



# Influence of $\kappa$ -carrageenan on the properties of a protein-stabilized emulsion

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## Abstract

We report on the influence of  $\kappa$ -carrageenan ( $\kappa$ -CAR) on the surface activity of bovine serum albumin (BSA) and on the properties of BSA-stabilized oil-in-water emulsions. Surface tension data at low ionic strength indicate an electrostatic interaction at neutral pH which becomes much stronger at pH 6. The effect of the attractive BSA- $\kappa$ -CAR interaction on the state of aggregation and creaming stability of protein-stabilized emulsions (20 vol% *n*-tetradecane, 1.7 wt% BSA, 5 mM) has been investigated at three pH values. At pH 6 the system behaviour is interpreted in terms of bridging flocculation leading to an emulsion droplet gel network over a certain limited polysaccharide concentration range. While the trend of behaviour is qualitatively similar to that reported recently for equivalent BSA +  $\iota$ -carrageenan ( $\iota$ -CAR) solutions and emulsions, the BSA- $\kappa$ -CAR interaction is clearly weaker than the BSA- $\iota$ -CAR interaction under similar pH and ionic strength conditions. This means that a higher polysaccharide content is required to induce flocculation in systems containing  $\kappa$ -CAR, and also that the resulting emulsion gel network is weaker. The behaviour is consistent with the lower density of charged sulfate groups on the  $\kappa$ -CAR as compared with the  $\iota$ -CAR. © 1998 Elsevier Science Ltd. All rights reserved.

## 1. Introduction

Proteins and polysaccharides are frequently present together in food systems, with both kinds of biopolymers contributing to product texture and shelf-life. In a protein-stabilized oil-in-water emulsion containing polysaccharide in the aqueous phase, attractive protein-polysaccharide interactions can significantly affect the stability of the system. At low polysaccharide contents, polymer chains can link together adjacent pairs of protein-covered oil droplets leading to bridging flocculation. However, at higher polysaccharide contents, sufficient to cover fully the surfaces of the protein-coated particles, the same emulsion may become restabilized as a result of combined electrostatic and steric stabilization (Dickinson & Eriksson, 1991).

We have been investigating properties of oil-in-water emulsions stabilized by bovine serum albumin (BSA) containing anionic sulfated polysaccharides. It was found previously (Dickinson & Pawlowsky, 1996b, 1997) that bridging flocculation can be induced at low ionic strength by addition of either (i) dextran sulfate, a convenient model polysaccharide of high charge density,

or (ii)  $\iota$ -carrageenan ( $\iota$ -CAR), a hydrocolloid commonly used in milk-based food products for stabilization, thickening and gelation. It was shown (Dickinson & Pawlowsky, 1996b, 1997) that the protein-polysaccharide interaction is electrostatic in nature, and that pH and ionic strength are important experimental variables controlling the strength of complexation. In systems containing dextran sulfate, a significant effect was observed at neutral pH. In systems containing  $\iota$ -CAR, however, the pH had to be reduced to  $\leq 6.5$  in order for the effect of the attractive protein-polysaccharide interaction to be detectable. This difference is presumably due to the higher charge density on the dextran sulfate molecule. The present paper compares the effect of  $\iota$ -CAR on the BSA-stabilized emulsion system with that of another important member of the carrageenan family, i.e.  $\kappa$ -carrageenan ( $\kappa$ -CAR).

The main difference between the two polysaccharides,  $\iota$ -CAR and  $\kappa$ -CAR, is in the number of sulfate groups on the polygalactose backbone. Whereas  $\iota$ -CAR carries about 32 % ester sulfate groups by weight,  $\kappa$ -CAR has only ~25% (Enriquez & Flick, 1989). Also, it is known (Norton, Goodall, Morris, & Rees, 1983) that  $\kappa$ -CAR binds  $K^+$  -ions more strongly than  $\iota$ -CAR, leading to an ordering of the structure from coil to helix (Piculell,

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1991). There is a greater charge density in the helical form due to shorter distances between ionized groups (Kvam & Grasdalen, 1989). In dilute solution in the absence of potassium ions, the  $\kappa$ -CAR helices have less tendency to aggregate than do the  $\iota$ -CAR helices (Van-neste, Sloommaekers, & Reynaers, 1996).

The interaction of  $\kappa$ -CAR with proteins has been widely studied, especially for the case of caseins (Snoeren, Both, & Schmidt, 1976; Keogh, Lainé, & O'Connor, 1995; Lynch & Mulvihill, 1994), but also for other proteins, e.g. soy protein (Kampf & Nussinovitch, 1997). Due to the higher charge density, the electrostatic interaction of the  $\kappa$ -CAR helix with positively charged amphiphilic drug molecules inferred from surface tension measurements has been shown (Persson & Caram-Lelham, & Sundelöf, 1996) to be stronger than for the case of the  $\kappa$ -CAR coil form. A qualitatively similar sort of effect has been observed (Dalgeish & Morris, 1988) for the  $\kappa$ -CAR-casein micelle interaction. Caseins can be stabilized against  $\text{Ca}^{2+}$  precipitation in the presence of carrageenan, and the effect is apparently more important for  $\iota$ -CAR than  $\kappa$ -CAR (Hansen, 1993). The presence of  $\kappa$ -CAR has also been found (Dalgeish & Hollocou, 1997) to protect milk protein-stabilized oil droplets from aggregating at low pH.

There have been various attempts in the recent colloid science literature to interpret the effectiveness of a polymer for inducing bridging flocculation in terms of factors such as molecular weight, charge density and conformational structure (Chaplain, Janex, Lafuma, Graillat, & Audebert, 1995; Gray, Harbour, & Dixon, 1997; Leong, Scales, Healy, & Boger, 1995; Lips, Campbell, & Pelan, 1991; Walker & Grant, 1996; Xu & Somasundaran, 1996). The two types of carrageenan compared in this study have different molecular weights and charge densities. We therefore want to explore how the effect of  $\kappa$ -CAR on BSA-stabilized emulsions compares with our previously obtained data for systems containing BSA +  $\iota$ -CAR (Dickinson & Pawlowsky, 1997). For this purpose we have also carried out surface tension measurements on BSA +  $\kappa$ -CAR mixed solutions, as well as measurements of electrophoretic mobility, particle-size distributions and gravity creaming on the equivalent emulsion systems. In order deliberately to enhance the influence of non-specific electrostatic interactions, the experiments were performed with the same low ionic strength buffer (5 mM imidazole) as used previously for the  $\iota$ -CAR experiments (Dickinson & Pawlowsky, 1997). This medium differs from the aqueous environment present in many carrageenan-containing foods, where the presence of potassium ions induces the formation of brittle gel networks from extended rod-like supermolecular aggregates (Sugiyama, Rochas, Turquois, Taravel, & Chanzy, 1994; Piculell, 1995; Borgström, Piculell, Viebke, & Talmon, 1996).

## 2. Materials and methods

### 2.1. Materials

Food-grade  $\kappa$ -carrageenan ( $\kappa$ -CAR) was kindly donated by Systems Bio Industries (Carentan, France). It was 60% in the potassium form and 40% in the sodium form. The weight-average molecular weight was given by the suppliers as  $7.2 \times 10^5$  Da and the z-average hydrodynamic radius as 100 nm. Bovine serum albumin (BSA) (lyophilized globulin-free powder,  $\geq 99\%$ , product no. A7638, lot no. 16H9314) and *n*-tetradecane were purchased from Sigma Chemicals (St. Louis, MO). All other reagents were AnalaR grade. The buffer solution (5 mM imidazole) was prepared with double-distilled water (0.02 wt% sodium azide added as antimicrobial agent in the creaming experiments). Polysaccharide solutions were prepared by dispersing the  $\kappa$ -CAR powder in buffer and continuously stirring for 30 min at 70°C. The resulting solutions of  $\kappa$ -CAR (up to 0.22 wt%) were close to Newtonian, with no signs of gelation under these electrolyte conditions. The protein sample was dissolved in aqueous pH 7 buffer solution (concentration: 4.6 wt% for emulsion preparation, 0.25 wt% for surface tension measurements) and the pH was adjusted in the range 5–7 with HCl or NaOH.

### 2.2. Surface tension measurements

Protein solutions were diluted to  $10^{-3}$  wt% BSA with buffer of the appropriate pH in the range 5–7. For measurements on mixed systems, the BSA to  $\kappa$ -CAR weight ratio was 1:4 while the BSA concentration was kept at  $10^{-3}$  wt%. The time-dependent change in the 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 already adsorbed molecules. The estimated experimental error in the tension measurements was ca.  $0.5 \text{ mN m}^{-1}$ .

### 2.3. Emulsion preparation and microelectrophoresis

Oil-in-water emulsions were prepared at room temperature using a laboratory-scale jet homogenizer (Burgaud, Dickinson, & Nelson, 1990) operating at 300 bar. Emulsions were made from 45 vol% *n*-tetradecane + 55 vol% aqueous phase containing 4.6 wt% protein. Particle electrophoretic measurements were recorded with a

Malvern Zetasizer 4 laser Doppler apparatus. A single droplet of BSA-stabilized stock emulsion was introduced into a set of buffered solutions of  $\iota$ -CAR or  $\kappa$ -CAR (concentration  $10^{-5}$ – $10^{-1}$  wt%) prepared by serial dilution, and the highly diluted emulsion samples were injected into the quartz capillary cell. Quoted values of the electrophoretic mobility ( $\pm 0.2 \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ ) are based on duplicate sets of experiments.

#### 2.4. Emulsion stability

Parts of the concentrated stock BSA-stabilized emulsion (45 vol% oil) were diluted with  $\kappa$ -CAR solutions to give final emulsion samples (20 vol% oil, 1.7 wt% BSA) containing various concentrations of  $\kappa$ -CAR in the range 0–0.44 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. The extent of gravity creaming was determined over a period of 9 days by following visually the change in thickness of the serum layer, a distinct (semi-transparent) region, at the bottom of the tube. The average error in the serum layer height was ca. 5%. 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  $d_i$ , was determined using a Malvern Mastersizer S2.01. Values of  $d_{32}$  were found to be reproducible to  $\pm 2\%$  for the original polysaccharide-free emulsions, but this increases to up to  $\pm 10\%$  for the most extensively flocculated emulsions.

### 3. Results and discussion

We first compare the surface activities of solutions of BSA and BSA +  $\kappa$ -CAR mixtures to confirm the presence of soluble complexes. Fig. 1 shows the time-dependent surface tension  $\gamma(t)$  for BSA alone and BSA +  $\kappa$ -CAR in 5 mM imidazole buffer at two different pH values. The  $10^{-3}$  wt% BSA solution exhibits very low surface activity at pH 7; the tension is only reduced by ca.  $2 \text{ mN m}^{-1}$  after 400 min. This result contrasts with previous surface tension measurements of BSA solutions under the same conditions (Dickinson & Pawlowsky, 1997) where  $\gamma$  was found to decrease by  $9 \text{ mN m}^{-1}$  units after  $t = 400$  min. This difference is attributable to the use of different BSA batches in the two studies, since the surface properties of globular proteins have been previously reported to vary between commercial batches of the same 'pure' protein (Clark et al., 1995; Dickinson & Iveson, 1993; Dickinson & Pawlowsky, 1996a).

Addition of  $\kappa$ -CAR to BSA at weight ratio 4:1, whilst keeping the BSA concentration at  $10^{-3}$  wt%, leads to a slight increase in surface tension, i.e. to a decrease in

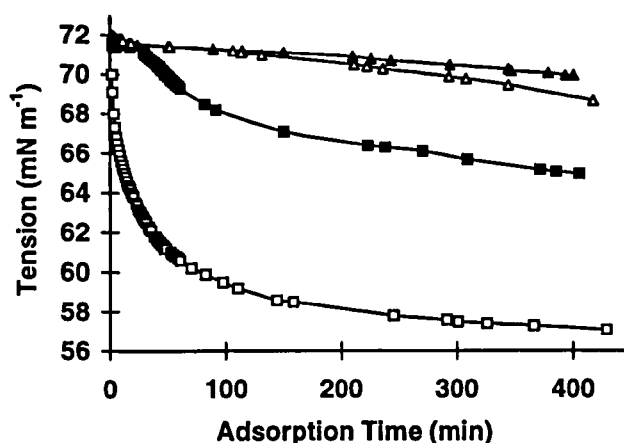


Fig. 1. Time-dependent surface tension  $\gamma(t)$  of biopolymer solutions (5 mM imidazole, 25°C):  $\triangle$  and  $\blacktriangle$ , pH 7;  $\square$  and  $\blacksquare$ , pH 6. Open and filled symbols refer, respectively, to  $10^{-3}$  wt% BSA and  $10^{-3}$  wt% BSA +  $4 \times 10^{-3}$  wt%  $\kappa$ -CAR.

solution surface activity, possibly indicating some protein–polysaccharide complexing, as was seen previously in the case of BSA +  $\iota$ -CAR (Dickinson & Pawlowsky, 1997). The chemical potential of the protein in the bulk aqueous phase solution is lowered and hence the effective concentration of free BSA available for adsorption reduced.

When the pH of the system was reduced to pH 6, the pure BSA solution was found to have a considerably higher surface activity, probably due to the lowered net charge on the protein reducing protein–protein repulsion and hence the electrostatic barrier to adsorption. Again, at this pH, we find a lower steady state value at  $t = 400$  min ( $\gamma = 58 \text{ mN m}^{-1}$ ) in comparison to the previous study with the different batch of BSA ( $\gamma = 54 \text{ mN m}^{-1}$ ) (Dickinson & Pawlowsky, 1997).

On addition of  $\kappa$ -CAR at pH 6, the surface tension of the BSA solution decreases slowly within the first 30 min, after which it declines more rapidly and finally levels off. The delay in surface tension reduction is attributed to the slow diffusion of protein–polysaccharide complexes to the surface and/or the masking of hydrophobic sites on the protein by the more bulky polysaccharide moiety (Dickinson & Pawlowsky, 1997). The higher equilibrium value of  $\gamma$  for the mixed solution as compared with the pure BSA solution is also consistent with an attractive interaction between the molecules. The existence of an electrostatic interaction above the isoelectric point of the protein, where both biopolymers carry net negative charge, is assumed to involve interaction of the anionic polysaccharide with small patches of positive charge on the BSA surface.

The influence of pH on the strength of complexation is clearly shown in Fig. 2. The difference in surface tension between the pure BSA solution and the mixed

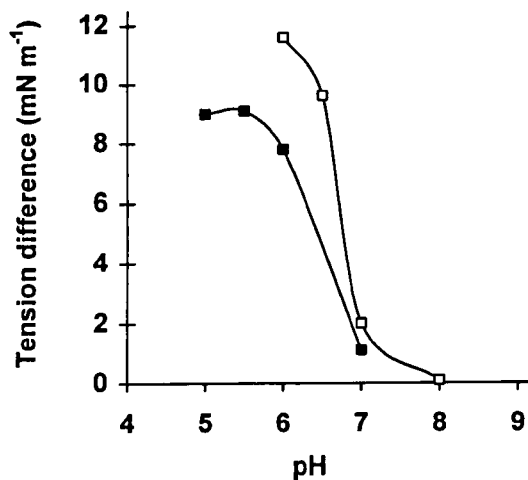


Fig. 2. Effect of pH on difference in surface tensions after 400 min for a  $10^{-3}$  wt% BSA +  $4 \times 10^{-3}$  wt% carrageenan solution and a  $10^{-3}$  wt% BSA solution (5 mM imidazole, 25°C): □,  $\iota$ -CAR; ■,  $\kappa$ -CAR.

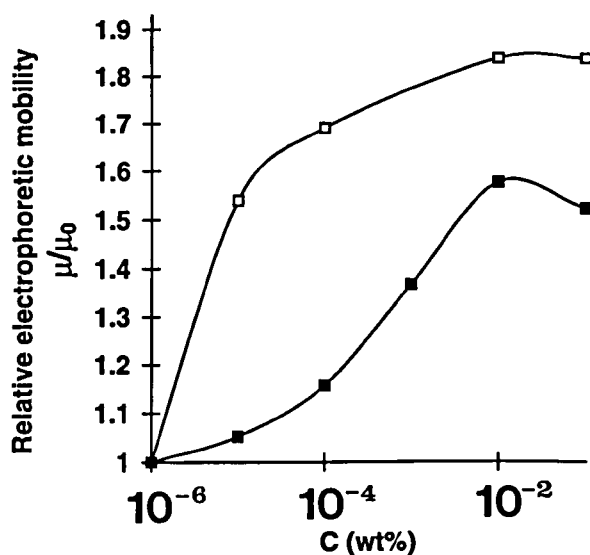


Fig. 3. Effect of carrageenan concentration  $C$  on the electrophoretic mobility  $\mu$  at 25°C of BSA-coated emulsion droplets (45 vol% oil, 3 wt% protein, 5 mM imidazole) dispersed in solutions of carrageenan at pH 5.5 (quoted values are relative to  $\mu_0$  at  $C=0$ ): □,  $\iota$ -CAR; ■,  $\kappa$ -CAR.

BSA + carrageenan solution after  $t=400$  min,  $\Delta\gamma(400$  min), is plotted against pH for both  $\kappa$ -CAR and  $\iota$ -CAR. The quantity  $\Delta\gamma(400$  min) increases with decreasing pH and appears to level off at the lowest pH. At the same pH, a larger value of  $\Delta\gamma(400$  min) for  $\iota$ -CAR than for  $\kappa$ -CAR is consistent with a stronger BSA–carrageenan interaction for the former than the latter. The behaviour is consistent with a protein–polysaccharide interaction that is electrostatic in nature and one which is therefore favoured when the protein molecule carries fewer negative charges at a pH near to its isoelectric point. The complexation becomes substantial only for  $\text{pH} < 7$ . The levelling off in  $\Delta\gamma(400$  min) for the BSA +  $\kappa$ -CAR system at the lowest pH may indicate a ‘saturation’ in complexing; possibly all the available BSA molecules have become attached to carrageenan chains at  $\text{pH} \approx 5.5$ , and so any further reduction in pH does not have a significant influence on  $\gamma(t)$ .

We now turn to properties of emulsion systems containing protein-coated oil droplets and polysaccharide added in various amounts to the aqueous phase. Fig. 3 shows the effect of polysaccharide concentration on the relative electrophoretic mobility  $\mu/\mu_0$ , where  $\mu_0$  is the mobility with no carrageenan present. To obtain each experimental point in Fig. 3, a single drop of BSA-stabilized emulsion was highly diluted in a pH 5.5 solution of  $\iota$ -CAR or  $\kappa$ -CAR. In both cases, the increase in  $\mu/\mu_0$  with increasing polysaccharide concentration is consistent with the carrageenan adsorbing onto the BSA-coated droplets. The higher relative mobility with the  $\iota$ -CAR samples is consistent with the higher charge density on this polysaccharide. (A minor additional factor is the

presumably slightly larger diameter of  $\kappa$ -CAR-covered droplets:  $\kappa$ -CAR has 1.5 times the molecular weight of  $\iota$ -CAR and a 20% larger hydrodynamic volume.) Comparing the effects of  $\iota$ -CAR and  $\kappa$ -CAR, we can see in Fig. 3 that the difference in  $\mu/\mu_0$  is most strongly apparent at the lower polysaccharide concentrations. This again supports the idea of a considerably weaker electrostatic interaction for BSA– $\kappa$ -CAR than for BSA– $\iota$ -CAR. Qualitatively similar trends were obtained by Dalglish and Morris (1988) for mixed solutions of casein micelles +  $\iota$ -CAR or  $\kappa$ -CAR.

To investigate the effect of  $\kappa$ -CAR content on stability properties, we carried out experiments on  $\kappa$ -CAR-containing BSA-stabilized 20 vol% oil-in-water systems. Samples were stored at 25°C for nine days, and the particle-size distribution and extent of gravity creaming were observed. Fig. 4 shows the *apparent* average droplet diameter  $d_{32}^*$  as a function of added  $\kappa$ -CAR concentration for three different pH values. (The asterisk on  $d_{32}^*$  is to denote that the parameter measures average floc size and not the sizes of actual individual particles). Emulsions with no polysaccharide added were found to have a constant average droplet diameter of  $d_{32}^* = 0.55 \pm 0.01 \mu\text{m}$  over the experimental timescale. Addition of  $\kappa$ -CAR to the system at pH 7 leads to no significant effect—except for a slight increase in  $d_{32}^*$  at the highest concentration (0.22 wt%) which is indicative of a very slight degree of flocculation.

Polymer-induced flocculation by  $\kappa$ -CAR becomes apparent when the pH value is reduced to 6. Fig. 4 has a maximum in  $d_{32}^*(C)$  at  $C \approx 0.1$  wt%. A similar trend obtained previously (Dickinson & Pawlowsky, 1997) for

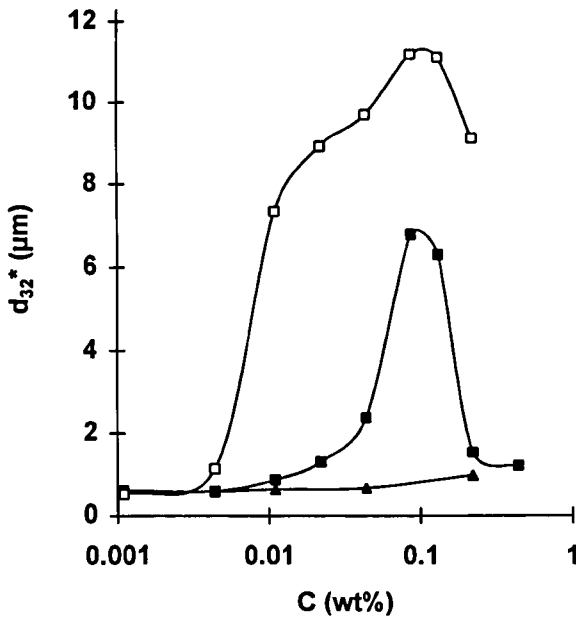


Fig. 4. Effect of pH on the extent of flocculation of  $\kappa$ -CAR-containing emulsions (20 vol% oil, 1.7 wt% BSA) stored at 25°C for nine days. Apparent average droplet diameter  $d_{32}^*$  is plotted against added  $\kappa$ -CAR concentration  $C$ :  $\blacktriangle$ , pH 7;  $\blacksquare$ , pH 6;  $\square$ , pH 5.5.

$\iota$ -CAR in BSA-stabilized emulsions was attributed to a bridging flocculation mechanism. The charged polysaccharide molecules interact with adsorbed protein on the surfaces of two (or more) emulsion droplets forming macromolecular bridges between them. The large increase in  $d_{32}^*$  reflects the association of the emulsion droplets into large flocs. When the concentration exceeds that required for full coverage of the droplet surfaces, the emulsion becomes partially restabilized again as a result of electrostatic repulsion between the polysaccharide-coated droplets. This is indicated in Fig. 4 by a substantial fall in  $d_{32}^*$  at large  $C$  for the pH 6 system, although the  $d_{32}^*$  value does not go right back down to that of the original BSA-stabilized emulsion. The incomplete nature of the restabilization is reflected in a particle-size distribution (not shown) indicative of a mixture of single droplets and flocs (Dickinson & Pawlowsky, 1997). This is due to the poorly reversible character of bridging flocculation. When polysaccharide is added to the emulsion at a relatively high concentration, some aggregates are formed locally before the polymer can become evenly distributed, and the droplet interactions originally formed are so strong as not to permit easy structural rearrangement.

The bridging flocculation of BSA-coated droplets by  $\iota$ -CAR has been discussed previously (Dickinson & Pawlowsky, 1997). Comparing directly the  $d_{32}^*(C)$  plots for the emulsions containing the two types of carrageenan at pH 6, we observe that the maximum in  $d_{32}^*$  is shifted from  $C = 2.2 \times 10^{-2}$  wt% for  $\iota$ -CAR to  $C = 0.1$

wt% for  $\kappa$ -CAR. Also, the maximum value of  $d_{32}^*$  reached for the  $\kappa$ -CAR emulsion ( $\sim 7 \mu\text{m}$ ) is only about half that for the  $\iota$ -CAR emulsion (see Fig. 5 of Dickinson & Pawlowsky, 1997). In accordance with previous inferences from the surface tension and electrophoretic mobility measurements, this comparison is consistent with the interfacial protein-polysaccharide interaction being weaker in the  $\kappa$ -CAR-containing emulsions than in the corresponding  $\iota$ -CAR-containing emulsions. The greater width of the flocculation peak in  $d_{32}^*(C)$  for the  $\kappa$ -CAR system is also consistent with a weaker interfacial interaction for BSA +  $\kappa$ -CAR interaction, since it has been suggested elsewhere (Gray et al., 1997; Lips et al., 1991) that a weak binding affinity of the polymer leads to a widening of the bridging window. The difference between the two polysaccharides may be due in part to the differing molecular conformations of the two carrageenans, with the  $\iota$ -CAR being more elongated due to its higher charge. More extended polymers are generally found to be more effective for bridging flocculation (Jin, Hu, & Hou, 1987; Xu & Somasundaran, 1996).

When the pH value is reduced further to 5.5, i.e. closer to the isoelectric point of BSA, the  $d_{32}^*(C)$  plot for the  $\kappa$ -CAR-containing emulsions changes shape (see Fig. 4). The putative maximum appears to have broadening considerably, especially at the lower polysaccharide concentration end. The  $d_{32}^*$  value increases strongly at  $C \approx 10^{-2}$  wt%, which is about a factor of 10 lower than at pH 6. We can infer therefore that the protein-polysaccharide interaction is considerably stronger at pH 5.5 than at pH 6, since at the lower pH much less  $\kappa$ -CAR is required to form a flocculated network of polymer linked droplets. The apparent absence of restabilization possibly indicates that the bridging interaction is too strong to allow rearrangement of the

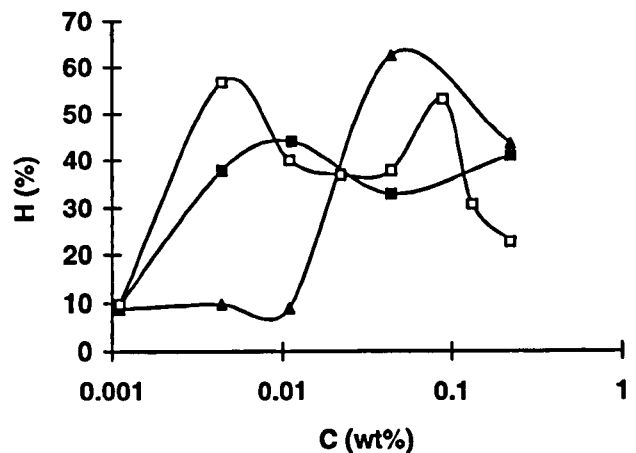


Fig. 5. Effect of pH on creaming of  $\kappa$ -CAR-containing emulsions (20 vol% oil, 1.7 wt% BSA) stored at 25°C for nine days. Thickness  $H$  of the serum layer (expressed as percentage of total sample height) is plotted against  $\kappa$ -CAR concentration  $C$ :  $\blacktriangle$ , pH 7;  $\blacksquare$ , pH 6;  $\square$ , pH 5.5.

polymer on the particle surface once flocs have been formed.

We now move on to consider the effect of pH and polysaccharide concentration on the creaming stability of the emulsions. Fig. 5 shows the influence of  $\kappa$ -CAR concentration on the serum layer thickness  $H$  after 9 days storage (expressed as a percentage of total emulsion sample height). In discussing the data, the effect of the added  $\kappa$ -CAR on the continuous phase viscosity will be neglected as the polysaccharide concentration is kept well below that required for gelling in this potassium-free buffer of low ionic strength. (This contrasts with the supermolecular aggregates and gels of  $\kappa$ -CAR formed at the highest polysaccharide concentration studied here (0.22 wt%) in the presence of  $K^+$  ions (Sugiyama et al., 1994; Piculell, 1995; Borgström et al., 1996).

For low concentrations of added  $\kappa$ -CAR ( $C \leq 10^{-2}$  wt%), the creaming stability is affected at both pH 5.5 and 6, but not at pH 7. The sudden increase in  $H(C)$  at  $C > 0.01$  wt% at pH 7 seems consistent with the slight increase in  $d_{32}^*(C)$  seen in Fig. 4. The implication is that there is some slight bridging flocculation under these conditions, with the result that after several days the small flocs have risen to form a compact cream layer leaving an extensive serum phase ( $H \approx 60\%$ ) at the bottom of the sample. An alternative explanation for the large increase in  $H(C)$  at  $C > 0.01$  wt%, without much significant change in  $d_{32}^*(C)$ , is that the enhanced creaming is due to reversible depletion flocculation of emulsion droplets by the non-adsorbed carrageenan. Such depletion flocculation, as demonstrated elsewhere (Dickinson, Ma, & Povey, 1994; Dickinson, Goller, & Wedlock, 1995) with other polysaccharides like xanthan and dextran, is not normally detectable on the basis of light-scattering of the diluted emulsion. The rather lower value of  $H$  at  $C = 0.22$  wt% can be attributed to the formation of a less compact cream layer from larger (more space-filling) flocs, as discussed already for  $\iota$ -CAR-containing emulsions (Dickinson & Pawlowsky, 1997).

At pH 6, the system behaves similarly to the BSA +  $\iota$ -CAR system (see Fig. 8 in Dickinson & Pawlowsky, 1997), in that there is a maximum in  $H(C)$  in Fig. 5 at  $C = 0.01$  wt%, followed by a reduction to  $H \approx 30\%$ , and then a renewed increase in serum height at higher concentrations. The  $d_{32}^*(C)$  plot in Fig. 4 and the evolving particle-size distributions (not shown) also indicate some flocculation at 0.01 wt%  $\kappa$ -CAR. Polymer bridging becomes more extensive at  $C = 4.4 \times 10^{-2}$  wt% and a network structure is formed which leads to improved creaming stability. On further increase of polysaccharide concentration, we assume that some restabilization takes place based on the droplet-size analysis already discussed (Fig. 4). But, as the restabilization is incomplete, aggregates coexisting with individual dispersed droplets still give rise to enhanced

creaming kinetics and hence a large serum layer after several days storage. As compared with the previous  $\iota$ -CAR experiments (Dickinson & Pawlowsky, 1997), more added polysaccharide is necessary to induce the formation of the gel-like network because of the weaker BSA- $\kappa$ -CAR interaction under the same conditions.

On reducing the pH to 5.5, the  $H(C)$  plot in Fig. 5 remains similar to that for pH 6 with a maximum reached at relatively low  $\kappa$ -CAR content. In this case we recall that the  $d_{32}^*(C)$  data in Fig. 4 show no obvious evidence of restabilization at high  $\kappa$ -CAR concentrations; instead, the particle network becomes more extensive leading to a less developed serum layer. The second maximum in  $H(C)$  at  $C = 0.1$  wt% is not easily explained.

In conclusion, we can infer from this study that  $\kappa$ -CAR forms electrostatic complexes with BSA in bulk aqueous solution as well as at the surface of emulsion droplets. The strength of the interaction increases as the pH is reduced from neutral towards the protein's isoelectric point. Protein-polysaccharide interaction in the emulsion samples leads to bridging flocculation. In comparison with our previous experiments involving the more highly charged  $\iota$ -CAR, the combination BSA +  $\kappa$ -CAR has a weaker interaction, which is clearly reflected in the differing flocculation and creaming behaviour. It would be interesting to extend these experiments to higher ionic strength buffers containing potassium and calcium ions, since such conditions are more representative of the ionic environment in many food systems.

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