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Study of the compatibility/incompatibility of gelatin/iota-carrageenan/water mixtures

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The incompatibility of acid gelatin/iota-carrageenan mixtures has been studied. Both these biopolymers undergo a conformational coil–helix transition under suitable conditions of temperature and salt. The aim of this work was to study the concentration at which mixtures are incompatible and the influence of pH, salt and temperature on the phase diagram. Incompatibility occurred over a wide range of concentrations for mixtures prepared in deionized water. Compatibility was increased by increasing the pH or the salt concentration. Temperature did not greatly influence the size of the incompatible region. This is in agreement with the hypothesis that attractive electrostatic interactions lead to associative phase separation (traditionally called complex coacervation). Copyright © 1996 Elsevier Science Ltd.

INTRODUCTION

Two macromolecular species in solution are usually incompatible, which leads to phase separation, except in very dilute solution. Incompatibility can lead to either microscopic or macroscopic phase separation. The incompatibility is called 'segregative' when each phase is enriched in one of the components. This is the case for mixtures of two non-ionic polymers, two similarly charged polyelectrolytes or a polyelectrolyte plus a nonionic polymer (Dickinson, 1987; Piculell & Lindman, 1992). When the majority of both polymers is in the same phase, the incompatibility is called 'associative' (traditionally called complex coacervation) (Dickinson, 1987; Piculell & Lindman, 1992). In this case, the attraction between the polymers leads to complex formation. Such complexes are obtained in mixtures of oppositely charged polyelectrolytes, for example, gelatin and anionic polysaccharides at pHs below the p*I* of the gelatin (Tolstoguzov, 1986).

The study of such complexes has a long history (Bungenberg de Jong, 1949). The formation of polyelectrolyte complexes cannot be easily interpreted using a thermodynamic approach. Frugier (1988) has shown that it is difficult to model this phase behaviour using modified Flory–Huggins theory, because of the strong attraction between the two macromolecular components. This type of aggregation is greatly dependent on the polymer structure and on the mixing conditions. The literature suggests that for gelatin/polysaccharide mixtures, either associative or segregative interactions can occur (Tolstoguzov, 1986), depending on the ionic strength, pH and polymer concentration. When the polymers gel, mixed systems may have different structures depending on each polymer behaviour alone and the nature of their interaction (Morris, 1986).

The aim of this work was to study the incompatibility of acid gelatin/iota-carrageenan mixtures. Both these polymers undergo a conformational coil–helix transition under suitable conditions of temperature and salt. Chains in the helical conformation can form thermoreversible gels. Previous rheological measurements of the thermoreversible sol–gel transitions of gelatin and iota-carrageenan separately allowed us to characterize them in both the sol and gel state and to determine the critical point parameters of their gelation and melting (Michon *et al.*, 1993, 1995).

In this work, we have studied the influence of three parameters on the areas of incompatibility and compatibility in the phase diagram: temperature, pH, and salt concentration. One sample of each polymer was studied. In a previous paper (Michon *et al.*, 1995) the acid gelatin and iota-carrageenan samples used here were referred to as g1 and i2, respectively. The molecular weights were obtained by gel permeation chromatography. M_w was 182,000 Da for gelatin and 700,000 Da for iota-carrageenan.

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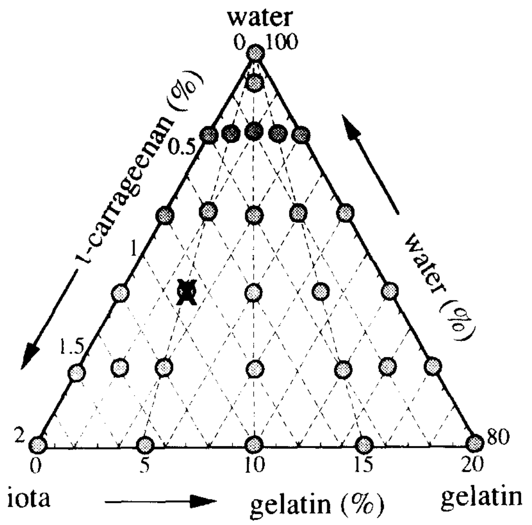

Fig. 1. Compositions of the 29 ternary gelatin/iota-carrageenan/water mixtures studied. ○ indicates the position of each system in the phase diagram. They were 1 solvent, 5 containing only gelatin, 5 containing only iota-carrageenan and 18 were mixtures containing various proportions of both polymers. **X** indicates the composition used to study the effects of salt concentration and pH.

MATERIALS AND METHODS

Preparation of mixtures

A 20% solution of gelatin and a 2% solution of iota-carrageenan were mixed in various proportions in deionized water, in order to obtain compositions whose concentrations covered the largest range practically accessible (Fig. 1). Each series of samples included all the possible mixtures of five gelatin concentrations (4, 8, 12, 16 and 20%) and five iota-carrageenan concentrations (0.4, 0.8, 1.2, 1.6 and 2.0%), together with a few other mixtures and the solvent alone, giving 29 tubes in all per series. Four hundred ppm of NaN_3 were added to prevent microbial attack. The hot solutions were mixed and stirred at 60–70°C for about 10 min before pouring into tubes which were then tightly capped and placed in a thermostatted water bath.

Three series with identical compositions were studied: (1) in deionized water without pH adjustment; (2) pH adjusted to 6.5; and (3) pH adjusted to 6.5 and in the presence of 0.2 M NaCl.

Phase diagrams

Phase diagrams were obtained by visual observation:

Mixtures were arbitrarily classified as 'clear' when we could see distinctly through them, 'cloudy' when objects viewed through the tube appeared blurred, 'opaque' when nothing could be seen through them and 'phase separated' when two distinct phases were visible. Samples were classified as 'gel' or 'sol' depending on whether they prevented the fall of a small glass sphere. On this basis, very weak gels were classified as being in the sol state and so the true gel zones of the phase diagrams were probably larger than described. Turbidity was due to incompatibility, since samples of gelatin and iota-carrageenan alone were transparent.

RESULTS

Compatibility and incompatibility domains in the phase diagrams

Phase diagrams for systems prepared in deionized water (series 1) show that gelatin and iota-carrageenan were incompatible at all concentrations: even the samples containing 1% gelatin and 0.1% iota carrageenan were turbid or phase separated (Figs 2 and 3). Chemical analysis of phase separated systems showed that the majority of the gelatin and iota-carrageenan were in the same phase, so the incompatibility was associative.

When the gelatin concentration increased, mixtures became clearer, as if the incompatibility decreased. This may have been due to an increase in the solubility of the complexes, whose composition and structure depend on polymer proportions (Tolstoguzov, 1986). An alternative explanation is that the systems were metastable, i.e. gelation prevented phase separation in systems that should have been two phase on thermodynamic grounds.

Influence of temperature on the phase diagram

Three thermal histories were tested:

I quenching from 70°C to 20, 30, 40, 50 or 60°C. Observation after 1 h.
II decreasing the temperature in 5°C steps from 70°C to 20°C. Observation at the end of each temperature step (20 min).
III following step 2, increasing the temperature in 5°C steps from 20°C to 70°C. Observation at the end of each step (20 min).

Note that at temperatures below the gelatin transconformation temperature (40°C), systems were not at equilibrium, because gelatin rheology evolves slowly and indefinitely. However, changes in appearance due to changes in temperature occurred quickly and no significant change in the phase diagram was observed even after several hours.

Whatever the thermal history (1, 2 or 3), temperature had little effect on the extent of incompatibility in the phase diagrams (Figs 2 and 3). Domains of incompatibility did not change when: (i) the temperature decreased; (ii) the temperature went through either the gelatin or iota-carrageenan transconformation temperatures (about 40 and 60°C,

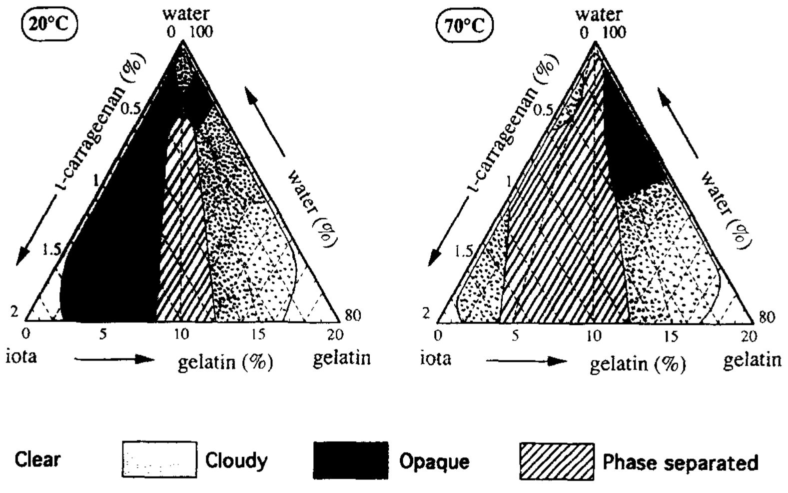

Fig. 2. Phase diagrams at 20 and 70°C for series 1 (deionized water, pH unadjusted). All mixtures were gelled at 20°C, except those containing less than 0.2% iota-carrageenan.

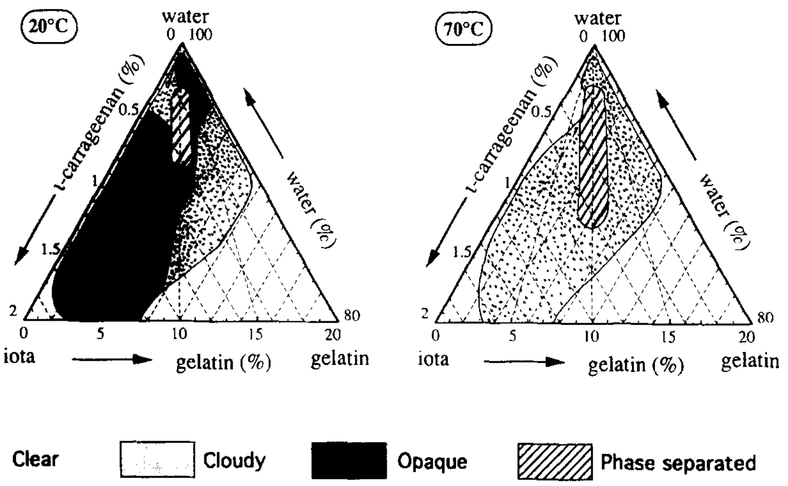

Fig. 3. Phase diagrams at 20 and 70°C for series 2 (deionized water, pH 6.5). All mixtures were gelled at 20°C except those containing less than 0.2% iota-carrageenan.

respectively, for our samples); and (iii) the temperature increased to 80–90°C (the two biopolymers were then in their disordered, coil, conformation). These observations are in accordance with the hypothesis that the interaction was electrostatic, as has been classically suggested for protein/polysaccharide mixtures (Bungenberg de Jong, 1949; Tolstoguzov, 1986), since such interactions have a low temperature dependence. The interaction occurred whatever the polymer conformation, although the turbidity was higher at low temperatures, where they were in helical form.

Influence of pH and salt concentration on the incompatibility

The pH of the samples in series 1 was close to 5, except at low gelatin concentrations ($C < 2$–3%) where the pH increased with decreasing gelatin concentration, reaching 7.6 for systems containing iota-carrageenan alone. A comparison of series 1 (in deionized water) and 2 (adjusted pH to 6.5) shows that, whatever the proportions of gelatin and iota-carrageenan, a pH decrease induced greater interaction at all temperatures (Figs 2 and 3).

A comparison of series 2 (pH 6.5, [NaCl] < 0.04 M) and 3 (pH 6.5, [NaCl] = 0.2 M) shows that an increase in salt concentration led to increased compatibility. The influence of pH and salt concentration were studied in more detail for a mixture containing 3% gelatin and 0.9% iota-carrageenan, chosen in a zone of strong incompatibility (see X in Fig. 1). Samples with pHs of 4.4–8.9 and NaCl concentrations of 0–1.2 M were prepared. The results are shown in Fig. 4.

At salt concentrations below 0.8 M the results confirmed the trends in phase behaviour shown in Figs 2 and 3. Without added salt, solutions were opaque, whatever the pH. At a given salt concentration, increasing the pH increased the compatibility. This observation is simply explained in terms of an attractive electrostatic interaction: the lower the pH, the more the

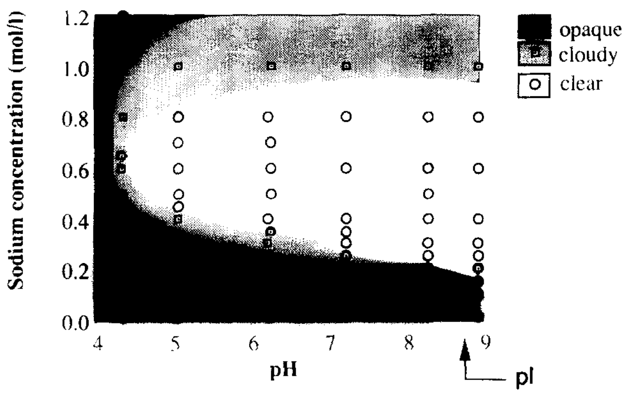

Fig. 4. Phase diagram of a 3% gelatin/0.9% iota-carrageenan mixture as a function of sodium chloride concentration and pH.

gelatin molecules are (net) positively charged. As iota-carrageenan molecules are negatively charged, they are attracted by gelatin. As the pH increases and approaches the p*I* of gelatin (close to pH 9), the protein and polysaccharide chains have less and less affinity for each other, due to the decreasing charge of the gelatin, so the compatibility increases. At a given pH, when the salt concentration increased, opacity decreased, the mixture went from opaque to cloudy and in some cases became transparent. No doubt this increased compatibility was due to screening of the electrostatic interactions.

At NaCl concentrations above 0.8 M, turbidity sometimes reappeared. At high salt concentrations, where the screening of electrostatic interactions is complete, turbidity could have been due to segregative phase separation. This sequence of associative phase separation → compatibility → segregative phase separation with increasing salt concentration has already been observed in anionic polymer/cationic surfactant mixtures (Thalberg *et al.*, 1991).

CONCLUSIONS

Iota-carrageenan and acid gelatin are incompatible. There are three concentration domains in the ternary phase diagram: (i) macroscopically phase separated systems; (ii) cloudy or opaque one-phase systems; (iii) clear one-phase systems. In each case, the mixture can be in either the sol or gel state, depending on the temperature. It would be interesting to determine how the phase behaviour modifies the rheological properties of the one-phase systems (work in this area is in progress). Incompatibility was associative and due to attractive electrostatic interactions. When the pH increases, gelatin becomes less positively charged and has less and less affinity for iota-carrageenan. Addition of salt screens the attractive electrostatic interactions and so increases the compatibility.

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