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Aqueous phase-separated biopolymer mixture compatibilized by physical interactions of the constituents

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Summary

A new way of compatibilization of an aqueous phase-separated two-component biopolymer system based on physical interactions of the constituents with a third biopolymer (compatibilizer) was suggested. The rheological behavior and the changes in morphology of the compatibilized mixtures were studied using a two-phase water-casein-alginate system, with the droplet morphology as a model. The time-dependent evolution of the microstructure of the compatibilized casein-alginate emulsion was investigated by image analysis using optical microscopy. Addition of 0.04–1.5 wt% of dextran sulfate as compatibilizing molecule to the emulsion leads to a considerable increase in the compatibility of casein with alginate and to a dramatic increase in storage and loss moduli (G' and G''). Rheological differences could be correlated with differences in morphology, visible by optical microscopy.

Keywords

Casein, alginate, compatibilization, interactions, viscoelasticity

Introduction

Mixtures of natural, modified natural and synthetic polymers are frequently used in the polymer, cosmetic, and pharmaceutical industries to generate materials with improved or even unique properties. To stabilize the fine microstructure of synthetic polymer blends and to increase the interfacial adhesion between their phases, compatibilizers can be added [1]. Normally, these compatibilizers are copolymers containing two blocks, each compatible with one of the polymers, or they contain both nonionic repeat units and a small amount of ion-containing repeat units (so-called ionomers). As was shown by several authors [2, 3], these agents have an influence on the morphology development of polymer mixtures.

Aqueous phase-separated mixtures of biopolymers are widely used in the food industry, and the morphology development in biopolymer emulsions is governed by the same physical principles as apply to immiscible polymer blends [4–6]. Although phase separation in biopolymer systems frequently allows control of the morphology and, hence, the rheology/texture of biopolymer gels [5–7], in many cases it leads also to a spontaneous separation into two layers, which is not desired in many food and biotechnology processes. In general, the compatibility of biopolymers in solution may be increased by decrease of their molecular weight, as well as dissociation or limited proteolysis of proteins. However, these ways lead to the loss of the functional properties of biopolymers. Note that an appropriate compatibilizer for such systems has not been described in the literature. Therefore, the increased application of natural polymer mixtures in food technology, as thickeners and stabilizers (morphology tuners) in yoghurt and mayonnaise, has now turned the attention to the search for an appropriate way to compatibilize these natural polymers [7].

Recently, it has been demonstrated [8] that a single-phase water-gelatin-pectin system containing interacting macromolecules undergoes phase separation in the presence of dextran. Phase separation of such a system can be explained by blockage of the reactive gelatin groups due to their competitive interactions with dextran. In general, the types of biopolymer interactions can vary widely due to wide variations in biopolymer structure and solvent conditions (pH, ionic strength, temperature) [9–13]. It can be supposed that inter-macromolecular interactions, caused by the presence of complexing agents in a two-phase biopolymer mixture, can have an influence on the phase equilibrium and the morphology in incompatible biopolymer mixtures.

The aim of this present study was to achieve a quantitative increase in biopolymer compatibility by a novel method based on the physical interactions of the biopolymer(s) with a complexing polysaccharide, and to study the rheological and morphological changes in such systems. The two-phase system under investigation here is an aqueous mixture of sodium caseinate (SC) and sodium alginate (SA), the phase equilibrium of which is not sensitive to changes in pH, ionic strength and temperature in the quiescent state [14] and under conditions of shear flow [15]. Therefore, the effect of compatibilization that can be reached for this system can be easily reproduced for other emulsions in which the phase equilibrium is more sensitive to physicochemical parameters. The casein-alginate system is typical with regard to thermodynamic considerations and it is relevant to applications in the food industry for its textural and structuring properties [7, 16].

Dextran sulfate sodium salt (DSS) was chosen as a possible compatibilizer forming a water-soluble complex with casein at pH values above the isoelectric point.

To understand how a sulfate polysaccharide can affect the phase equilibrium in an aqueous casein-alginate system and to estimate the efficiency of this way of compatibilization, we investigated the effects of the compatibilizer concentration on the phase volume ratio of the system, after its separation by centrifugation, as well as on the morphological changes of the system.

Experimental

Materials

SC (95.8% protein, 3.3% ash, 97.3% dry weight, 0.5% lactose, <0.05% fat, and 0.02% calcium) was purchased from Acros Organics. The weight average molecular mass of

SC under the conditions studied is 320 kDa [17]. SA (block content: 70% guluronic acid, 30% mannuronic acid, heavy metals <40 ppm) extracted from brown seaweed (*Macrocystis pirifera*) was purchased from Acros Organics (Lot No. A0207320). The molecular weight of the studied sample reported by the manufacturer was 200 kDa. DSS (M_W = 500,000 Da, M_n = 166,000 Da, η (in 0.01 M NaCl) = 50 mL/g, 17% sulfate content, free SO_4 less than 0.5%) was produced by Pharmacia Fine Chemicals, Uppsala, Sweden (Reg. No. 15520 A, Lot No. IE 96231). DSS is prepared by sulfating a selected fraction of dextran with chlorosulfonic acid in pyridine, followed by careful purification. Each glucose unit in the dextran chain has approximately two sulfate groups.

To prepare molecularly dispersed solutions of SC, SA and DSS with the required concentrations, deionized water was gradually added to the weighed amount of biopolymer sample at 298 K, and stirred, first for 1 h at this temperature and then for 1 h at 318 K. The final solutions were centrifuged in a Sigma centrifuge at 23,000 × g for 2 h at 313 K to remove insoluble particles. The pH values of all solutions were close to 7.0. Concentrations of solutions were determined by drying at 373 K until constant weight.

Methods

The ternary water (W)-SC-SA system and the quaternary W-SC-SA-DSS systems with different compositions were prepared by mixing binary solutions of each biopolymer at 298 K. After mixing for 1 h, the systems were centrifuged at 23,000 × g for 2 h at 318 K using a temperature-controlled rotor. An emulsion containing 90 wt% of the casein-enriched phase and 10 wt% of the alginate-enriched phase was mainly used in the present study. The initial and phase compositions of this emulsion, determined by the phase volume ratio method [18, 19], are presented in Table 1.

The rheological measurements, including the viscosity measurements, were performed using a Modular Advanced Rheometer System (HAAKE MARS) equipped with a cone/plate rotor C60/1Ti geometry (A-factor, 17,583 Pa/N m; M-factor, 57.39 rad/s), at 298 K. At this temperature, the ratio of dispersed phase viscosity over matrix phase viscosity was about unity. Samples were subjected to a preshearing of 500/s for 900 s. Such a preshearing aims at generating a reproducible initial morphology prior to the start of the rheological or morphological experiments. After preshearing, the shear rate is stopped for 50 s and the evolution of the morphology as a result of flow-induced coalescence is monitored by periodically interrupting the shear flow and conducting dynamic mechanical measurements at 25% strain. It has been verified here that the morphology does not change during the dynamic measurements.

Compatibilized W-SC-SA systems were prepared by adding the desired amount of compatibilizer to the W-SC-SA emulsion containing 10 wt% alginate-enriched dispersed phase obtained after centrifugation and separation of the coexisting phases of the W (93%)-SC (6.0%)-SA (1.00%) system. All mixing was performed by hand, using a spatula.

Droplet sizes were determined with an Axioplan 2 Imaging Zeiss optical microscope connected to a digital camera (AxioCam HRC). Samples were subjected to a preliminary preshearing of 500/s for 900 s by a Modular Advances Rheometer System (HAAKE MARS) equipped with a cone/plate rotor C60/1Ti geometry and then immediately studied by optical microscopy. All images were treated using Axiovision 4.0 software.

Results and discussion

Characteristics of the emulsion

In this study, the water (95.4%)-casein (14.4%)-alginate (0.24%) emulsion was used. It consisted of 90 wt% of the caseinate-enriched phase and 10 wt% of the alginate-enriched phase. This emulsion is located in the two-phase region, far from the critical point. The emulsion was hand-mixed prior to loading into the rheometer device. The temperature used in this study was 298 K, *i.e.* both phases were in the liquid state. The interfacial tension of the system, determined by a rheo-optical method, amounted to 5.2×10^{-5} N/m [20]. Similar values (8×10^{-6} N/m) were obtained by Capron *et al.* [21] and by Simeone *et al.* [22] (8.8×10^{-6} N/m) for the SC (6 wt%)-SA (1.0 wt%) system located far from the bimodal. It can also be noted that the alginate-enriched and the casein-enriched phases have similar viscosities at 298 K and a shear rate of 10/s, *i.e.* 3.97 and 3.30 Pa s, respectively. The phase compositions of the emulsion and its coexisting phases are presented in Table 1.

Table 1 Initial and phase compositions of the system studied

System	Composition		
	Solvent (%)	Casein (%)	Alginate (%)
Emulsion	95.40	14.40	0.24
Alginate-enriched phase	97.97	0.12	1.91
Casein-enriched phase	83.98	15.98	0.04

Effect of the presence of sulfate polysaccharide on phase separation and morphology of the water-casein-alginate system

Figure 1 shows the effect of the addition of 1.4 wt% DSS to the two-phase W (91%)-SC (8.0%)-SA (1.0%) system. In the absence of compatibilizer, the system separated into two coexisting phases, with preferential concentration of casein in the bottom phase and alginate in the upper phase (Fig. 1A). The volumes of these coexisting phases were equal to each other. The presence of 1.4% DSS in the system led to a considerable decrease in volume of the alginate-enriched phase (from 50 to 15%). At the same time, the concentration of casein in the casein-enriched phase changed insignificantly.

Both these facts give an indication of the considerable increase in compatibility of casein and alginate in the middle part of the phase diagram or, more precisely, of the significant increase in solubility of casein in solutions of SA. The solubility of alginate in concentrated solutions of casein does not change noticeably (Fig. 2).

The increase in compatibility of casein and alginate is especially surprising when taking into account that the phase composition of this system is weakly dependent on many physicochemical factors, such as pH (in the pH range from 7 to 10), ionic strength and temperature (from 5 to 60°C) [23].

The presence of DSS in the emulsion results in dramatic changes in its morphology (Fig. 3). In the presence of a small amount of DSS (0.25%), the emulsion becomes finer. The average size of the droplets decreases considerably. At 0.5 wt% DSS, the droplet morphology of the emulsion disappears and the morphology is presented by

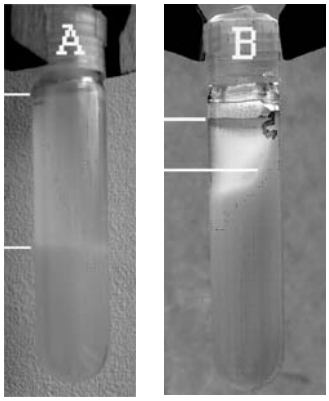

Figure 1. The water-casein (8.0%)-alginate (1.0%) system after centrifugation at 23,000 × g for 2 h at 298 K.
(A) Without DSS, (B) in the presence of 1.4% DSS. Marks refer to the phase border and the top level of the two-phase system.

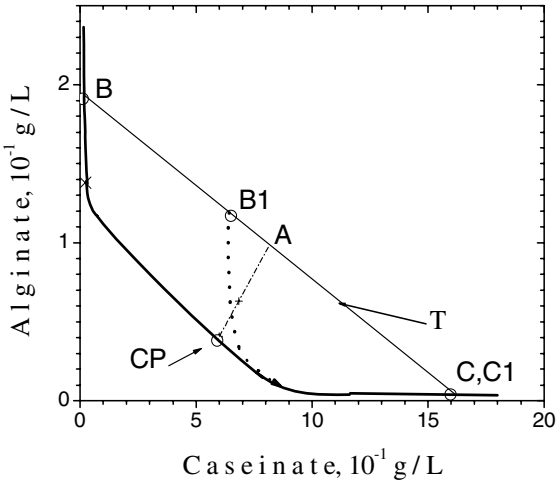

Figure 2. Phase diagram of the water-casein system (pH 7.0, 298 K). The dotted line shows a possible shift of the bimodal line in the presence of 1.4 wt% DSS. T1 is the line connecting the composition of coexisting phases in the uncompatibilized emulsion. A, Composition of the uncompatibilized emulsion; B, composition of the alginate-enriched phase of the uncompatibilized emulsion; C, C1, composition of the casein-enriched phases of both the uncompatibilized and the compatibilized emulsions; B1, composition of the alginate-enriched phase of the compatibilized emulsion; CP, critical point of the uncompatibilized emulsion.

irregular lamellae. At higher DSS concentrations, the optical density of the emulsion increases considerably (data not shown) and a continuous phase occupies almost all the volume (Fig. 3D), which is in agreement with the results of the phase volume measurements (see also Fig. 1B).

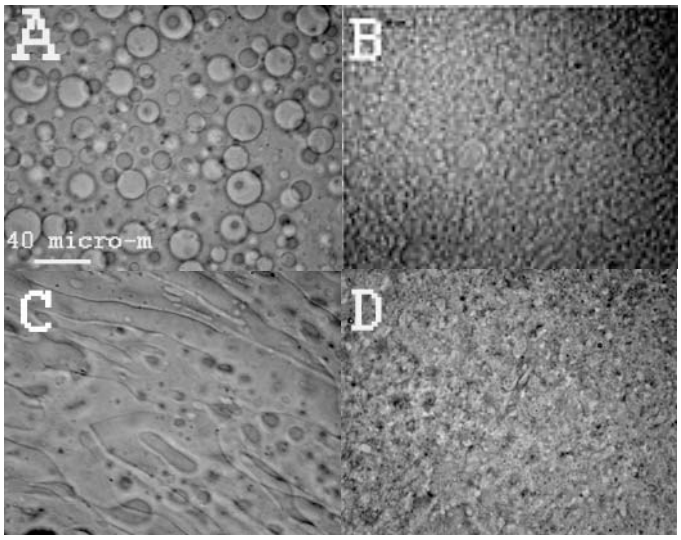

Figure 3. Steady-state morphology of the casein (10.4%)-alginate (0.24%) two-phase system. (A) Uncompatibilized system; (B–D) in the presence of 0.25, 0.5 and 0.75% DSS. The scale for all images is indicated in Fig. 3A. Samples were subjected to a preliminary preshearing of 500/s for 900 s by a Modular Advances Rheometer System (HAAKE MARS) equipped with a cone/plate rotor C60/1Ti geometry, and then immediately studied by optical microscopy.

Rheology of uncompatibilized water-casein-alginate systems

For the rheological investigations of the two-phase W-SC-SA system, we separated the two coexisting phases by centrifugation. The coexisting phases and the emulsion containing 10% alginate-enriched dispersed phase were then characterized through their viscoelastic behaviors. It has been shown [20–22] that at moderately low shear rates, the biopolymer emulsion can be regarded as a conventional emulsion, and various structural models that are available in the literature for prediction of the morphology in these emulsions can also be used for prediction of the structure in aqueous biopolymer emulsions.

The experimental flow protocol applied was in accordance with data obtained before for a W-SC-SA system [21]. First, the mixture was presheared at 500/s for 900 s, in order to wipe out any previous mixing history. The sample was allowed to relax for 60 s, leaving enough time for full relaxation of the deformed droplets. Finally, a dynamical spectrum was measured in order to characterize the state of the emulsion. The obtained results are presented in Fig. 4.

In the case of the investigated emulsion, the appearance of a "shoulder" in the low-frequency region of G' was noticed. A shoulder in the mechanical spectrum of a casein-alginate system has been shown and studied in detail before, by Capron and coworkers [21]. The "shoulder" indicates the terminal relaxation time of the blend of viscoelastic liquids and is attributed to the shape relaxation of the droplets. Changes in size of the droplets induce a shift of the characteristic frequencies or relaxation times. It is important to note that our previous experiments show [15] that the casein-alginate emulsion remains as a two-phase system over a wide concentration range of biopolymers, at all shear rate values measured (from 0.3/s to 500/s). This point is mentioned in order to clarify the comparison with other phase-separated biopolymer emulsions that undergo shear-induced mixing at shear rates above 60/s.

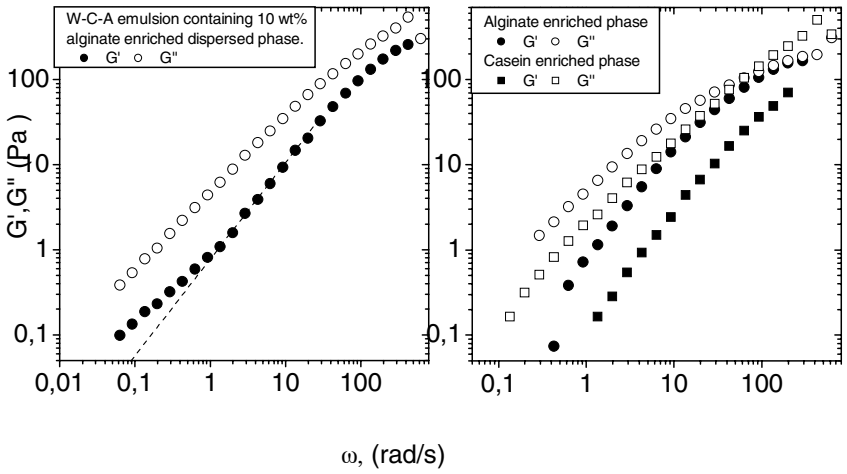

Figure 4. The dynamic moduli in dependence on the frequency of the water-casein (14.4%)-alginate (0.24%) system (point T1 on the phase diagram; see Fig. 2) containing 10% alginate-enriched phase and 90% casein-enriched phase (left) and the coexisting phases of this system (right), after shearing at 500/s for 900 s and subsequent stop in shearing for 60 s.

Rheology of compatibilized water-casein-alginate systems

The evolution of the mechanical spectrum in a W-SC-SA system was investigated as a function of DSS concentration. For this purpose, we prepared W-SC-SA emulsions containing 10 wt% of the alginate-enriched phase in the presence of different amounts of DSS. Their viscoelastic behaviors were monitored and compared with the behavior of the W-SC-SA system without DSS. The obtained data are presented in Fig. 5.
As reported in Fig. 5, in the presence of even small amounts of compatibilizer (0.19 wt%), a dramatic increase in the viscoelasticity of the emulsion takes place. The growth of viscoelasticity is more significant for a higher concentration of DSS and it is especially outstanding at low frequency. Thus, in the presence of 0.5 wt% of DSS and at frequency values equal to 0.1 rad/s, G' and G'' values are more than 300 and

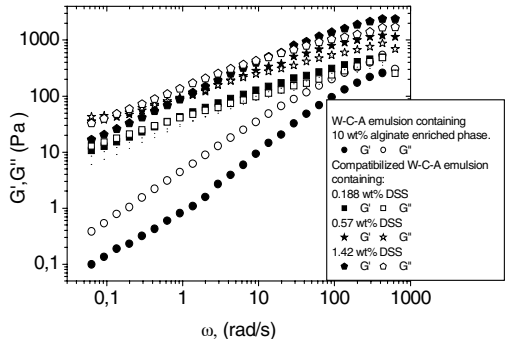

Figure 5. Dynamic spectra of emulsions: 10 wt% alginate-enriched phase + 90 wt% casein-enriched phase containing different amounts of DSS. The dynamical spectrum of the same emulsion without the presence of DSS is shown for comparison.

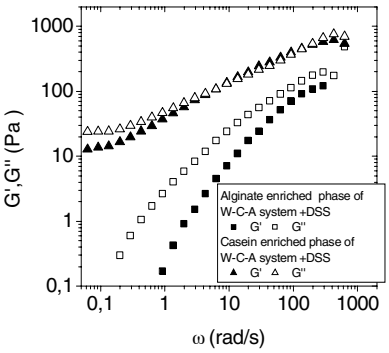

Figure 6. Dynic spectrof the coexisting phases of the water-casein (14.4 wt%)-alginate (0.24 wt%)-DSS (1.42 wt%) emulsions, at 298 K.

70 times, respectively, higher compared with those of the uncompatibilized emulsion. The morphology of such a system (Fig. 3C) is characterized by the presence of lamellae. The behavior of the coexisting phases in the W-SC-SA emulsion in the presence of sulfated polysaccharide is different (Fig. 6); the casein-enriched phase is extremely sensitive to the presence of DSS, while the viscoelastic properties of the alginate-enriched phase in the presence of DSS remain almost the same as before compatibilization. Similar changes were observed for viscosity (data not presented). It can be suggested that casein forms some sort of gel with DSS, and this gel may have a lamellar structure (Fig. 3C).

To understand the reasons for such dramatic effects, the viscosity of the casein-DSS systems was measured as a function of the DSS/casein weight ratio (Fig. 7). As can be seen from Fig. 7, the dependence of the viscosity on the DSS/casein ratio has an

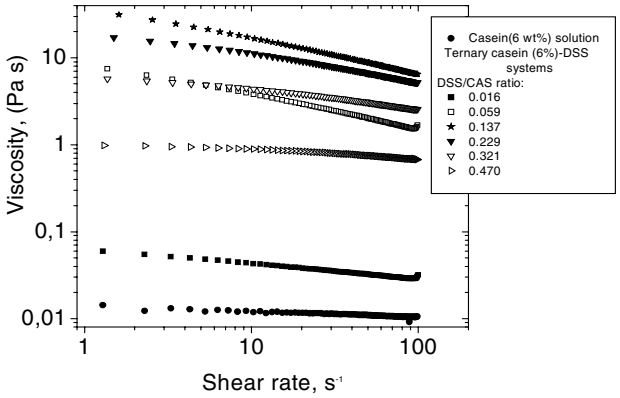

Figure 7. Viscosity of the ternary water-casein (6%)-DSS (var) system as a function of the DSS/casein ratio and the shear rate (at 298 K).

extreme character, with a maximum at a DSS/casein ratio equal to 0.137. From theory, we know that such dependences are typical for the formation of inter-polymer complexes [24]. Therefore, it can be assumed that the dramatic changes in rheological behavior of the casein-alginate system in the presence of DSS are due to interactions of the casein molecules with the DSS molecule. Structure, composition, and conditions of formation and dissociation of such complexes need to be investigated in the future.

At the same time, from the theory of thermodynamic behavior of systems containing a neutral and polyelectrolyte chains, developed by Khokhlov and Nyrkova [25] and confirmed by Joanny and Leibler [26], we know that a decrease in polyelectrolyte charge of one polyelectrolyte leads to considerable increases in its compatibility with other polyelectrolytes. Therefore, it is reasonable to assume that, in the case of the casein-alginate-DSS system, a decrease in charge of the casein molecules due to their electrostatic interaction with DSS can be responsible for the increase in compatibility of casein with alginate. The mechanism of compatibilization in such a system will be clarified in our next study.

Conclusion

According to the results obtained in this study, the main conclusions are: The presence of even a small amount of DSS in the W-SC-SA system (0.04–1.5 wt%) leads to a considerable increase in the compatibility of casein with alginate and a dramatic increase in viscosity, storage and loss moduli (G' and G''). Rheological differences are characteristic mainly of the casein-enriched phase and could be correlated with differences in morphology, visible by optical microscopy. This can be an indication of an interaction (complexing) of the casein molecules with DSS. Such peculiarities of thermodynamic and rheological behaviors allow us to consider sulfate polysaccharides as a new type of compatibilizer for biopolymer emulsions.

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