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Phase separation in aqueous casein- guar gum systems

Yurij Antonov* (✉), Jacques Lefebvre, Jean-Louis Doublier****

* N.M. Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, Kosigin Str. 4, 119991 Moscow GSP-1, Russia
** I.N.R.A., Centre de Recherchers Agro-Alimentaires - Laboratoire de Physico-Chimie des Macromolecules. BP 1627, 44316, Nantes Cedex 03, France

E-mail: chehonter@yandex.ru; Fax: (7) 495.137-41.01

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Summary

Phase separation in aqueous mixtures of guar gum and casein, containing micellar casein, sodium caseinate or its β and k-fractions was investigated, and the effects of the state of casein [colloidal-dispersed (~0.3 μm), molecular-dispersed associated, and dissociated] were established by the determination of the phase diagrams. Phase separation occurring in moderately concentrated mixtures was depended on ionic strength and was not depended on the state of protein. Passing from dissociated to molecular-dispersed associated and then to colloidal-dispersed casein result in a decrease of the total concentration in the threshold point (C^*_t) in accordance to $C^*_t \propto M_w^{0.27}$, and in increasing asymmetry of phase diagrams. In the two-phase region, the degree of compatibility was dependent on pH and ionic strength of mixtures, varying according to the change of casein -solvent interaction.

Keywords

Casein, guar gum, phase separation, incompatibility

Introduction

The phase behavior of proteins and polysaccharides (PS) in aqueous solutions is studied both for practical and fundamental reasons [1]. At definite conditions, these biopolymers are usually incompatible, that leads to phase separation, except in dilute solution [2]. The purpose of a ternary phase diagram is to determine the conditions of incompatibility and thereby establish how the solvent partitioning between the equilibrium phases. Most studies in this area are concern with the effects of the main physico-chemical parameters on the phase equilibria in mixed solutions of proteins and polysaccharides (1). The role of the structural features of these biopolymers and state of protein molecules on the phase separation and phase equilibria begin to study only recently [3-5].

The aim of this work is to study the effects of state of casein molecules (molecular-dispersed, associated colloidal dispersed, or dissociated), as well as ionic strength (μ) on the phase diagrams of aqueous casein –guar gum systems. Micellar

casein, sodium caseinate , the β and k-fractions of caseins, and sodium caseinate subjected dissociation in 8 M urea, which are similar by origin, but differing in a state of molecules, as well as commercial guar gum samples were used. It is well known that guar gum forms very viscous solutions at relatively low concentrations, which are almost unaffected by pH, salts, or heat processing. We assumed that using uncharged guar gum, whose association behavior is not depended on pH and ionic strength, allow to obtain information on the effect of casein -solvent interaction on phase separation.

The data obtained have been analysed using general theoretical concepts of the phase separation in ternary polymer 1-polymer 2 –solvent systems [6-8].

Experimental

Materials

Sodium caseinate (95.6% protein; 0.7% fat; 3.7% ash; 580 mg calcium per kg) was provided by Kerry Ingredients (Ireland). The β-casein (97% protein; 3.0% ash) was purchased from the National Dairy Products Research Centre at Moorepark, Ireland. The k-casein sample was obtained chromatographically by the INRA Centre in Jouy-en-Josas; it has not been characterised. Solutions of β- and k- casein samples in water had a pH 6.8 and 6.7, respectively. Micellar casein was a native calcium phosphocaseinate sample purified by ultrafiltration and freeze-dried, prepared at the INRA Centre in Rennes. It had the following characteristics: total protein content: 90.71%; non -casein protein: 5.05%; lactose: 0.52%; mineral salts: 8.33%; pH of a 3% solution in water: 7.29. The size distribution of the micelles has been determined in imidazole buffer pH 7, μ= 0.25 /NaCl/ with a Malvern Mastersizer laser granulometer 2000, Malvern Instr., UK, using a 45 mm lens; the volume/surface average diameter found after 20 min centrifugation of the dispersion under 1700 g was 0.47mm.

Guar gum sample was purchased from the Meyhall Chemical AG (Switzerland); its intrinsic viscosity an molecular weight in water at 293 K estimated from Mark-Houwink parameters obtained for galactomannans [9] were 1130 ml/g, and $1.22 \cdot 10^6$ Da accordingly.

Polyethyleneglycol, (PEG-20000), from Fluka Chemie AG. (Switzerland), M_η =17 KDa, was used without additional purification.

Urea, purity >99.9%, was from BDH Chemicals Ltd. (England).

Methods

Binary solutions of casein, guar gum and PEG in solvent were prepared at room temperature and fixed values of NaCl concentration and pH. The solutions of casein and guar were then centrifuged for 60 min under 100,000 g at 303 K to remove undissolved particles and air bubbles and dialysed against solvent during 24 h at 277 K. “Solutions” of micellar casein were purified by centrifugation for 60 min under 1,700 g at 303 K. The ternary water-casein-guar gum systems with different compositions were prepared by mixing binary solutions of each biopolymers. After mixing during 1 h, the mixtures were centrifuged at 50,000 g (the mixtures comprising solutions of caseins) or 1,700 g (the mixtures comprising micellar casein) for 1 h at 303 K and phase diagrams were determined. The phase diagrams of the ternary systems have been obtained under isothermal conditions described elsewhere [3].

The mistake of determination of the critical point and phase composition by this method doesn't exceed 5-7%.

The compatibility of caseins and guar gum was characterised by the co-ordinates of the threshold (C_2^*; C_3^*), and critical (C_{2c}; C_{3c}) points, as well as by the values of maximum solubility of guar gum in a concentrated solution of casein - $\overline{C}_3$ - and maximum solubility of casein in a concentrated solution of guar gum - $\overline{C}_2$ /limit of solubility [10]. The threshold point was determined on the plot as the point where the line with the slope -1 is tangent to the binodal. The critical point of the system was defined as the point where the binodal intersects the rectilinear diameter [11] .

Due to the incomplete phase separation of mixed solutions containing more than 1.2% of guar gum, the determination of $\overline{C}_2$ was impeded. Therefore, the maximum solubility of casein in a concentrated guar gum solution was taken as the value of the maximum solubility in a 1.2-% polysaccharide solution - $\overline{C}_2^{1.2}$.

The effect of ionic strength on solvent quality with respect to casein was estimated by the method of Middaugh *et al* [12] , which consists in determination the dependence of protein solubility in the given aqueous solvent on the concentration of PEG in the Water -1 - Protein 2 - PEG 3 system. Extrapolation of this dependence to C_{PEG} =0 gives the value for the effective activity of the protein in its saturated solution ($\ln C^o_{PEG}$).

Concentrations of casein and PEG in initial binary solutions were 2 wt % and 40 wt %, respectively. The weight concentration of the protein in supernatant (W_2) was determined spectrophotometric measurement at 280 nm, considering absorption of the PEG solution as the reference.

Results and discussion

Phase diagrams of the ternary solvent- protein –polysaccharide systems containing casein in colloidal (micellar casein), fully dissociated (in 8 M urea) and molecular dispersed (sodium caseinate) states were compared with each other at the same pH =7.0, ionic strength (μ) = 0.25, and temperature (293 K) (Figure 1).

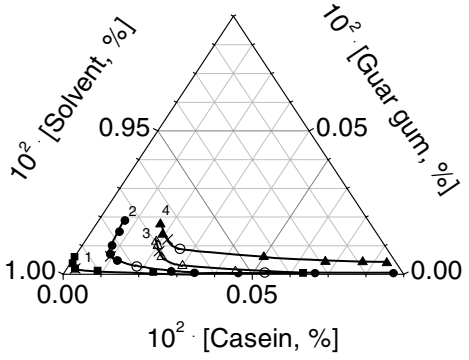

Figure 1. Isothermal phase diagram of water-casein-guar gum systems. pH=6.7; μ=0.25 /NaCl/; 293K. curve 1-micellar casein; curve 2,3-sodium caseinate; 3-in 8 M urea.
–binodal; ○ critical point; × - threshold point.

The major parameters of all the phase diagrams obtained are listed in Table I.

Table I. Parameters of the phase diagrams obtained

System	pH	μ	C_{2c} wt %	C_{3c} wt %	K_s	C_2^{12} wt %	$\bar{C}_3$ wt %	C^*_2 wt %	C^*_3 wt %	C^*_t wt %
$Cas^{(mic)}$-guar gum	7.0	0.25	5.30	0.06	88.3	10^{-4}	0.03	0.21	0.20	0.41
$Cas^{(sod)}$-guar gum-8M urea	6.9	0.25	2.65	0.89	6.05	2.14	0.40	2.15	1.22	3.37
$Cas^{(sod)}$-guar gum	6.9	0.25	1.81	0.28	6.46	0.78	0.03	0.93	0.63	1.56
$Cas^{(sod)}$-guar gum	6.9	0.15	-	-	-	1.14	0.05	1.37	0.73	2.10
$Cas^{(sod)}$-guar gum	6.9	0.08	-	-	-	1.94	0.09	2.17	0.79	2.96
$Cas^{(sod)}$-guar gum	9.5	0.25	-	-	-	1.91	0.05	2.13	0.81	2.94

C_{ic} - Weight concentration at the critical point (i=2 – Casein; i=3-Guar gum,).
K_s - casein/guar gum weight ratio at critical point.
$\bar{C}_2^{1.2}$ - Limit of solubility of casein in 1.2 wt % solution of guar gum.
$\bar{C}_3$ - Limit of solubility of guar gum in concentrated solution of gelatin.
$C^*_{2,3}$ -Concentration of biopolymer in the threshold point (2-Casein, 3-Guar gum);
C^*_t - Total concentration of biopolymers in the threshold point.

Small C^*_t, $\bar{C}_2^{1.2}$, and $\bar{C}_3$ values are peculiarities of the phase diagrams obtained in the absence of urea. This gives indication on a low compatibility of these biopolymers. Compatibility of guar gum with casein increases considerably in the following sequence : micellar casein< sodium caseinate< fully dissociated casein. Phase diagrams obtained are asymmetric (the critical point corresponds to a much lower concentration of guar gum than casein), and their asymmetry decreases in the same sequence. It is known from the general theoretical concepts of the phase separation in ternary polymer 1-polymer 2 –solvent systems [6,7] that so high asymmetry is result from significant differences between the second virial coefficients, characterising interactions of polymers with solvent.

Table II shows the effect of molecular mass of casein on the total concentration of biopolymers in the threshold point of the systems (C^*_t).

Table II. Dependence of the total concentration of biopolymers in the threshold point (C^*_t) on the molecular weight of casein.

Molecular weight, Da	C^*_t, wt%	$C^*_t \cdot M^{0.27}$
25,000^1	3.37	51.9
243,000^2	1.56	44.4
160,000,000^3	0.41	67.3

1) Molecular weight of sodium caseinate in the presence of 8 M urea [13] .
2) Molecular weight of β-casein at μ =0.15 /NaCI/ according to [13].
3) Molecular weight of micellar casein, according to [14].

Since casein is compatible with guar gum in the absence of salt [14] the comparative data concerning C^*_t values were evaluated in the presence of 0.25 M NaCl). One can see that, the higher molecular weight of casein, the lower C^*_t value. The dependence obtained is determined through:

$$C^*_t \propto M_W^{-(0.27)} \qquad (1)$$

Systematic experimental data concerning dependence of C^*_t upon the radius or molecular weight of synthetic or natural polymer´s particles are unknown till now. Exception was the results of Clarke and Vincent [15] obtained for silica particles sterically stabilized by polystirene.

According to these data $C^*_t \sim \propto a^{-(0.5)}$, where a is a size of particles.

Here it should be take into account that above mentioned theoretical dependence is truly in the conditions when radius of particle is much more than size of neutral polymer [16].

The dependence of C^*_t from molecular weight of casein, plotted in double logarithm co-ordinates in Figure 2 is rectilinear. This gives us a possibility to evaluate roughly the critical value for molecular weight of casein (M_c*) at which phase separation of the casein-guar gum systems doesn't occur. Taking into account that solubility of guar gum in water doesn't exceed 2% wt (see figures 1 and 2), and assuming that the relative content of guar gum in the threshold point for the systems comprising low molecular weight casein (C^*_3 / C^*_t =0.36).

Taking into account that solubility of guar gum in water doesn't exceed 2% wt (see figures 1 and 2), and assuming that the relative content of guar gum in the threshold point for the systems comprising low molecular weight casein (C^*_3 / C^*_t =0.36) don't depends from molecular weight of casein, we can assume that the value C^*_t = 2/0.36 =5.6 wt% is maximal at which phase separation doesn't occur. Simple extrapolation shows that, if the average molecular weight of the degraded casein is about 3.5 kDa, it will be compatible with guar gum in all the concentration range studied (figure 2). Obviously, molecular weight of the casein samples is not the critical parameter, which determine two-phase state of the casein-guar gum system.

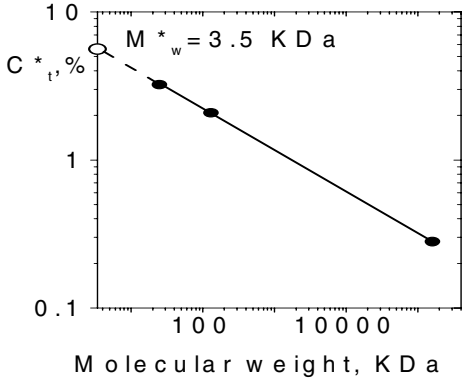

Figure 2. Dependence of the total concentration of biopolymers in the threshold point of the casein –guar gum system on the effective molecular weight of casein

The binodals of the systems, containing guar gum and β or k fractions of casein are practically the same with other conditions being equal (Figure 3), that is indication that these casein fractions are similar thermodynamically.
The effect of the presence of NaCl on incompatibility of casein with guar is different (Figure 4).

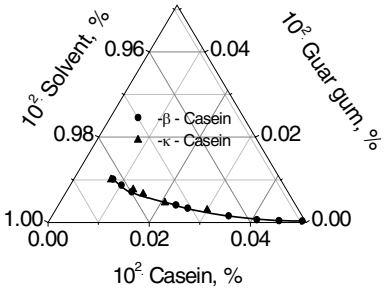

Figure 3. Isothermal phase diagram of water-casein-guar gum systems. pH=6.7; μ=0.25 /NaCl/; 293K

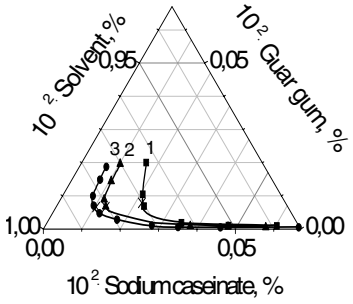

Figure 4. Isothermal phase diagram of water-casein-guar gum system.
μ=0.08 (curve 1); μ=0.15 (curve 2); μ=0.25 (curve 3). pH 6.7

In the range of NaCI concentrations from 0.25 to 0.15 M the compatibility changes relatively little. A further decrease in NaCl concentration results in a considerable rise in biopolymers compatibility. These features are reflected in the co-ordinates of the critical and threshold points for 0.25, 0.15 and 0.08 M NaCL concentration (Table I). Decrease in NaCI concentration result in a decrease in asymmetry of the phase diagram. It is important to note that ionic strength of solution, $\mu = 0.5 \Sigma$ mi Z^2i , where Z is a charge of ion, and mi- its molal concentration. It is easy to see that at equal weight concentrations, ionic strength created by low molecular salt (MW_{NaCI} is 58,5) will be thousand times higher than that created by polyelectrolytes (MWcasein is 243,000^2).Therefore the only low molecular weight salts are normally used to create ionic strength in protein -containing solutions.

This allows to consider ionic strength values of the protein solution in the presence, for example, 0.2 M NaCI as μ = 0.2. Moreover, considering the data presented in Figure 4 one can see that phase separation become to show itself when concentration of NaCI is equal or higher 0.08 M, (curve 1) while without NaCI phase separation was nor observed in all concentration range of biopolymers studied/ [14]. If to suppose that casein effect noticeably on the ionic strength the phase separation should develop itself at high concentration of biopolymers in the absence of NaCI but it does not occur.

According to general theoretical concepts [6-8] a sharp increase in the compatibility of casein with guar gum, when ionic strength decreases from 0.25 to 0.08 (in the range of μ corresponding biopolymers´incompatibility), or pH of the systems increases can be explained by the disappearance of the difference in the thermodynamic interaction parameter between each of the biopolymers and water. Therefore, the method of Middaugh *et al* [12] was employed here to examine the effect of ionic strength and pH on the interaction of casein with the solvent. Figure 5 shows the effects of ionic strength (curves 1 and 2) and pH (curves 2 and 3) on solubility of casein in the PEG solutions

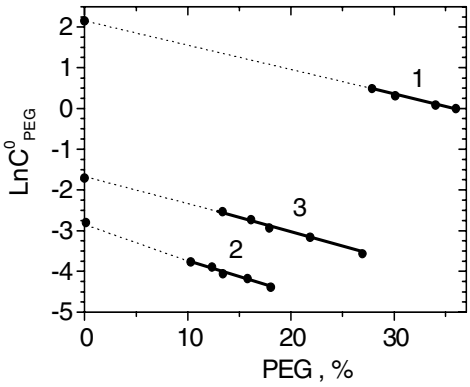

Figure 5. Effect of PEG on solubility of caseinate at different pH values and ionic strength. Measurements were made at 293 K.
curve 1. pH 6.86, μ= 10^{-4}; curve 2. pH 9.50, μ=0.25; curve 3. pH 6.86, μ=0.25
The *solid line* is a least squares fit of the data to a straight line.
The *dotted line* gives a linear extrapolation of the solubility in the absence of PEG

The dependences obtained proved to be rectilinear and almost parallel each other. We have seen before that a decrease in ionic strength, as well as an increase of pH of the systems, increases considerably the compatibility of the two kinds of macromolecules. The results obtained show that a decrease of ionic strength, as well as an increase of pH leads to dramatic increase in activity of saturated casein solutions (lnC^0_2) apparently in consequence of casein dissociation [14] .

Thus, taking into account that interaction of guar gum with water depends insignificantly on pH and ionic strength, strong effect of ionic strength and pH on compatibility of guar gum with casein is the result of a sharp change in the thermodynamic interaction parameter of caseins with solvent at given conditions (figure 5).

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