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Gelation and flocculation of casein micelle/carrageenan mixtures

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The specific attractive interaction between carrageenan and casein micelles is widely exploited in the dairy industry to tailor the texture of dairy desserts. However, in industrial practice it has been observed that the addition of carrageenan can cause casein micelles to sediment rapidly when the mixture is above the gelation temperature. To better understand this undesirable phenomenon, carrageenan/skim milk mixtures have been observed over a wide range of compositions at 65 and 25°C. Interactions occurred for iota, kappa and lambda carrageenan at both temperatures. At 65°C sedimentation was observed when the carrageenan concentration was above 0.2%. At 25°C either phase separation or gelation could occur. Depletion flocculation is suggested as a partial explanation for these observations. The effects of casein micelles on the viscoelastic properties of iota carrageenan have been measured in the absence of flocculation. Their presence decreases carrageenan's gelation concentration and increases both the gelation temperature and the elastic modulus.

Introduction

The unique interaction between casein micelles and carrageenans is used widely in the food industry to make milk gels and to 'stabilize' products such as milk fat emulsions, ice cream and chocolate milk (1–6).

Carrageenans are sulphated polysaccharides extracted from red seaweed, well known for their gelling and thickening properties. The different types of carrageenan vary in the number and position of the sulphate groups on the galactose dimer. Iota- and kappa-carrageenan undergo a coil (disordered state) to helix (ordered) transition, depending on the temperature and the ionic environment (6). The junction zones formed by the double helices between carrageenan chains lead to the formation of a tri-dimensional network and gelation. Helix formation is prevented in lambda-carrageenan by its $^4C_1$ conformation (rather than $^4C_1$–$^1C_4$ alternation in iota- and kappa-carrageenan) and the extra sulphate group not present in iota- and kappa-carrageenan.

Milk is ~3.3 wt% protein, of which 2.6% is in the form of caseins (α, β and κ), and 0.65 wt% mineral components. Its pH is 6.7. The pI of caseins is 4.6 (8). Caseins in milk have the peculiarity of self-associating into 'micelles'. One of the most frequently cited models [Schmidt and Payens, in Dickinson and Stainsby (9)] represents the casein micelle as being formed by the association of submicelles via calcium phosphate bridges. The submicelles are spherical aggregates of several casein molecules linked by hydrophobic and electrostatic bonds. The hydrophilic zones of the caseins are oriented to the exterior of the submicelles. The κ-caseins are mostly located on the periphery of the micelles, with their hydrophilic C-terminal part behaving as flexible chains in the solvent, forming a hairy layer around the casein micelles and giving them colloidal stability. Part of the κ-casein chain is positively charged, even at pH values above the pI (2); this part is located between residues 97 and 112 (3), and so is not in the hydrophilic region. Presumably, it is inside the micelle and not in the hairy layer. The diameter of micelles varies between 20 and 300 nm.

Snoeren showed that an electrostatic attraction occurs between carrageenan and κ-casein, but not α_s- and β-casein. He assumed that this was due to the positive charges between residues 97 and 112 of κ-casein, which are absent from the α_s- and β-caseins. He further assumed that the same interaction occurred when the κ-casein is part of a casein micelle, despite the latter's overall negative charge (3).

Despite their economic importance, few studies of the behaviour of carrageenan–milk mixtures have been published.

The first aim of this work was to describe the interaction phenomena through the observation of carrageenan–milk mixtures and to determine the conditions of flocculation, previously studied by Elfak *et al.* (4) and Dalgleish and

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Morris (5), and of gelation (temperature, milk and carrageenan concentrations, type of carrageenan). The second aim was to outline some effects of the presence of casein micelles on the rheological properties of iota-carrageenan systems.

Materials and methods

Preparation of systems

Carrageenans

Experimental samples of iota-, kappa- and lambda-carrageenan in sodium form provided by SBI were used. The ion and water contents (wt%) were as follows: for iota, Na: 7.51, K: 0.57, Ca: 0.25, Mg: 0.33, Cl: 0.1, water: 11.31; and for kappa, Na: 4.7, K: 0.51, Ca: 0.88, Mg: 0.034, Cl: 0.35, water: 10.80. The carrageenans were used without further purification.

The carrageenan solutions were prepared by first dispersing the carrageenan powder in deionized water or in permeate under stirring for 10 min at room temperature, and then heating at 70°C for 20 min.

Milk

Two samples of skim milk powder were used: Gloria and Nilac (supplied by Nizo). Milk concentrated 1.5-fold [on the basis of 3.3% proteins corresponding to 2.6% caseins for native milk (10)] was prepared by mixing 1000 g of deionized water with either 174.6 g of Gloria powder or 157.9 g of Nilac powder and then stirring for 30 min at 60 or 40°C, respectively.

Solvents

Two different solvents were used in order to keep the same ion composition whatever the milk concentration. For the study of phase diagrams, the milk was diluted by using a modified Jenness and Koops (11) medium which could be heated without unwanted precipitation. After dilution, this solvent had the same ionic strength and major cation concentrations as milk (cation chlorides, in mmol/dm^3: Na, 17.81; K, 37.22; Ca, 9.98; Mg, 3.20; and 146.10 mmol/dm^3 lactose). It was 1.5-fold concentrated. For the rheological study, Nilac milk permeate obtained by filtration at 50°C through an Amicon hollow fibre membrane (cut-off 0.1 μm) was used. The amount of permeate represented 30% of the initial milk which was reconstituted at a standard concentration (100 g powder was used for 1000 g of deionized water).

Sodium azide (0.4 g/l) was added to each system to prevent microbial growth.

Carrageenan–milk mixtures

For the study of phase diagrams, 45 ml mixtures were prepared by 'hot mixing' 1.5-fold concentrated milk (or milk and solvent) and 3-fold concentrated carrageenan solutions in water. Both were previously heated at 70°C (20 min). The mixing was followed by 3 min stirring at 70°C. The pH of the mixtures was kept constant, and was equal to that of milk (6.7). The final concentrations in the mixtures, in wt%, were: carrageenan: 0.01, 0.02, 0.05, 0.1, 0.3, 0.5; milk: 0 (solvent), 10, 20, 60, 100 (corresponding to native milk).

For the rheological study, 45 ml mixtures of 0.056 and 0.560% carrageenan in milk (hot mixing) and in permeate were prepared at 70°C, and poured directly into the measuring system at 65°C.

Method of study

Phase diagrams

Phase diagrams were based on visual observation. Mixtures were poured just after mixing (70°C) into 10 ml tubes, put in water baths at 25 and 65°C, and observed for 24–48 h. When phase separation occurred, a two-phase system was obtained and their appearance was 'clear' when we could see distinctly through them, 'translucent' when objects viewed through them appeared blurred, 'cloudy' or 'opaque'.

Study of viscoelastic properties

Dynamic viscoelastic measurements of carrageenan–milk and carrageenan–permeate mixtures were performed using a Rheometrics Fluids Spectrometer (RFSII) fitted with coaxial cylinders (R1 = 32 mm, R2 = 33.8 mm, h = 34.4 mm). The samples were transferred at 65°C. All measurements were made in the linear viscoelastic domain.

To study gelation, mixtures were cooled from 65 to 25°C at 0.5°C/min and G' and G'' were measured at 1 rad/s. The strain amplitude was automatically adapted during the test in order to have a measurable torque (in the range from 0.005 to 0.5 g·cm). The crossing point of G' and G'' ($\tan\delta = G''/G' = 1$) was used as an estimate of the gel temperature. Then, the evolution of G' and G'' as a function of time was recorded at constant temperature (25°C), frequency (1 rad/s) and strain amplitude (5%) for the systems containing 0.5% iota-carrageenan in milk or in permeate. In order to see whether the mixtures containing 0.05% iota-carrageenan were in the sol or in the gel state at 25°C, frequency sweeps were carried out at a fixed strain amplitude.

Results

Phase diagrams

At 65°C, all the mixtures were liquid and sedimentation could occur (Fig. 1). The appearance of the supernatants depended on the type and concentration of carrageenan. We confirmed by protein concentration measurements that the appearance of the supernatant (from clear to opaque) was correlated with increasing protein concentration.

At 25°C, depending on the carrageenan and milk concentrations, the mixtures were either liquid, with or

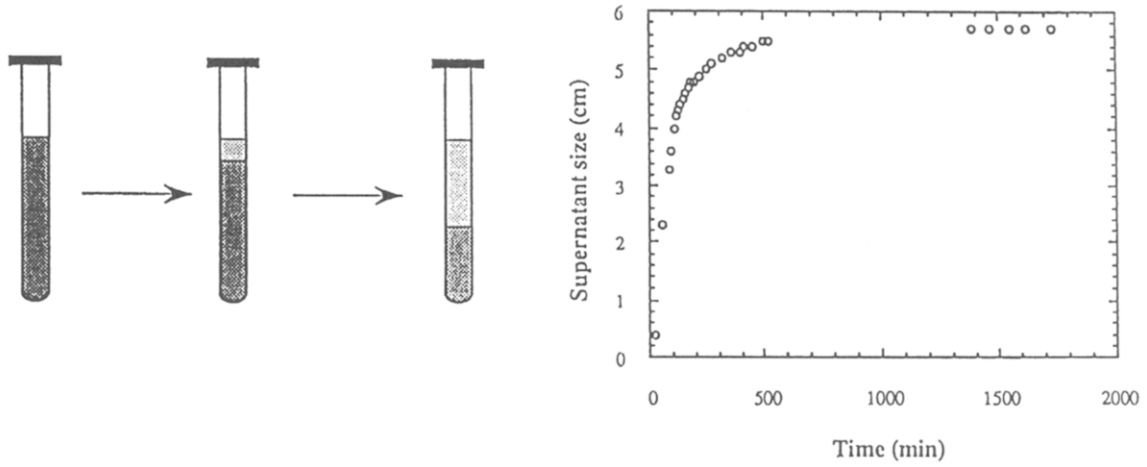

Figure 1 Sedimentation at 65°C of 0.45% iota-carrageenan in milk as a function of time.

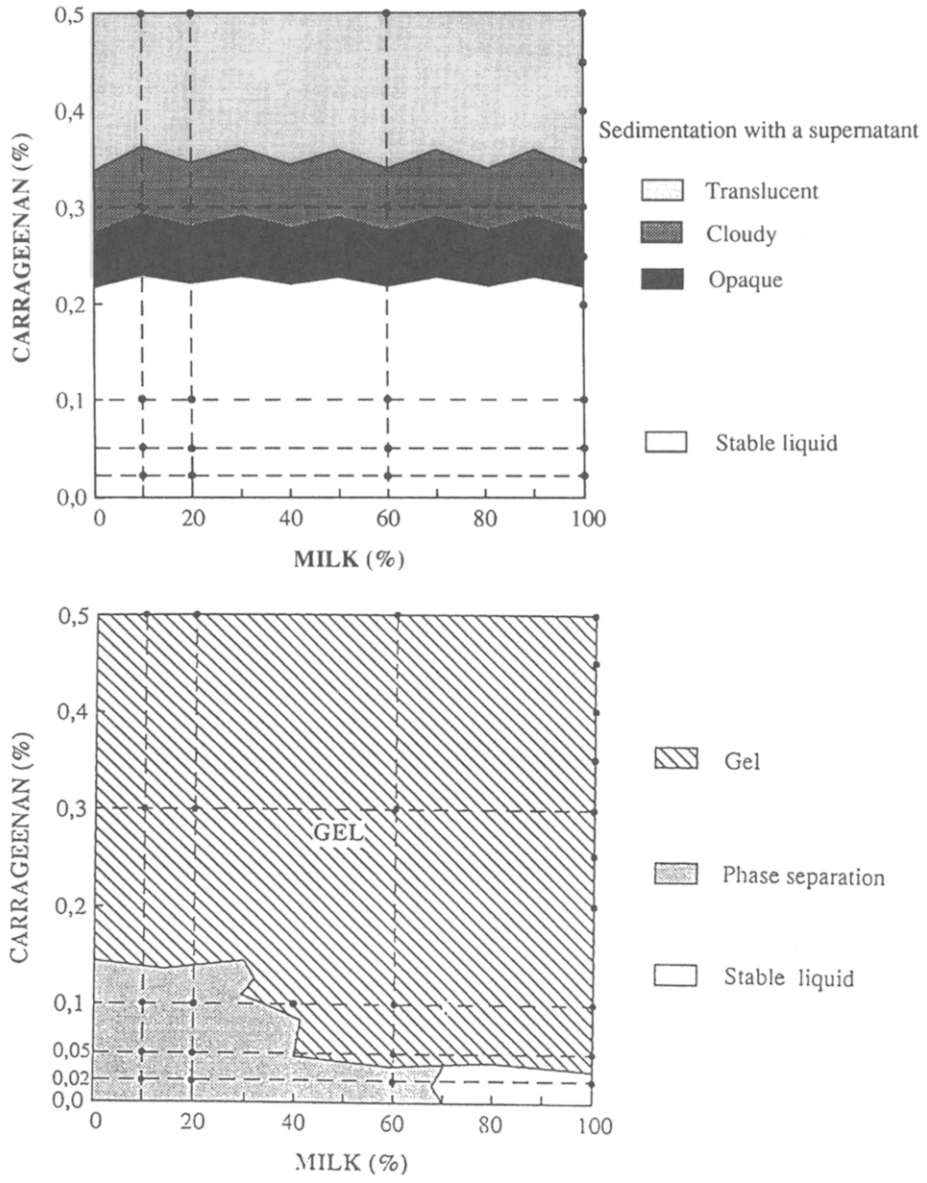

Figure 2 (a) Phase diagram of iota-carrageenan/milk mixtures at 65°C. (b) Phase diagram of iota-carrageenan/milk mixtures at 25°C.

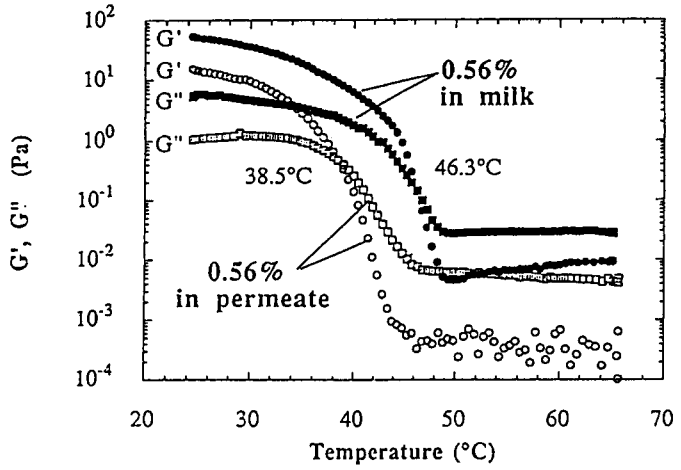

Figure 3 Evolution of G' and G'' during gelation of mixtures of 0.56% iota-carrageenan in milk and permeate. Temperature gradient between 65 and 25°C, 0.5°C/min, 1 rad/s. Strain amplitude: from 38 to 1% for iota in milk and from 50 to 6% for iota in permeate.

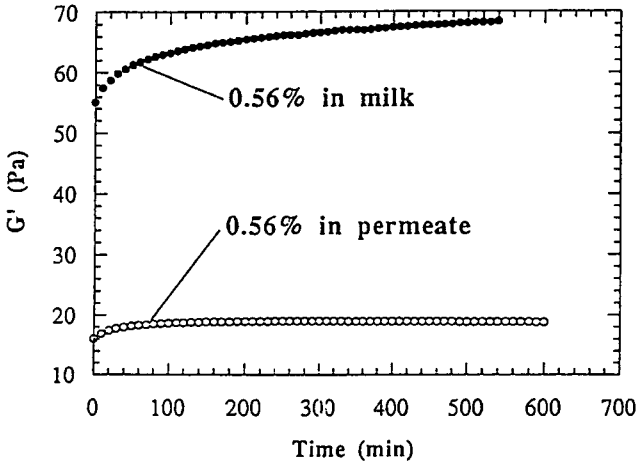

Figure 4 Evolution of G' at 25°C of 0.56% iota-carrageenan in milk and permeate (1 rad/s).

without phase separation, or gelled, with or without syneresis.

Phase diagrams obtained for mixtures containing iota-carrageenan

At 65°C (Fig. 2a). As previously mentioned, the mixtures showed two types of behaviour: either stable or sedimented, the latter implying that casein micelles may interact with disordered carrageenan chains. Sedimentation occurred at carrageenan concentrations above 0.2% and seemed to be independent of the milk concentration. The higher the carrageenan concentration, the greater the amount of casein micelles sedimenting (and the clearer the supernatant), but the slower the rate of sedimentation.

At 25°C (Fig. 2b). There were three domains: (i) one phase gelled (at high carrageenan concentration), (ii) one phase liquid or (iii) two phase liquid. Gelation depended on both

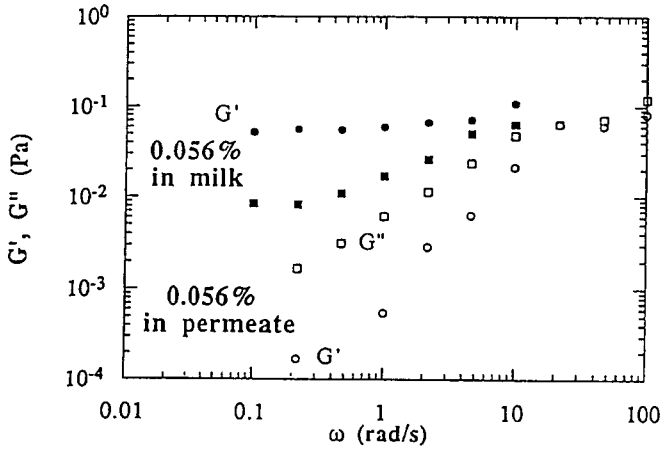

Figure 5 Frequency spectra at 25°C of the mixtures of 0.056% iota-carrageenan with milk [a gel (5% strain amplitude)] and permeate [a liquid (50% strain amplitude)].

the carrageenan and milk concentrations: the strength of the gels appeared to decrease when the amount of carrageenan or milk decreased, and the critical iota-carrageenan concentration for gelling decreased from ~0.1% in 100% solvent to 0.05% in 100% milk. Gel syneresis was observed below 0.3% carrageenan. At low carrageenan concentrations (0.01 and 0.02%), the liquid mixtures were only stable above 60% milk.

Similar behaviour was observed for kappa-carrageenan. With lambda-carrageenan, some differences were noticed. At 65°C, phase separation was observed above 0.3%, the supernatants were clear in milk, but became less transparent when the milk concentration decreased, and at 25°C no gelation occurred and almost all the samples phase separated.

Viscoelastic behaviour

Compared to permeate, the presence of casein micelles increased G' and G'' at all temperatures, and increased the gelation temperature by almost 10°C (Fig. 3).

For both carrageenan–permeate and carrageenan–milk mixtures, we found that G' increased with time at 25°C (Fig. 4), but in milk the increase was more pronounced and took longer to reach a plateau (>500 min compared to ~100 min).

For 0.056% iota-carrageenan, the viscoelastic spectrum at 25°C was typical of a sol in permeate and typical of a gel in milk (Fig. 5). This is consistent with the lower gelling concentration for iota-carrageenan in the presence of casein micelles.

Discussion

These results and, in particular, the effect of temperature give some insight into the mechanisms involved in the interactions between casein micelles and carrageenan chains. Note that the following speculative interpretation is only concerned with the casein micelle–carrageenan interactions

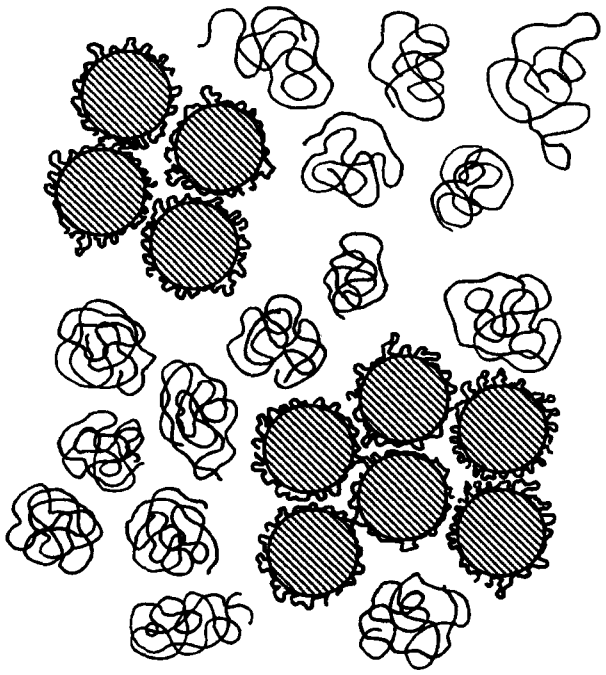

Figure 6 Schematic representation of depletion flocculation of the carrageenan-coated micelles in carrageenan–milk mixtures at 65°C (above 0.2% carrageenan).

and not with the carrageenan network. The exact mechanism of carrageenan gelation is still under debate, and is not the subject of this study, so the representation of the carrageenan network should be taken as schematic.

At 65°C, all carrageenan chains are disordered. When carrageenan is added to milk, it induces sedimentation of the casein micelles above a critical carrageenan concentration (0.2% in our experimental conditions with iota-carrageenan). Two mechanisms can be imagined, both implying *depletion flocculation* of the casein micelles, negatively charged particles, by addition of polymer chains (negatively charged) (12). (i) Depletion flocculation could be considered alone in order to explain the sedimentation of casein micelles. At 65°C, the sedimentation appears to be reversible: when a sedimented mixture of 0.5% carageenan in milk is stirred, it becomes homogeneous and sediments again at rest at this temperature. If such a phase-separated system is diluted with milk to obtain a carrageenan concentration of 0.1%, it remains stable at 65°C, which is in agreement with the depletion flocculation hypothesis. (ii) If we consider the evidence of Snoeren (2) and Dalgleish and Morris (5) for adsorption of the carrageenan chains on the casein micelles, we would have a progressive coverage of the surface of the casein micelles by the carrageenan chains until full coverage is achieved. The system is stable up to a critical carrageenan concentration. In that case, we suggest (Fig. 6) that above this concentration excess carrageenan is present in solution, which flocculates the carrageenan-coated micelles by depletion, causing sedimentation. According to Snoeren (2,3), adsorption of carrageenan onto casein micelles is due

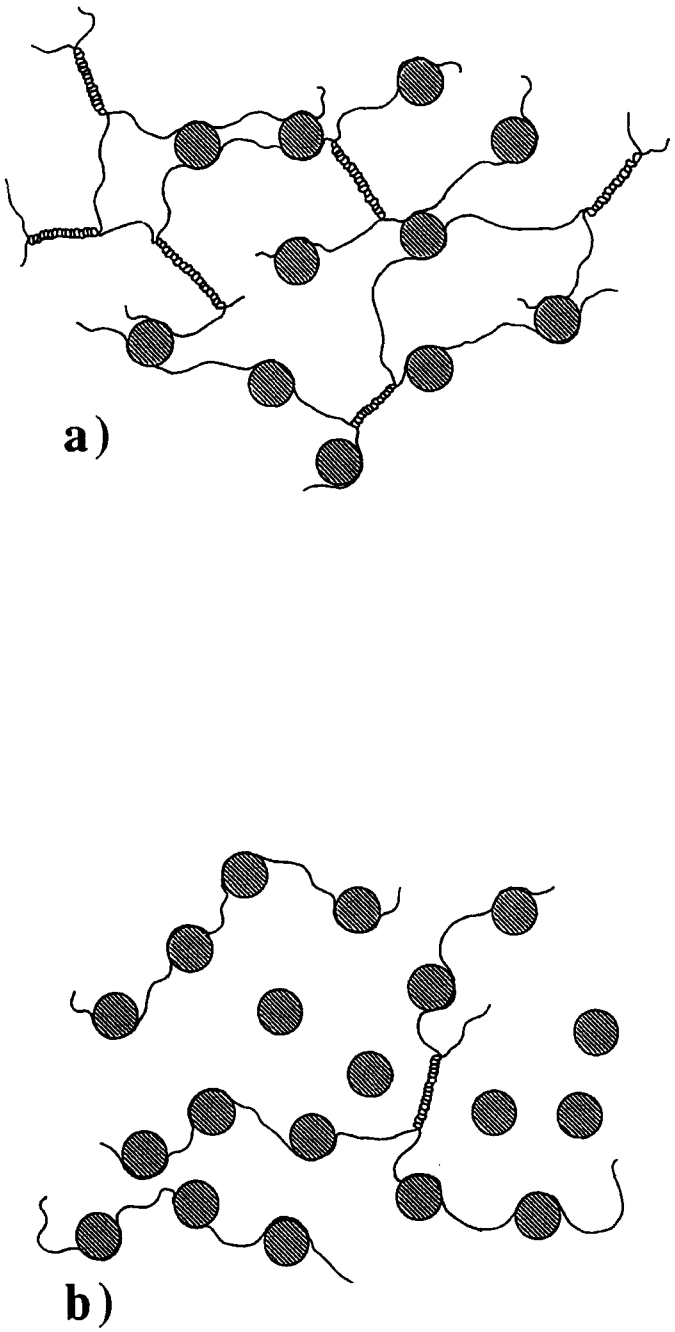

Figure 7 (a) Schematic representation of bridging of carrageenan chains by casein micelles at 25°C (at intermediate carrageenan concentrations). (b) Schematic representation of carrageenan chains mostly covered by casein micelles at 25°C (at low carrageenan concentrations and high milk concentrations).

to electrostatic attraction, by analogy with the interaction that he demonstrated between carrageenan and κ-casein. However, it has to be pointed out that this mechanism of electrostatic interaction implies the penetration of the carrageenan chains into the hairy layer of the micelle to interact with the positively charged region of the κ-casein.

At 25°C, the iota- and kappa-carrageenan chains can form double helices, becoming more rigid and undergoing

self-association. At this temperature, adsorption of carrageenan on casein micelles could also be involved, as demonstrated by Snoeren (2) and Dalgleish and Morris (5).

At levels of carrageenan above 0.1%, the systems gel at all milk concentrations: a carrageenan network is formed and the casein micelles may interact with the parts of the carrageenan chains which are not implied in junction zones.

Below 0.1% carrageenan, the system may be liquid and unstable due to the formation of small aggregates of carrageenan chains partly covered by casein micelles. Since the critical gel concentration is lowered by the presence of milk, it may be assumed that there is additional *bridging* of the carrageenan chains by casein micelles (Fig. 7a). At low carrageenan concentrations (below 0.05%), the system is stable above a certain milk concentration: the carrageenan chains could be mostly covered by the casein micelles so that there are insufficient unbound carrageenan chains to create the junction zones necessary for the formation of large aggregates. In this case, the bridging of carrageenan chains by casein micelles may be statistically unfavourable (Fig. 7b).

Conclusions

The phase diagrams and rheological properties of carrageenan–milk mixtures clearly demonstrate the existence of interactions between casein micelles and carrageenan chains at all concentrations and temperatures. We have shown that at high temperature, where carrageenan chains are in a disordered conformation, there is a critical carrageenan concentration above which the system becomes unstable. We suggest that, at this concentration, the destabilization is due to depletion flocculation of casein micelles promoted by carrageenan chains in solution. Snoeren's hypothesis of carrageenan adsorption onto the casein micelles implies that the latter must first be covered by carrageenan chains before depletion flocculation can occur.

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