Spinodal decomposition in a food colloid–biopolymer mixture: evidence for a linear regime

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Abstract
We investigate phase separation and structural evolution in a complex food colloid (casein micelles) and biopolymer (xanthan) mixture using small-angle light scattering. We demonstrate that phase separation is induced by a depletion mechanism, and that the resulting coexistence curve can be described by osmotic equilibrium theory for mixtures of colloids and polymer chains in a background solvent, taking into account interactions between the polymer chains in the excluded volume limit. We show that the light scattering pattern of an unstable mixture exhibits the typical behaviour of spinodal decomposition, and we are able to confirm the validity of dynamic similarity scaling. We find three distinct regimes (initial or linear, intermediate and transition stage) for the decomposition kinetics that differ in the time dependence of the peak position of the structure factor. In particular we find clear evidence for the existence of an initial linear regime, where the peak position remains constant and the amplitude grows. The existence of spinodal-like decomposition and the validity of universal scaling in the intermediate and transition stages have been found in previous studies of phase separation in attractive colloidal suspensions. However, to our knowledge the initial linear regime has never been observed in colloidal suspensions, and we attribute this at least partly to the effect of hydrodynamic interactions which are efficiently screened in our system due to the fact that the measurements were performed at high polymer concentrations, i.e. in the semi-dilute regime.

Colloidal suspensions have frequently been used as ideal model systems to address fundamental issues in condensed matter physics such as liquid ordering, crystallization and glass formation. The analogies and differences between the phase diagrams of colloidal suspensions and atomic and molecular systems and the corresponding structural and dynamic properties of the various

systems as a function of the interaction potential have been thoroughly investigated [1–3]. In recent years, attention has turned to non-equilibrium phenomena and the effect of an attractive interaction between colloidal particles. This current emphasis on out-of-equilibrium properties of soft matter is not only driven by the curiosity of soft matter scientists, but also by their enormous industrial importance. It is obvious that these recent advances in our theoretical understanding of colloidal suspensions can have a direct impact in areas such as food sciences and technology. Food is indeed a classical example of complex soft condensed matter, and a basic understanding of the interactions and different non-equilibrium situations encountered in food systems has thus become important for improving product quality as well as for designing novel food materials [4, 5].

In this letter we now demonstrate that a complex food system such as a polydisperse mixture of skim milk and a high molar mass biopolymer (xanthan) exhibits equilibrium and non-equilibrium properties very similar to those found for well-defined mixtures of almost monodisperse colloidal particles and synthetic polymers. We focus on the observation that the addition of xanthan to skim milk can lead to phase separation reminiscent of a colloidal gas–liquid phase separation in binary mixtures. We show that the resulting coexistence curve in the phase diagram can be understood as the result of a xanthan-induced depletion interaction [6] similar to the situation encountered in colloid–polymer mixtures [7]. Moreover, we demonstrate that the resulting phase separation kinetics closely follows the theoretical predictions for spinodal decomposition, and we are able to confirm the validity of dynamic similarity scaling. In particular we find clear evidence for the existence of an initial linear regime, where the peak position remains constant and the amplitude grows.

The existence of spinodal-like decomposition in attractive colloidal suspensions has already been demonstrated in previous studies [8, 9]. However, to our knowledge the initial linear regime has never been observed in colloidal suspensions. Previous theoretical investigations of phase separation and spinodal decomposition in colloidal suspensions have indicated the importance of the intrinsic dynamic asymmetry and the existence of long-range hydrodynamic interactions [10–12]. As pointed out recently by Tanaka and collaborators [11, 12], phase separation of a fluid mixture whose components have a very different internal dynamics is essentially different from that of classical fluid mixtures. It has also been shown by Dhont [10] that hydrodynamic interactions between the colloidal particles can strongly influence both the initial as well as the intermediate and transition stage of spinodal decomposition and result in a diminishing linear regime with increasing strength of hydrodynamic interactions. Our experiments now demonstrate that for our system, where hydrodynamic interactions are efficiently screened due to the fact that we work at high polymer concentrations, i.e. in the semi-dilute regime, we indeed observe a well-defined initial linear regime.

Mixtures of proteins and polysaccharides are widely used in the food industry as they play a vital role in determining the structure, texture and stability of many food products [5]. Particularly well-investigated examples of food proteins are casein micelles, the major protein component of milk. Casein micelles are association colloids with an average diameter of about 200 nm and quite polydisperse. They have been shown to closely follow theoretical descriptions originally developed for colloidal model systems [13, 14]. While casein micelles follow simple hard sphere behaviour at low to intermediate concentrations, at high concentrations they behave very similarly to soft particles such as microgels [15]. They even exhibit an apparent divergence of the zero shear viscosity and the formation of a jammed solid-like state in a way that closely resembles that found for synthetic microgels [16]. Xanthan is a high molecular weight bacterial exo-polysaccharide used in many milk products whose solution properties can be described quite well using theoretical descriptions for excluded volume polymers and polyelectrolytes [9].

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Although the individual properties of caseins and xanthan are well investigated, there are not many studies involving mixtures of casein and xanthan [17]. Here we now focus on the phase behaviour of skim milk upon the addition of xanthan. In this case the biopolymer induces phase separation at higher xanthan concentrations due to depletion interactions [6] similar to the situation encountered in colloid–polymer mixtures [7, 9]. We determined the location of the coexistence curve using visual inspection and turbidity measurements, and used time-resolved small-angle light scattering (SALS) experiments to study the phase separation kinetics and structural evolution. We also compared our light scattering data with confocal and optical microscope results.

Details of the sample preparation procedures and the design of the small-angle light scattering instrument that allows us to follow the onset of phase separation and the decomposition kinetics over a broad range of time and length scales are given elsewhere [18]. Food-grade low-heat skim milk powder was provided by Nestlé Research Center (Lausanne, Switzerland). The skim milk powder contained 36% (w/w) total protein, the majority being in the form of casein micelles (28%). The average radius (R_c) of casein micelles obtained from our light scattering measurements is 100 nm. Xanthan (Keltrol, CP Kelco Co. Ltd) was also a kind gift from Nestlé Research Center. It is an anionic polysaccharide with a molecular weight of 4×10^6 g mol^{-1}. Light scattering measurements provided a value of 230 nm for the radius of gyration (R_g) and 0.008 wt% for the overlap concentration c^*. These values are very close to those reported by Koenderink *et al* [9].

MilliQ water was used as the solvent. A stock solution of the skim milk was prepared by dissolving 20 wt% of the powder in the solvent at 55 °C using a magnetic stirrer at moderate speeds. After mixing, the solution was brought to room temperature (22 °C) by constantly stirring for 1 h. Milk-permeate was obtained from the stock solution using a Vivaflow 50 ultra-filtration unit (Sartorius AG, Switzerland) with a 10 kDa molecular weight cut-off. The final pH of the stock solution and permeate was around 6.8. Stock solutions of xanthan were prepared by dissolving 0.2 wt% of the powder in MilliQ water at 85 °C in the presence of 0.1 M NaCl under constant stirring for about 2 h using a magnetic stirrer. The added NaCl results in a relatively strong screening of xanthan, and thus reduces the polyelectrolyte effect, i.e. a theoretical treatment can be done primarily on the basis of simpler polymer models. The solution was brought to room temperature and again stirred for about 4 h. 2 mM of sodium azide (NaN$_3$) was added in the stock solutions of milk and xanthan and it was refrigerated for about 14 h before being used for experiments.

Skim milk/xanthan mixtures were obtained by mixing appropriate amounts of skim milk, xanthan and milk permeate using a vortex mixture (Vortex Gene II, model G560E) at the highest speed for about 1–3 min. All measurements were done at room temperature of 22 °C and a pH of 6.8 for the final mixture. Figure 1 shows the resulting phase diagram for skim milk/xanthan mixtures using visual observations, confocal microscopy and turbidity measurements carried out with our SALS setup [18, 19].

Figure 1 immediately reveals that an increasing xanthan concentration results in an increasing strength of the depletion-induced attraction. We see that at lower concentrations of skim milk and xanthan, one generally observes gas–liquid type phase separation with a phase inversion taking place at the equi-phase volume region where bicontinuous structures are formed. On either side of this point, the phase with the maximum phase volume forms the background with the other phase forming droplets. Depending upon the quench depth, i.e. the xanthan concentration, the phase separation in this lower concentration region proceeds through a spinodal decomposition or a nucleation and growth scenario [20].

Before we proceed with the analysis of a time-resolved investigation of the decomposition kinetics after a deep quench, i.e. in the spinodal regime, we see whether the location of
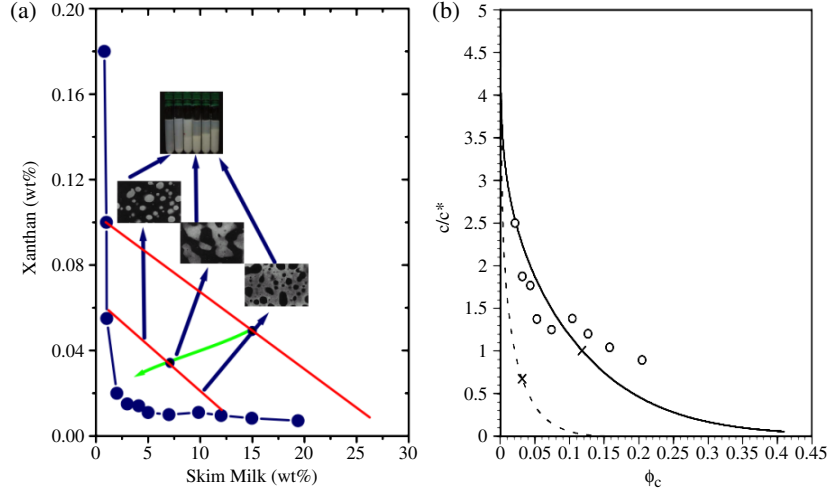
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Figure 1. (a) Phase diagram for a skim milk/xanthan mixture at a pH of 6.8 obtained at room temperature. At lower concentrations of skim milk and xanthan one observes gas–liquid phase separation with phase inversion on either side of the equi-phase volume point where a bicontinuous structure is observed. (b) Comparison of experimentally determined (open circles) and calculated coexistence curve (solid line) for skim milk–xanthan mixtures. Also shown is a comparison with the corresponding result for ideal chains mixed with hard spheres as the dashed line (see text for details). The location of the critical point is indicated by a cross.

the coexistence curve for this complex and polydisperse food mixture can be rationalized in terms of a theoretical prediction using osmotic equilibrium theory for mixtures of colloids and polymer chains in a background solvent. This theoretical approach was first proposed for hard spheres plus polymers described as freely overlapping spheres by Lekkerkerker *et al* [21]. In mixtures of xanthan and casein micelles the polymer concentrations at the coexistence curve are near and beyond the coil overlap concentration, so we have to take into account interactions between the polymer chains. Hence we use a theory for mixtures of hard spheres plus polymer chains in the excluded volume limit [2], which is an extension of [21]. The osmotic pressure of the polymer solution is described using renormalization group theory as in [22] with the correct scaling ($\sim c^{2.25}$) in the semi-dilute regime. The depletion thickness δ around the casein micelles in solutions containing xanthan is calculated from [23]:

$$\frac{1}{\delta^2} = \frac{1}{\delta_0^2} + \frac{1}{\xi^2} \tag{1}$$

where δ_0 is the depletion thickness at infinite dilution, which depends only on the chain length, and ξ is a (chain-length independent) correlation length. Xanthan is a stiff polymer chain with a persistence length of about 120 nm, and we thus describe it as a Kuhn chain consisting of effectively eight segments. Since casein micelles have an average radius close to 100 nm [24], we cannot treat the chains as being Gaussian on the colloid length scale as has been done in most theories. We calculate the depletion thickness around a casein micelle in a dilute xanthan solution, δ_0, from the theory of a sphere immersed in a solution of freely jointed chains. Therefore the segment to sphere size ratio can be chosen arbitrarily [25] and we find $\delta_0 = 123$ nm, which is much smaller than the radius of gyration. For the correlation length we use $\xi = R_{\mathrm{g}}(c/c^*)^{-3/4}$ (which has been used in the past for xanthan, see [9, 26]), which in the case of a casein micelle surrounded by xanthan polymers then leads for equation (1) to $\delta^{-2} = \delta_0^{-2} + R_{\mathrm{g}}^{-2}(c/c^*)^{3/2}$. This choice ensures the correct scaling of the depletion thickness in
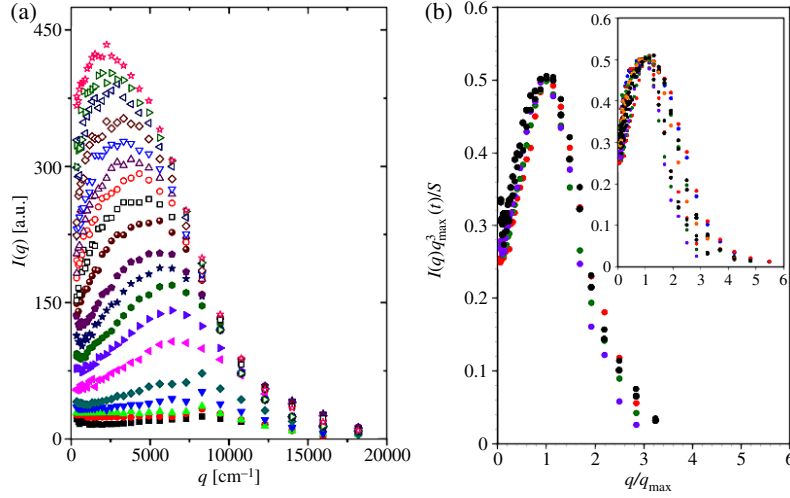
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Figure 2. (a) Static light scattering patterns obtained using small-angle light scattering for skim milk (3.125 wt%) and xanthan (0.1 wt%) at $T = 22\,^\circ$C and pH = 6.8. The first curve (■) is taken 5 s after mixing and the last curve (○) is after 1 h of mixing. (b) Dynamic scaling of the data shown in (a) for a period between 180 and 500 s, i.e. in the early part of the intermediate regime. The inset shows a dynamic scaling plot of all the data in the intermediate regime, and we observe considerable broadening at later times due to the scattering from sharper interfaces that form in the course of the separation process.

the semi-dilute regime, $\delta \sim (c/c^*)^{-3/4}$. Note that for small polymer concentrations ($c \ll c^*$) $\delta = \delta_0$, and for concentrations in the semi-dilute regime ($c \gg c^*$) $\delta = \xi$. The depletion thickness and osmotic pressure are then inserted in the many-body theory of [22] for hard spheres plus interacting chains, resulting in the fluid–fluid coexistence (full) curve shown in figure 1(b). The dashed curve is the corresponding result for ideal chains mixed with hard spheres (the theory for hard spheres mixed with ideal chains is also reported in [22]). We see that while the simple model of hard spheres and ideal chains strongly underestimates the stability of our skim milk–xanthan mixtures, the improved model of hard spheres and semiflexible chains is capable of reproducing the location of the coexistence curve of such a complex food mixture surprisingly well. It is only at high biopolymer or casein concentrations, where we cannot expect our simple model to still be correct, that we observe significant deviations between the experimental phase diagram and the theoretical calculations. Figure 1 demonstrates that the phase separation observed in skim milk–xanthan mixtures is indeed due to a xanthan-induced depletion effect, and that relatively simple models from classical soft condensed matter theory are capable of reproducing the phase behaviour in complex food colloid–biopolymer mixtures. In this context it is useful to mention that the excluded volume limit would provide an upper estimate of the location of the binodal; for arbitrary solvency (segments will never be pure hard spheres but always attract one another somewhat) one expects demixing between the limits of ideal and excluded volume chains, respectively (see also [27]).

In a next step we can now follow and analyse the phase separation process in an unstable skim milk–xanthan mixture. Here we profit from recent developments in instrumentation for ultra small-angle light scattering [18, 19], which permits us to follow phase separation with a time resolution of a few milliseconds and look, for example, for the classical hallmarks of spinodal decomposition. Figure 2(a) shows the corresponding static scattering intensity patterns for a sample with a concentration of skim milk (3.125 wt%) and xanthan (0.1 wt%)

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that is situated in the region of the phase diagram where we expect droplet-like spinodal decomposition to occur.

A peak is observed in the scattering pattern at a scattering vector q_{max} that corresponds to a characteristic length scale $\xi \approx 2\pi/q_{max}$. With time the peak increases in amplitude and moves towards lower scattering vectors, indicating a coarsening of the structures formed in the phase separation process. The scattering patterns shown in figure 2(a) are typical for spinodal decomposition seen for example in binary mixtures, polymer blends or in classical colloid polymer mixtures [8, 20]. This is illustrated in figure 2(b), where the data are shown to follow universal scaling typical for spinodal decomposition processes given by

$$\frac{I(q)q_{max}^3(t)}{S} = F\left(\frac{q}{q_{max}(t)}\right) \tag{2}$$

as proposed by Furukawa [28] and derived by Dhont for the spinodal decomposition of colloids in the initial and intermediate stage including hydrodynamic interactions [10]. Here $S = \int_{q_1}^{q_2} I(q,t)q^2\,dq$, where the normalization integral is performed for a finite interval $q_1 \ll q_{max} \ll q_2$, and $F(x)$ is a universal scaling function that depends on the ratio $x = q/q_{max}$ only [10, 29]. We see that all the data obtained in the beginning of the intermediate regime collapse onto a single universal scaling function, but with time the peak starts to broaden (see inset), most likely due to the increase of the scattering intensity at higher q-values caused by the formation of sharp interfaces.

The relationship $\xi = 2\pi/q_{max}$ between the peak position q_{max} and the characteristic length scale or domain size ξ now allows us to investigate the temporal evolution of the domain size. Commonly one distinguishes between at least three characteristic regimes during the spinodal decomposition process: (i) early, (ii) intermediate and (iii) late stage [20]. The early stage is described by linear Cahn–Hillard theory and in essence predicts an increase of the amplitude at constant q_{max} until the peak spatial concentration fluctuations have reached the final coexisting concentrations and domain growth and coarsening sets in. In a simple picture coarsening is expected to occur according to a simple power law $\xi \propto t^\alpha$, where the scaling exponent is $\alpha = 1/3$ in the intermediate (or diffusive) and $\alpha = 1$ in the late (or flow) stage of the coarsening regime [29, 30]. A detailed calculation for the intermediate stage including hydrodynamics reveals that the exponent α is between 0.2 and 1.1, depending upon the relative importance of hydrodynamic interactions [10]. While intermediate and late stages in agreement with the theoretical predictions for α have been found in previous studies of colloid–polymer mixtures (see [8] and references therein), these studies were generally not able to find evidence for the linear regime.

Figure 3 shows the evolution of microstructure as a function of time derived from the data obtained with a skim milk–xanthan mixture undergoing spinodal decomposition (figure 2). A notable feature of our light scattering data is that our CCD camera-based setup records even the fast evolving linear regime of spinodal decomposition. This is further supported from an analysis of the scattered intensity $I(q_{max}, t)$ at the peak position shown in the inset of figure 3. The logarithm of $I(q_{max}, t)$ shows a linear behaviour at short times ($t < 100$ s), indicating an exponential growth of the intensity in agreement with Cahn–Hillard theory. To our knowledge the initial linear regime has not been observed in colloidal suspensions previously, and we partly attribute this to the effect of hydrodynamic interactions which are efficiently screened in our system due to the fact that we work at high polymer concentrations, i.e. in the semi-dilute regime. However, it is clear that this screening effect is not the only reason, as previous experiments with mixtures of well-defined colloidal Teflon particles of a similar radius ($R = 93$ nm) and xanthan under comparable conditions, i.e. also in the semi-dilute regime, were not able to provide evidence for a measurable linear regime [9]. We have
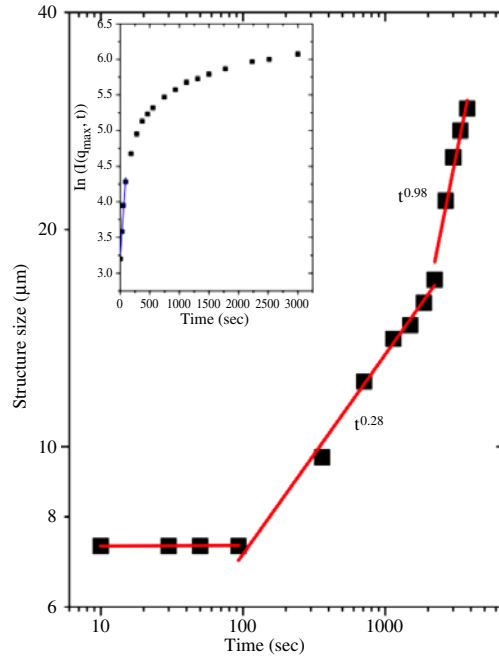
Figure 3. Evolution of the characteristic domain size $\xi = 2\pi/q_{max}(t)$ obtained from the position $q_{max}(t)$ of the peak of the scattered intensity as a function of time. The inset shows the time evolution of the logarithm of the scattered intensity $I(q,t)$ at q_{max}.

made some test experiments with a similar system and also found the decomposition kinetics substantially accelerated when compared to those found for skim milk and xanthan (data not shown). Other possible reasons for such a distinctly different behaviour could be the higher polydispersity and lower surface charge density of the casein micelles, and in particular the very different density mismatch between particles and solvent. While the Teflon particles have a significant density mismatch, the casein micelles are extremely open, porous structures with a high voluminosity, a low particle density of approximately 1.06 g cm^{-3} and a correspondingly low density mismatch.

Figures 2 and 3 clearly demonstrate that phase separation in complex food systems such as skim milk–xanthan mixtures follow universal rules originally derived for much simpler systems. This lends support to future attempts of using analogies to colloids in order to understand and improve food mixtures. Moreover, these mixtures have allowed us to verify for the first time the existence of a linear regime in the spinodal decomposition of attractive colloidal particle suspensions. It will be interesting to compare the results obtained with the casein micelles to other more well-defined colloidal model systems under different background solvent conditions in order to test out the contributions from hydrodynamic interactions and polydispersity to the existence and extension of a linear regime.

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