and gelling agents in milk-based products (Enriquez and Flick, 1989). They have a strong electrolyte character due to their sulfate groups. The various types of carrageenan mainly differ in the number and position of the sulfate groups on the polygalactose backbone. Aqueous solutions of ι-carrageenan yield transparent thermoreversible gels upon cooling in the presence of various cations (effectiveness $Ca^{2+} > K^{+} > Na^{+}$) (Morris and Belton, 1982). The gelation mechanism arises from the formation and association of right-handed double helices. In calcium-induced ι-carrageenan gels, network formation arises from bridging of polysaccharide chains by the divalent cations. As compared with κ-carrageenan, bridging is enhanced in ι-carrageenan due to the higher ester sulfate content (Enriquez and Flick, 1989).

There have been many studies of protein−carrageenan interactions, in particular involving the caseins (Snoeren *et al*., 1976; Elfak *et al*., 1979; Keogh *et al*., 1995; Lynch and Mulvihill, 1996; Drohan *et al*., 1997) but also involving other proteins (Chakraborty and Randolph, 1972; Ipsen, 1995; Fernandes, 1996; Michon *et al*., 1996). Several groups have studied the interaction between carrageenan and milk protein particles (casein micelles) (Dalgleish and Morris, 1988; Heertje, 1993; Langendorff *et al*., 1997), but much less is known about the details of protein−carrageenan interactions in protein-stabilized emulsion systems. Hansen (1982) has presented a model for the creaming stabilization of processed milk by carrageenan involving interaction of polysaccharide with casein submicelles on the fat globule surface. More recently, Dalgleish and Hollocou (1997) reported on the stabilizing effect of κ-carrageenan in emulsions prepared with sodium caseinate or whey protein.

This paper is concerned with systems containing BSA and ι-carrageenan under electrolyte conditions favoring strong electrostatic interaction. The choice of BSA as the protein emulsifier allows direct comparison with our recent work on BSA plus dextran sulfate systems (Dickinson and Pawlowsky, 1996a,b). Surface tension measurements are used here to demonstrate the presence of the BSA−ι-carrageenan complexation, and gravity creaming, rheology and droplet-size distribution measurements are used to explore its influence on emulsion properties.

## MATERIALS AND METHODS

**Materials.** Food grade ι-carrageenan samples were kindly donated by Systems Bio Industries (Carentan, France). It was in almost pure sodium form with a small amount of contami-

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nation by κ-carrageenan (about 5%). The weight-average molecular weight was given by the suppliers as $5.6 \times 10^5$ Da and the *z*-average hydrodynamic radius as 80 nm. BSA (lyophilized globulin-free powder, ≥99%, product A7638, lot 14H9350) and *n*-tetradecane (99%) were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical grade. The buffer solution (5 mM imidazole, pH 7) was made with double-distilled water (0.02 wt % sodium azide added as antimicrobial agent in the creaming experiments). Polysaccharide solutions were prepared by dispersing the ι-carrageenan powder in buffer and continuously stirring at 70 °C for 30 min. BSA was dissolved in pH 7 buffer at the desired concentration and pH then adjusted as required.

**Surface Tension Measurements.** Protein solutions were prepared containing $10^{-3}$ wt % BSA, and the pH was adjusted to a value in the pH range 6−8. For measurements on mixed systems, the BSA to ι-carrageenan weight ratio was 1:4 and the BSA concentration was $10^{-3}$ wt %. The time-dependent change in air−water surface tension $\gamma$ at 25 °C was monitored by the static Wilhelmy plate method using a Krüss digital tensiometer K10ST (Krüss Instruments, Germany). To ensure effective removal of surface-active contaminants, all glassware in contact with the sample was previously cleaned in a nitric acid bath and rinsed with plenty of double-distilled water. The platinum plate was washed with double-distilled water, heated in a Bunsen burner flame, and left to cool to room temperature. Before the start of the experiment, the surface of the sample solution was "sucked" to remove any already adsorbed molecules. The estimated experimental error in the tension measurements was *ca.* 0.5 mN $m^{-1}$.

**Emulsion Preparation and Stability.** Oil-in-water emulsions were prepared at room temperature using a laboratory-scale jet homogenizer (Burgaud *et al.*, 1990) operating at 300 bar. Emulsions were made from 45 vol % *n*-tetradecane plus 55 vol % aqueous phase containing 4.6 wt % protein. Polysaccharide solutions of the appropriate concentrations were mixed with freshly prepared emulsions to give final emulsion samples (20 vol % oil, 1.7 wt % BSA) containing various concentrations of ι-carrageenan in the range 0−0.22 wt %. Half of each emulsion sample was stored quiescently in a sealed glass tube at 25 °C. The other half was kept in a tube at 25 °C with periodic gentle agitation for the purpose of monitoring time-dependent change in average droplet diameter. Rates of gravity creaming were determined over a period of 9 days by following visually the change in thickness of the serum layer [a distinct (semi-)transparent layer] at the bottom of the tube. The droplet-size distribution $P(d)$ and the average volume−surface diameter $d_{32} = \Sigma_i n_i d_i^3 / \Sigma_i n_i d_i^2$, where $n_i$ is the number of droplets of diameter $d_i$, were determined using a Malvern Mastersizer S2.01.

**Rheology.** Parts of the concentrated stock BSA-stabilized emulsion (45 vol % oil) were diluted with ι-carrageenan solutions to give samples of concentrated polysaccharide-containing emulsion (40 vol % oil, 2.7 wt % BSA). Shear viscoelastic properties were analyzed by dynamic small-deformation rheology using a controlled-stress Bohlin CS-50 rheometer with a concentric cylindrical cell. The maximum shear strain (0.5%) was set to lie within the linear viscoelastic regime. Oscillatory experiments at 30 °C were used to determine the dynamic moduli over the frequency range $10^{-3}$−10 Hz. To facilitate comparison of samples with differing compositions, attention was mainly focused on values of the complex modulus $G^*$ at the single frequency of 1 Hz.

Steady-state apparent shear viscosities of ι-carrageenan solutions were measured at 30 °C using the same apparatus over the stress range 0.15−10 Pa.

## RESULTS AND DISCUSSION

We first attempt to confirm the presence of soluble protein−polysaccharide complexes in mixed aqueous solutions of BSA plus ι-carrageenan at low ionic strength. Surface tension measurements have previously been shown to be useful for this purpose (Dickinson and Galazka, 1991). Figure 1 shows the decrease in air−water surface tension $\gamma$ with time at 25 °C for a $10^{-3}$ wt % BSA solution, a $10^{-3}$ wt % ι-carrageenan solution, and a mixed system of BSA plus ι-carrageenan ratio (1:4 weight ratio, BSA concentration $10^{-3}$ wt %), all in 5 mM imidazole buffer at pH 7.0. The protein solution exhibits the typical behavior of a slowly adsorbing macromolecular surfactant, *i.e.*, lowering the interfacial free energy by diffusion to the interface followed by rearrangement in the surface film. Even after 400 min, the steady state condition is still not reached. The tension values determined here are slightly higher than those previously reported for BSA (Paulsson and Dejmek, 1992; Suttiprasit *et al.*, 1992; Patino and Nino, 1995), but it is difficult to compare our absolute values directly with those in the literature because of the sensitivity of protein adsorption behavior to solution conditions (pH, ionic strength) and to the well-recognized variability in surface properties between different batches of commercial "pure" globular proteins (Dickinson and Iveson, 1993; Clark *et al.*, 1995; Dickinson and Pawlowsky, 1996a). What is relevant here is the relative change in surface activity caused by the presence of the polysaccharide.

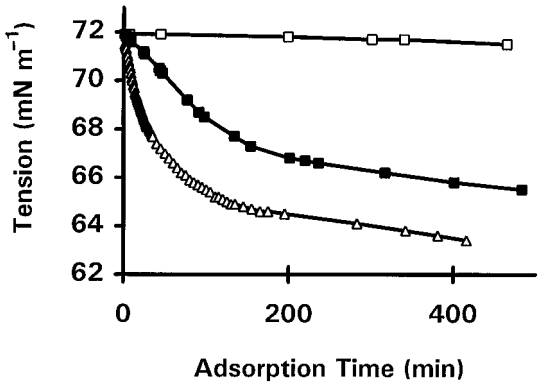


**Figure 1.** Time-dependent surface tension $\gamma$ of biopolymer solutions (5 mM imidazole, pH 7.0, 25 °C): △, $10^{-3}$ wt % BSA; □, $10^{-3}$ wt % ι-carrageenan; ■, $10^{-3}$ wt % BSA + $4 \times 10^{-3}$ wt % ι-carrageenan.

Most (pure) polysaccharides are not surface-active because they lack the hydrophobic side chains that provide the strong driving force for protein adsorption. This is clearly seen in Figure 1 to be the case for the ι-carrageenan solution, which has an essentially constant tension value (71.8 mN $m^{-1}$), close to that for pure water, over the whole experimental time scale.

The mixed biopolymer solution shows time-dependent tension data intermediate between pure protein and pure polysaccharide behavior. The $\gamma$ value reached after 6−7 h is *ca.* 66 mN $m^{-1}$, which is about 2 units higher than for BSA alone. The effect of the polysaccharide in reducing the rate of lowering of the surface tension by the protein is consistent with a substantial net attractive interaction between BSA and ι-carrageenan molecules in bulk solution, which lowers the chemical potential of the protein in the bulk aqueous solution and hence reduces the effective concentration of free BSA available for adsorption. In other words, the BSA−ι-carrageenan complex is less surface-active than BSA. The smaller rate of lowering of $\gamma$ is attributable to the smaller diffusion coefficient (*i.e.* larger hydrodynamic volume) of the protein−polysaccharide complex than for BSA alone and to the blocking of hydrophobic adsorption sites on the complexed protein by the bulky polysaccharide. [A reduction in protein surface hydrophobicity has been demonstrated (Galazka *et al.*, 1997) by probe spectrofluorometry for the analogous system of BSA plus dextran sulfate at pH 7.] As not all of the BSA is necessarily bound to the polysaccharide, the surface film

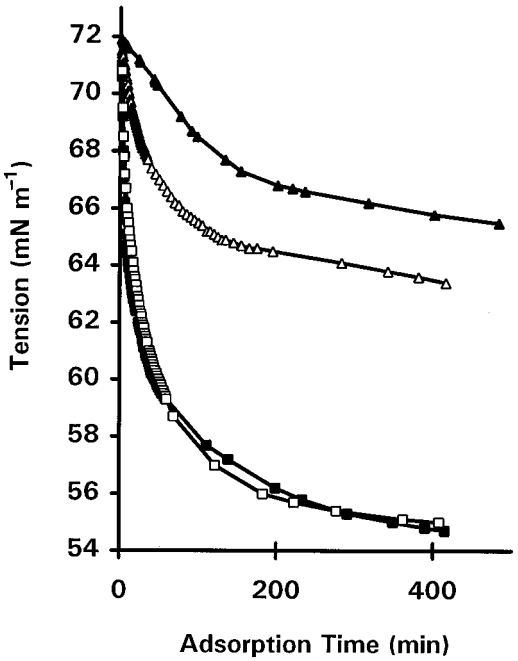


**Figure 2.** Effect of ionic strength on time-dependent surface tension $\gamma$ of biopolymer solutions (pH 7.0, 25 °C): △ and ▲, 5 mM imidazole; □ and ■, 100 mM NaCl. Open and solid symbols refer, respectively, to $10^{-3}$ wt % BSA and $10^{-3}$ wt % BSA + $4 \times 10^{-3}$ wt % $\iota$-carrageenan.

probably consists of a mixture of individual BSA molecules and BSA−$\iota$-carrageenan complexes. The protein−polysaccharide interaction is presumably mainly of an electrostatic nature, as previously proposed for BSA plus dextran sulfate at interfaces (Dickinson and McClements, 1995; Dickinson and Pawlowsky, 1996a,b) and for $\iota$-carrageenan plus caseinate molecules in solution (Keogh *et al.*, 1995). The net attraction at neutral pH is due to interacting patches of positively charged residues on the protein surface with the highly charged backbone on the anion polysaccharide.

The effects of salt concentration on the surface activity of the pure BSA system and the mixed BSA plus $\iota$-carrageenan system at pH 7.0 are shown in Figure 2. Increasing the ionic strength by addition of 0.1 M NaCl leads to a much more rapid drop in surface tension for the BSA solution over the first 30 min. This is probably attributable to a substantial reduction in the electrostatic barrier to adsorption arising from the screening of repulsive protein−protein electrostatic interactions. The surface tension value reached after 6−7 h in the presence of 100 mM NaCl is nearly 10 mN m$^{-1}$ lower than that in the 5 mM buffer solution. The data are consistent with air−water surface tension measurements for BSA in simulated milk ultrafiltrate of ionic strength 80 mM (Paulsson and Dejmek, 1992). At the higher ionic strength, we can see from Figure 2 that $\gamma$-($t$) for the mixed BSA plus $\iota$-carrageenan solution is identical (within the combined experimental error) to that for the BSA alone. This would seem to confirm the electrostatic character of the protein−polysaccharide interaction; when simple salt ions are present to screen the local molecular charges, the evidence for complexation disappears.

To investigate further the effect of solution conditions on the protein−polysaccharide complexation, we consider the influence of pH. Figure 3 shows the time-dependent surface tensions for solutions of BSA alone and BSA plus $\iota$-carrageenan at pH 6.0, 6.5, and 8.0. The change in pH strongly affects the adsorption behavior of the BSA sample itself, with the highest surface

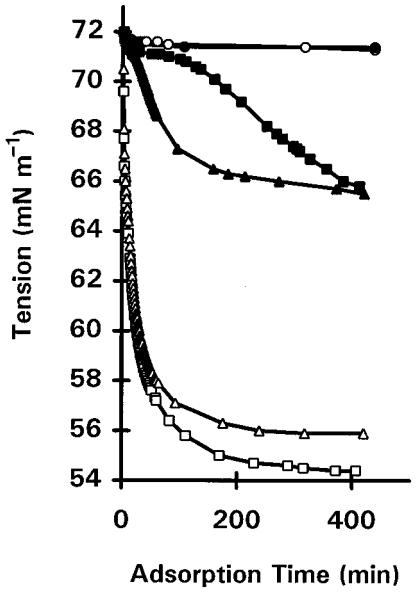


**Figure 3.** Time-dependent surface tension $\gamma$ of biopolymer solutions (5 mM imidazole, 25 °C): ○ and ●, pH 8.0; △ and ▲, pH 6.5; □ and ■, pH 6.0. Open and solid symbols refer, respectively, to $10^{-3}$ wt % BSA and $10^{-3}$ wt % BSA + $4 \times 10^{-3}$ wt % $\iota$-carrageenan.

activity occurring at the lowest pH value ($\gamma$ < 55 mN m$^{-1}$ after 6−7 h at pH 6). At pH 8 the protein has practically no adsorption at all. A similar trend in BSA surface activity as a function of pH was observed by Paulsson and Dejmek (1992) over the pH range 4−6.6. As for the case of increased ionic strength discussed above, the increase in extent and speed of lowering of the surface tension on lowering the pH toward the protein's isoelectric point (p$I \approx 5$) can be explained predominantly in terms of a reduction in the electrostatic barrier to adsorption due to charge screening. On addition of $\iota$-carrageenan to these BSA solutions of different pH values, the most dramatic effect is seen with the solution at pH 6. The difference in surface tension after 6−7 h between the BSA alone and the BSA plus $\iota$-carrageenan mixture is *ca.* 12 mN m$^{-1}$. The $\gamma-t$ plot for the mixed polymer system exhibits a large shoulder indicative of an initial "lag phase" arising from the very slow adsorption of high molecular weight complexes. At pH 6.0 the protein−polysaccharide interaction appears to be very strong, so that the shape (especially at short times) of the mixed biopolymer adsorption curve $\gamma(t)$ resembles more that of the pure polysaccharide curve rather than the typical hyperbolic shape characteristic of rapid protein adsorption.

Figure 4 shows the effect of pH on the difference in surface tension after 400 min, $\Delta\gamma$, between the BSA sample alone and the mixed biopolymer system. The strength of the protein−polysaccharide interaction is clearly sensitive to pH, becoming substantial only for pH ≤ 6.5. It is also interesting to note, however, that although the rate of adsorption for the protein plus polysaccharide mixtures changes with pH, the $\gamma(t)$ curves at pH 6−7 converge to similar $\gamma$ values after several hours. It would appear that the strength of the BSA−carrageenan interaction does not much affect the surface free energy of the final mixed biopolymer film so long as sufficient time is allowed for adsorption and rearrangement.

We now turn our attention to the effect of polysaccharide on the BSA-stabilized emulsion system, which by itself does not show any evidence of flocculation or coalescence. The average diameter of dispersed droplets

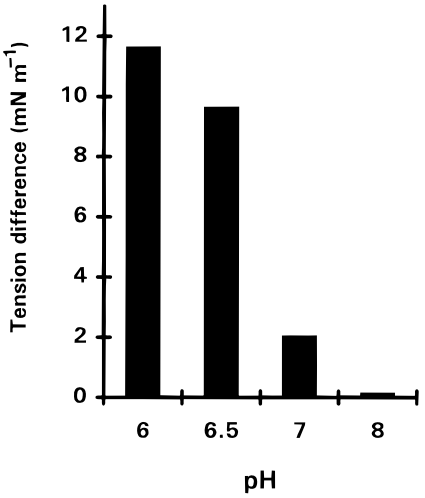


**Figure 4.** Effect of pH on the difference in surface tension values after 400 min, $\Delta\gamma$, for a $10^{-3}$ wt % BSA + $4 \times 10^{-3}$ wt % $\iota$-carrageenan solution and a $10^{-3}$ wt % BSA solution (5 mM imidazole, 25 °C).

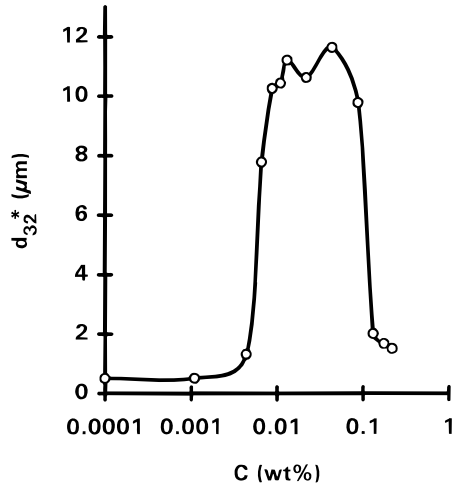


**Figure 5.** Effect of $\iota$-carrageenan on droplet size of emulsions (20 vol % oil, 1.7 wt % BSA, 5 mM imidazole, pH 6.0) stored at 25 °C for 41 h. The apparent average droplet size $d_{32}$* is plotted against the added polysaccharide concentration $C$.

in the emulsion not containing polysaccharide (20 vol % oil, 1.7 wt % BSA, pH 6.0) remains constant at $d_{32} = 0.55 \pm 0.01$ $\mu$m over a period of up to 10 days. Flocculation due to added polymer, which is not reversed on dilution, can be detected by a large increase in the *apparent* particle diameter ($d_{32}$*) measured by multi-angle static light scattering in the Malvern Mastersizer. (The asterisk on $d_{32}$* denotes that the parameter obtained from the Mastersizer is really an average floc size and not the size of the individual particles in flocs.) Figure 5 records $d_{32}$* for a BSA-stabilized emulsion stored for 41 h as a function of the $\iota$-carrageenan concentration. Under these conditions (5 mM imidazole, pH 6, 25 °C) only a modest increase in $d_{32}$* is noticeable (on this scale) up to an added polymer concentration of $C \approx 5 \times 10^{-3}$ wt %. Increasing the polysaccharide concentration further, however, leads to a sharp rise in notional particle size from $d_{32}^* \approx 1$ $\mu$m to $d_{32}^* > 10$ $\mu$m. When the polysaccharide content is above *ca.* 0.1 wt %, $d_{32}$* drops again to lower values, indicating some restabilization. The corresponding droplet-size distributions are shown in Figure 6 for five different $\iota$-carrageenan concentrations. In Figure 6a the narrow distribution $P(d)$ of the original BSA emulsion is first broadened and then split into two peaks on the addition of a small amount of polysaccharide ($C = 0.0044$ wt %).

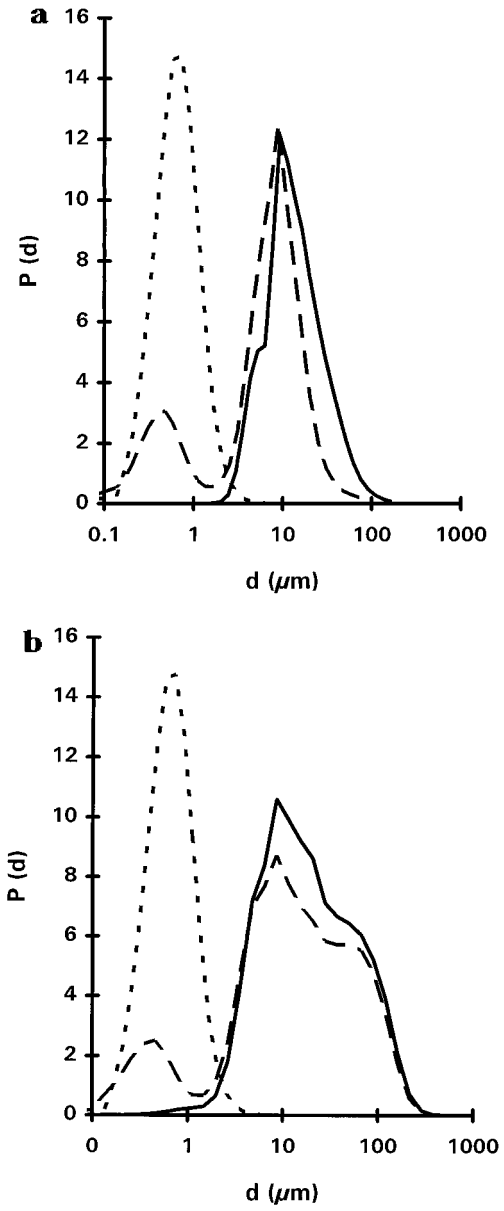


**Figure 6.** Effect of $\iota$-carrageenan on the droplet-size distribution of emulsions (20 vol % oil, 1.7 wt % BSA, 5 mM imidazole, pH 6.0): (a) - - -, no added polymer; — —, $4.4 \times 10^{-3}$ wt %; —, $2.2 \times 10^{-2}$ wt %; (b) - - -, no added polymer; —, $8.8 \times 10^{-2}$ wt %; — —, $1.3 \times 10^{-1}$ wt %.

Adding more polymer leads to a single peak at high droplet size (modal value ~10 $\mu$m). It appears that all of the primary droplets are now incorporated into large flocs. At the highest indicated polysaccharide content of $C = 0.13$ wt % (in Figure 6b), there is a reappearance of the peak in $P(d)$ centered around $d \approx 0.5$ $\mu$m, which is characteristic of the primary droplets, and, although large flocs are still clearly present also at this polysaccharide concentration, it is evident that the flocculation is less extensive than at $C = 0.088$ wt %.

Figure 7 compares the gravity creaming of emulsions at pH 6.0 containing various concentrations of $\iota$-carrageenan. The serum layer thickness estimated by eye is plotted against the storage time. Addition of $1.1 \times 10^{-3}$ wt % $\iota$-carrageenan to the BSA-stabilized emulsion does not affect its stability significantly. At $C = 4.4 \times 10^{-3}$ wt %, however, the aggregation of the emulsion droplets as indicated by the increase in $d_{32}$* and the widening of the size distribution leads to strongly enhanced creaming. The creaming rate plot (Figure 7) shows a sudden increase after an initial lag time of *ca.*

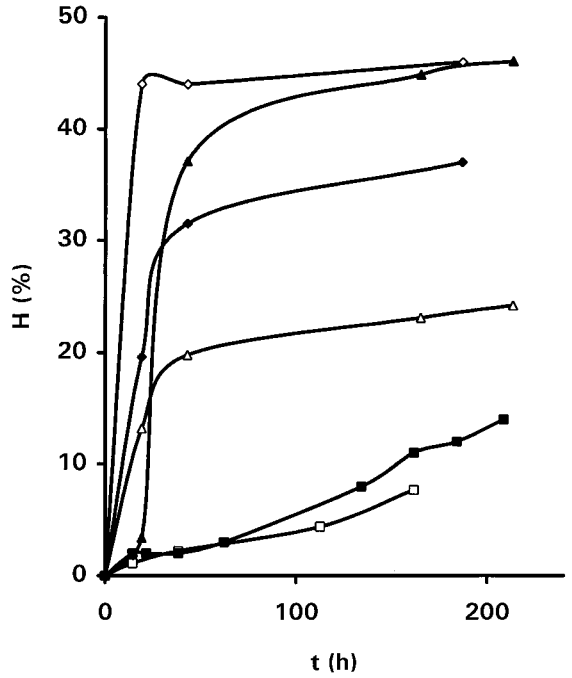


**Figure 7.** Creaming stability of emulsions (20 vol % oil, 1.7 wt % BSA, 5 mM imidazole, pH 6.0) containing various concentrations of ι-carrageenan at 25 °C. The thickness $H$ of the serum layer (expressed as percentage of total sample height) is plotted against storage time $t$: □, no added polymer; ■, $1.1 \times 10^{-3}$ wt %; ▲, $4.4 \times 10^{-3}$ wt %; △, $2.2 \times 10^{-2}$ wt %; ◇, $8.8 \times 10^{-2}$ wt %; ◆, $1.3 \times 10^{-1}$ wt %.

18 h. The formation of aggregates appears to be rather slow, but, once formed, the flocs steadily rise under gravity to the top of the emulsion, possibly dragging along with them most of the single unflocculated droplets and leaving behind a clear serum. It was shown previously (Dickinson and Pawlowsky, 1996a,b) with the BSA plus dextran sulfate system, that, under conditions where there is some interfacial protein−polysaccharide complexation, the addition of polymer at a concentration below that required for total surface coverage can lead to bridging flocculation. We assume that the same phenomenon is occurring in our BSA plus ι-carrageenan systems. Somewhat similar behavior has also been found in studies of interactions between carrageenan and casein micelles (Langendorff *et al.*, 1997); in this case, bridging flocculation caused enhanced sedimentation.

Figure 8 shows the influence of polysaccharide concentration on the serum layer thickness $H$ after 9 days of storage (expressed as a percentage of the total emulsion sample height). With increasing polysaccharide content, there is a systematic reduction in $H$ from a maximum at $C = 4.4 \times 10^{-3}$ wt % to a minimum at $C = 2.2 \times 10^{-2}$ wt %. At this latter concentration, our interpretation is that we have enough ι-carrageenan present to form a polysaccharide-bridged droplet network throughout the whole emulsion. This is illustrated schematically in Figure 9, in which panels a and b are meant to represent the situations at $C \approx 0.004$ and 0.02 wt %, respectively. Were a network of droplets like that drawn in panel b to be sufficiently strong and permanent, it could confer long-term creaming stability (Dickinson *et al.*, 1997). In our case here, however, we still observe some creaming instability over the time scale of a few days, which indicates that the droplet network structure is not very permanent. The network seems partially to break down when the emulsion is diluted into the water bath of the Mastersizer for particle-size determination. The measured distribution $P(d)$ for

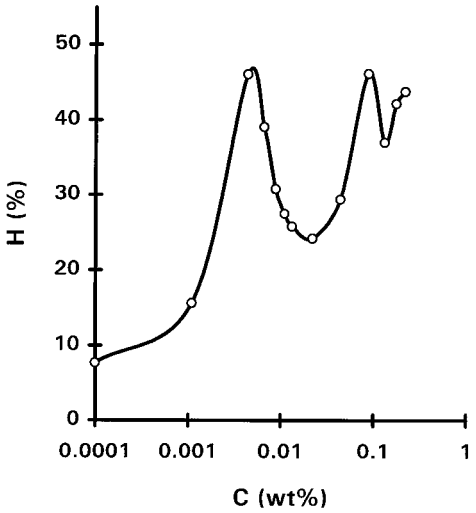


**Figure 8.** Effect of ι-carrageenan on extent of creaming of emulsions (20 vol % oil, 1.7 wt % BSA, 5 mM imidazole, pH 6.0) stored at 25 °C for 8.8 days. The thickness $H$ of the serum layer (expressed as percentage of total sample height) is plotted against the added polysaccharide concentration $C$.

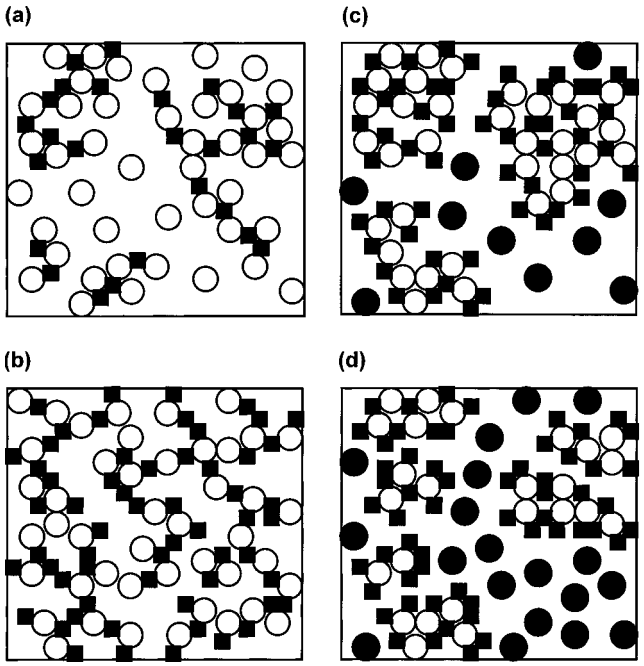


**Figure 9.** Representation (highly schematic) of likely states of flocculation in emulsions studied in creaming experiments at pH 6 as a function of polysaccharide content $C$ (see Figure 8). Open circles represent protein-coated droplets, solid squares represent polysaccharide (and protein−polysaccharide complex) bound to droplets at below saturation surface coverage, and large solid circles represent emulsion droplets fully saturated with secondary stabilizing polysaccharide layer (not to scale). The four states are as follows: (a) $C \approx 0.004$ wt % (dispersion of separate bridging flocs); (b) $C \approx 0.02$ wt % (network of polysaccharide bridged droplets); (c) $C \approx 0.08$ wt %, (dispersion of sterically stabilized droplets plus separate bridging flocs); (d) $C \geq 0.13$ wt %, like (c), but with smaller bridging flocs.

these emulsions (Figure 6b) indicates large aggregates only; there is no evidence of particles of the size of the original emulsion droplets, implying that all of the primary oil droplets are incorporated into polymer-bridged aggregates. For $C > 2.2 \times 10^{-2}$ wt %, $H$ increases again to a maximum value of 43% of the total emulsion height at $C = 8.8 \times 10^{-2}$ wt %. The corresponding $d_{32}$* values are similar to those found for the "networked" emulsions ($4.4 \times 10^{-3}$ wt % $\leq C \leq 2.2 \times 10^{-2}$ wt %), although at $C = 8.8 \times 10^{-2}$ wt % the value

is slightly lower ($d_{32}$* ≈ 10 μm). It was also noticed that $d_{32}$* values for these emulsions tend to decrease with storage time. On extended storage, polysaccharide bridges can become gradually flattened out on the surface of emulsion droplets (Dickinson and McClements, 1995). We are presumably here approaching the polysaccharide concentration region at which the amount of added polymer is sufficient to cover some of the droplet surfaces fully, as illustrated in Figure 9c. Bridging flocculation is now replaced by a restabilization mechanism due to combined steric and electrostatic repulsion between ι-carrageenan-covered particles. This seems to be a transition region because the droplet-size distribution still indicates large aggregates of particles (as for the network structure regime), but the increase in creaming rate and the fall in $d_{32}$* with storage time are indicative of a weakening of the interdroplet interactions. Also, the creaming rate at $C = 8.8 \times 10^{-2}$ wt % is very fast. Taking all of these observations together, it can be speculated that the polymer bridges are rearranging themselves, eventually breaking down the emulsion network structure into separate more compact aggregates, which then cream quickly due to their large size (see Figure 9c).

In going from the "transition regime" ($C \approx 9 \times 10^{-2}$ wt %) to even higher polysaccharide concentrations, we find $d_{32}$* falling to values much closer to that of the original emulsion and the distribution $P(d)$ showing the presence of some individual restabilized droplets (see Figure 6b). There is a slight minimum in $H(C)$ at $C \approx$ 0.1 wt % in Figure 8, which may or may not be significant. From its still turbid appearance at the end of the experiment, isolated dispersed particles could be inferred to exist in the residual serum of the emulsions with $C > 4.4 \times 10^{-2}$ wt %. (At low polysaccharide contents the residual serum was quite clear.) At the highest ι-carrageenan concentrations studied ($C \geq 0.13$ wt %), the serum height after 9 days appears to be increasing again (Figure 8). The particle-size distributions of these systems show the presence of more restabilized single droplets (Figure 9d) along with flocs of droplets, which are the reason for continued strong creaming. As more droplets exist separately at the high polysaccharide contents, less are necessarily involved in the more space-filling aggregates; this results in a denser cream layer (*i.e.* an increase in $H$). A rather similar mechanism of stabilization of milk protein-coated emulsion droplets through full adsorption of κ-carrageenan was postulated elsewhere (Dalgleish and Hollocou, 1997).

In this description of how added ι-carrageenan influences emulsion stability, we have neglected any effect of the rheology of the continuous aqueous phase. We consider this to be legitimate because the low-stress apparent viscosities of BSA plus ι-carrageenan solutions at the same concentrations as in the aqueous phase of the studied 20 vol % emulsions are all below 10 mPa s (at a shear stress of ~0.1 Pa). The solutions form gels only at much higher concentrations. A significant increase in continuous phase viscosity due to added polysaccharide could be expected to enhance emulsion stability (Cao *et al.*, 1990), but such behavior was not apparent in the present systems.

On the assumption that the attractive BSA−ι-carrageenan interaction is predominantly electrostatic in character, one expects emulsion stability to be sensitive to pH. Table 1 shows the pH dependence (in the range 5.5−9.0) of the effect of polysaccharide addition (at three levels) on the apparent average droplet size $d_{32}$*. At pH 9 the freshly made protein-stabilized emulsion is

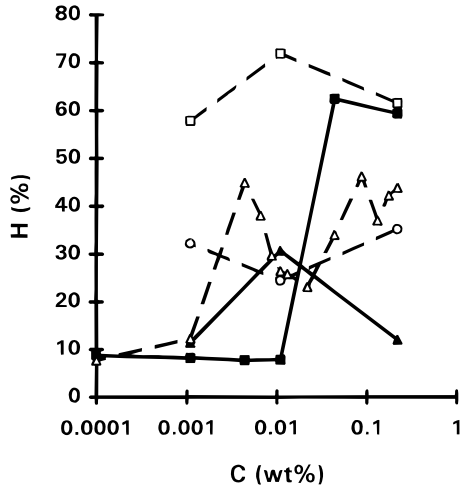


**Figure 10.** Effect of pH on extent of creaming of polysaccharide-containing emulsions (20 vol % oil, 1.7 wt % BSA, 5 mM imidazole) stored at 25 °C for 8 days. The thickness $H$ of the serum layer (expressed as percentage of total sample height) is plotted against the added ι-carrageenan concentration $C$: □, pH 9.0; ■, pH 7.0; ▲, pH 6.5; △, pH 6.0; ○, pH 5.5.

**Table 1. Effect of pH and ι-Carrageenan Concentration $C$ on the Apparent Average Droplet Size $d_{32}$* of Emulsions (20 vol % Oil, 1.7 wt % BSA, 5 mM Imidazole) Stored at 25 °C for 8 Days**

| | $d_{32}$* (μm) | | |
|---|---|---|---|
| pH | $C = 0.0011$ wt % | $C = 0.011$ wt % | $C = 0.22$ wt % |
| 9.0 | 0.64 | 0.65 | 1.01 |
| 7.0 | 0.75 | 1.6 | 1.07 |
| 6.5 | 0.73 | 1.3 | 0.93 |
| 6.0 | 0.53 | 10.6 | 1.4 |
| 5.5 | 1.09 | 11.0 | 15 |

itself flocculated due to the low surface activity of the protein (see Figure 3). Under these conditions BSA molecules are shared between adjacent droplet surfaces, as in the case of insufficient emulsifier. The flocculation induces extensive creaming as shown in Figure 10. In this system it was noted that the aggregated droplets tended to redisperse into individual droplets with time, possibly due to further gradual protein adsorption and associated interfacial reorganization.

The creaming behavior of the BSA-stabilized emulsion at pH 7.0 is rather different from that at pH 9.0 or 6.0. A thick clear serum layer forms very rapidly at ι-carrageenan concentrations above $1.1 \times 10^{-2}$ wt %, whereas at lower added polymer levels the emulsion forms an ill-defined thin cloudy serum layer. The particle-size distributions (not shown) suggest a restabilization of the individual droplets (especially at $C = 4.4 \times 10^{-2}$ wt %), which is not observed at lower concentrations. Possibly, the ι-carrageenan molecules cover the emulsion droplet surface more effectively at pH 7 than at pH 6, due to the mixed biopolymer interaction being weaker at the higher pH, thereby giving the polymers more opportunity for rearrangement at the surface. (Conversely, at pH 6, droplets will tend to become stuck together locally as the concentrated ι-carrageenan solution is introduced.) Once polysaccharide covers the oil droplets fully, thereby giving them a high net negative charge, additional ι-carrageenan could lead to destabilization by a depletion mechanism, as demonstrated independently for sodium caseinate (Dickinson *et al.*, 1997). This would be consistent with the creaming behavior because depletion flocculation is known to lead rapidly to a clear serum (Dickinson *et al.*, 1994). At polysaccharide concentrations below that required for total coverage, the biopolymer interaction at pH 7 does

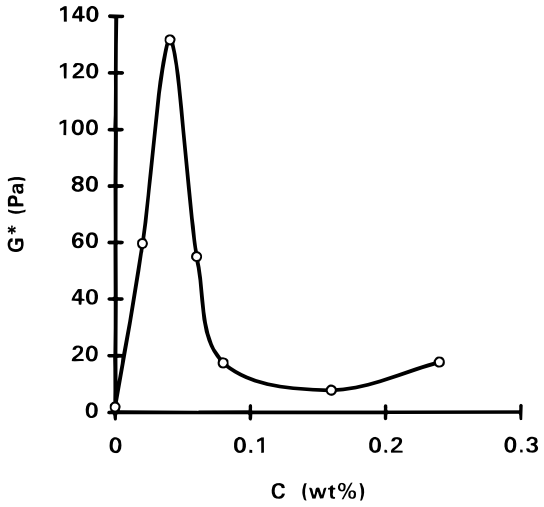


**Figure 11.** Effect of ι-carrageenan on the viscoelasticity of emulsions (40 vol % oil, 2.7 wt % BSA, 5 mM imidazole, pH 6.0). The complex shear modulus $G^*$ at 1 Hz and 30 °C is plotted against added polysaccharide concentration $C$.

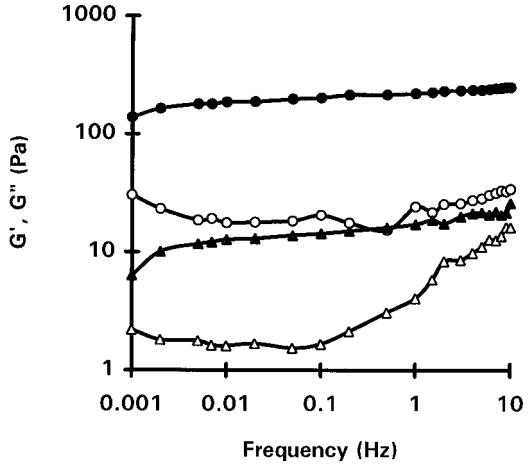


**Figure 12.** Effect of ι-carrageenan concentration on the dynamic shear rheology of emulsions (40 vol % oil, 2.7 wt % BSA, 5 mM imidazole, pH 6.0). Storage and loss moduli, $G'$ and $G''$, are plotted against frequency on a log−log scale: ● and ○, $C = 0.04$ wt %; ▲ and △, $C = 0.24$ wt %. Solid and open symbols refer to $G'$ and $G''$, respectively.

not seem to be strong enough to result in the same strong bridging network as seen at pH 6.

As shown in Figure 10, the creaming stability plots of $H$ against $C$ at pH 6.5, 6.0, and 5.5 are all quite similar. At the highest ι-carrageenan concentration considered there is a thinner serum layer at pH 6.5 than at pH 6. The particle-size distribution at pH 6.5 (not shown) indicates that the restabilization is more complete, but there is no suggestion of any depletion flocculation as at pH 7. The lower $d_{32}$* value in Table 1 for $C = 0.011$ wt % at pH 6.5 as compared to pH 6 suggests easier break-up of a weaker network under dilution in the Mastersizer. On the contrary, at pH 5.5 (approaching the isoelectric point of BSA), the mixed biopolymer interaction appears to be stronger, as indicated (especially) by the large value of $d_{32}$* at $C = 0.22$ wt %. No restabilized particles were apparent in the particle-size distribution (not shown), although the serum was somewhat cloudy. Figure 10 shows a serum thickness $H$ for the smallest addition of ι-carrageenan (∼0.001 wt %) at pH 5.5 which is double that at pH 6. Again, this is attributable to stronger interparticle bridging giving larger space-filling flocs.

We turn now to the small-deformation viscoelastic properties of the ι-carrageenan-containing emulsions. Figure 11 shows the dependence on ι-carrageenan content of the complex shear modulus $G^*$ at 1 Hz for a concentrated BSA-stabilized emulsion (40 vol % oil, 2.7 wt % protein, ionic strength 5 mM, pH 6). There is a steep increase in $G^*$ up to >100 Pa at low concentrations of polysaccharide followed by a drop and subsequent levelling off to *ca.* 20 Pa at higher concentrations. This correlates well with the creaming results and is fully consistent with the formation of a network of polymer-cross-linked droplets with gel-like rheology at polysaccharide concentrations well below saturation coverage. At increased ι-carrageenan concentrations, a restabilization process takes place and the system becomes less elastic and more viscous (see Figure 12). The ι-carrageenan concentration (weight percent) at which the degree of flocculation is maximized cannot be expected to be exactly identical in both the creaming and the rheology experiments since the balance of factors affecting the two measured properties is not the same. In addition, to give improved sensitivity, the emulsions used in the rheology experiments were deliberately of higher oil volume fraction. When expressed in terms of the polysaccharide available per unit area of surface, the two sets of results in Figures 5 and 11 do coincide reasonably well. The plot of $G^*$ against polysaccharide concentration is similar to that obtained previously for the BSA plus dextran sulfate emulsion system (Dickinson and Pawlowsky, 1996a), except that in the case of ι-carrageenan the maximum is shifted more toward lower values of $C$. Although both polysaccharides are of similar molecular weight (∼$5 \times 10^5$ Da), the larger hydrodynamic size of the ι-carrageenan molecule (as compared with the more compact branched dextran sulfate) means that it can be potentially more effective as a bridging polymer. Any direct comparison of the magnitude of the maximum $G^*$ value for the two sets of systems is not considered worthwhile, since different batches of protein were used for the two sets of experiments, and it has been shown previously (Dickinson and Pawlowsky, 1996b) that the rheology of concentrated BSA-stabilized emulsions varies with the batch number of the commercial BSA sample.

Figure 12 shows the frequency dependence of the storage and loss moduli, $G'$ and $G''$, for two concentrated emulsion samples with low ($C = 0.04$ wt %) and high ($C = 0.24$ wt %) polysaccharide contents, corresponding to the situations of maximum bridging flocculation and restabilization, respectively. It can be seen that polymer bridging does lead to a strong gel-like structure as indicated by $G' \gg G''$ and frequency-independent moduli. At the higher polysaccharide concentration, the system exhibits rheological characteristics more like those of a weak gel or a viscoelastic suspension, with moduli having a stronger frequency dependence, especially $G''$ at the higher frequencies.

We observed no evidence for a bridging maximum in $G^*$ with the corresponding emulsions at pH 7. If any polysaccharide bridges exist at this pH, they are apparently too weak to be detected by dynamic oscillatory rheology measurements. This is in contrast to the BSA plus dextran sulfate emulsion system (Dickinson and Pawlowsky, 1996a,b), which does possess a distinct maximum in shear modulus at pH 7. The difference is presumably attributable to the higher charge density on the dextran sulfate polymer.

## CONCLUSIONS

It has been clearly demonstrated that the anionic food grade polysaccharide ι-carrageenan forms an electro-

static complex with BSA in aqueous solutions at pH ≤7 and low ionic strength. The interaction appears weak at pH 7 but strengthens substantially at lower pH values. The effect of the interaction is detectable in the creaming behavior of 20 vol % oil-in-water emulsions, with strong bridging flocculation destabilizing the system at pH 6 over a certain range of carrageenan concentration. The small-deformation rheological behavior of the ι-carrageenan-containing concentrated emulsions (40 vol %) also supports the concept of solid-like emulsion structure formation through bridging flocculation. Comparison with previous work on the mixed BSA plus dextran sulfate system shows a comparable rheological behavior for both emulsion systems, but, possibly because of its larger molecular size, it is found that a lower amount of ι-carrageenan than dextran sulfate is required to form a bridging network.

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