

Colloidal zein particles at water-water interfaces



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ABSTRACT

We synthesize colloidal zein particles using the anti-solvent precipitation method and study particle behavior at water-water interfaces. When added to phase-separating aqueous mixtures of fish gelatin and dextran, particles accumulate at the interface. In order to explain the mechanism of particle accumulation at the water-water interface, we investigate how zein particles interact with polymers (i.e. fish gelatin and dextran). We show that both polymers adsorb similarly on particle surface, which can explain why particles form contact angles close to 90°. Moreover, we show that particle accumulation is accompanied by aggregation. Those aggregates are able to arrest the late stage of the demixing process of the emulsion by the formation of a stable particle-rich layer at the water-water interface. This layer is referred to as a 'foam-like layer' due to its morphology similar to that of a wet (non-drained) foam, and contains droplets of one phase, surrounded by particle-stabilized lamellae of the other phase.

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1. Introduction

Phase separation of moderately concentrated aqueous solutions of thermodynamically incompatible polymers leads to a dispersed system called a water-in-water emulsion (Tolstoguzov, 1986). The situation is often encountered in food systems containing a mixture of protein (e.g., gelatin) and polysaccharide (e.g., dextran, malto-dextrin), under conditions where the protein-polysaccharide interaction is net repulsive (Butler & Heppenstall-Butler, 2003; Firoozmand, Murray, & Dickinson, 2009; Loren, Langton, & Hermansson, 2002; Syrbe, Fernandes, Dannenberg, Bauer, & Klostermeyer, 1995; Tromp, Rennie, & Jones, 1995), usually due to a difference in affinity for the solvent. The interfacial tension between the aqueous phases is extremely low ($\gamma \approx 0.001$ – 0.01 mN m⁻¹) (Stokes & Frith, 2002; Wolf, Scirocco, Frith, & Norton, 2000), compared to the oil-water tension which is generally 10–30 mN m⁻¹. As a consequence of the low interfacial tension, the driving force for coalescence in water-in-water emulsions is low. Still, however, gravity-induced layer formation is observed within hours. A stabilizer is therefore needed. Kinetically stable water-in-water emulsions would be of great commercial interest,

since they could lead to fat-free food or capsules of sensitive materials such as proteins.

Recently it was shown that water-in-water emulsions can be stabilized by the addition of particles. Particle-stabilized emulsions, so-called Pickering emulsions, have been studied extensively for oil-in-water emulsions. In water-in-water emulsions, though, the behavior of particles can be quite different, due to the much smaller interfacial tension of these systems, and the presence of interactions of particles both with the polymers in the mixture and with each other at the interface. These interactions play a crucial role in the understanding of stabilization of water-in-water emulsions and should be addressed in the study of these systems.

Repulsive interactions between particles and polymers were suggested by Firoozmand et al. (2009) as the driving force for the accumulation of particles at the water-water interface. However, this explanation does not apply in every case, since particles and polymers do not always repel each other. In this study we show that polymers tend to adsorb on particle surface. Such an adsorption can affect the contact angle, and have consequences on the stability of the particle dispersion. Moreover, in the case of particles formed by aggregates during nucleation and growth, polymer adsorption could affect the aggregation state. Except for the particle – polymer interactions, one should also take into account the particle – particle interactions. Specifically, aggregation of particles may be induced as a result of increased particle

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concentration locally after their adsorption at the water-water interface. Due to this effect, aggregates of particles – instead of single particles – can interact in a stabilizing way with the water-water interface, forming an elastic layer around the droplets that cannot shrink or expand anymore (Murray & Phisarnchananan, 2014, 2016). The formation of the viscoelastic particle layer at the liquid-liquid interface has the potential to arrest the process of layer formation (Firoozmand et al., 2009), although does not necessarily prevent droplets from coalescing. This has been proved lately to be one of the most important differences between oil-in-water and water-in-water emulsions: whereas for the former the presence of particles at the interface leads invariably to stabilization, this is not the case for water-in-water emulsions where coalescence of fully-covered droplets can still be observed. During coalescence, it has been reported that particles may even be driven off the droplet surface into the solution (Balakrishnan, Nicolai, Benyahia, & Durand, 2012; de Freitas, Nicolai, Chassenieux, & Benyahia, 2016).

This paper is an effort to study the interactions between particles and polymers, and particles with each other at the water-water interface, and to show how they can contribute or complicate the Pickering stabilization of water-in-water emulsions. In other words, first, we want to show the relevance of particle-polymer interactions, which will help us to better understand the driving force for particle accumulation at the interface. Second, we aim to show particle aggregation at the interface which does occur even at low particle concentrations (where is no crowding of particles at the interfaces of dispersed droplets) and complicates the simple picture of a monolayer of adsorbed particles. For this study, we chose zein, an extensively studied, food-grade and abundant protein that can be used as a particle emulsifier. The main advantage of zein over other food-grade proteins is its solubility properties: it is very hydrophobic (insoluble in water), but soluble in some organic solvents (e.g. ethanol), which allows the controlled fabrication of colloidal particles through the anti-solvent precipitation method without the use of any crosslinker. Furthermore, zein particles do not require any additional chemical surface modification or pretreatment to assure interfacial affinity, as opposed to other particle emulsifiers (e.g. chemically modified starch granules) (Yusoff & Murray, 2011).

2. Experimental details

2.1. Materials

As a model system, we use the polymers cold water fish gelatin and dextran. The system gelatin/dextran/water is a well-documented model system for phase separation studies (Scholten, Sprakel, Sagis, & van der Linden, 2006; Vis et al., 2015). Fish gelatin (type A or acid-extracted, gelling temperature 8–10 °C, approximately 100 kDa) was obtained from Norland Products Inc., via FIB Foods (Harderwijk, the Netherlands). Gelatin has an isoelectric point at approximately pH 8 (Karim & Bhat, 2009). Dextran (from *Leuconostoc* spp., 450–650 kDa) was obtained from Sigma-Aldrich. As a source of particle material, we use the protein zein extracted from corn. Zein was obtained from Sigma-Aldrich. Absolute ethanol (99.9%) was obtained from Sigma-Aldrich as well. Phosphate and citrate buffer are used to control the pH. Water purified by a RO (reverse osmosis) system of pH 7 is used for all the experiments. The dialysis tubing used for the removal of ethanol in particle dispersions (MWCO 12–14000 Da) was obtained from Medicell Membranes Ltd.

2.2. Methods

2.2.1. Synthesis of zein particles

Zein is a hydrophobic protein, poorly soluble in water except in the presence of polar organic solvents (i.e. ethanol). Therefore, it can be used as a stabilizer only in the form of particles. These are dispersible in water due to charge, but still have a hydrophobic surface. In order to synthesize zein particles, the classic anti-solvent precipitation method was used; this method consists of adding a non-solvent to a solution to induce supersaturation, which provides a driving force for solute nucleation (Baars et al., 2015; de Folter, van Ruijven, & Velikov, 2012; Joye & McClements, 2013).

First, 0.2 g zein was dissolved in an aqueous ethanol mixture (80 (w/v) % EtOH), prepared by mixing 80 g ethanol with 20 g RO water. The solution was stirred for 1 h at 40 °C to achieve the complete dissolution of zein, and centrifuged at 9,000 g for 30 min. Afterwards, 30 g of the supernatant was added dropwise to a beaker which contained 70 g RO water, under gentle stirring. A turbid dispersion with a milky appearance was formed. To remove ethanol and obtain an aqueous dispersion of zein particles, dialysis was performed against acidic water of pH 3.7. The final aqueous dispersion had pH 3.7.

2.2.2. Characterization of zein particles in aqueous suspension, in gelatin solution and in dextran solution

The size and shape of zein particles in aqueous suspensions were analyzed by taking cryogenic transmission electron microscopy (cryo-TEM) photographs using a Tecnai 200 (FEI Company, The Netherlands), operating at 200 kV. One drop of a particle dispersion was placed on a carbon grid, which was then freeze-dried into liquid ethane at the temperature of liquid nitrogen. Larger size fractions of particle dispersions were observed with the optical microscope Reichert-Jung (Leica Microsystems, Switzerland). The size of zein particles was confirmed by dynamic light scattering measurements carried out with the ALV-CGS4 Compact Goniometer System with 4 detectors. Particle dispersions were diluted in buffer of pH 3.2, 7.1 and 8.1.

The electrophoretic mobility of the particles was measured with a Zetasizer Nano, employing cuvettes equipped with an electrode, using the Smoluchowski equation for converting measured mobilities to zeta potentials. The surface charge properties of the particles were investigated as a function of pH. At least two separate measurements were used for each pH value.

Dynamic light scattering measurements were performed to determine the effect of addition of polymer on the stability of particles. In other words, possible interactions between particles and polymers were investigated. The measurements were carried out using the ALV-CGS4 Compact Goniometer System with 4 detectors at pH 3.2, 7.1 and 8.1 at 20 °C for zein particles (0.003% w/w) in buffer without polymer and in suspensions of dilute gelatin (0.01%, w/w) and dilute dextran (0.01%, w/w) solutions. Low polymer concentrations were chosen to avoid the depletion and subsequent attractive interactions between the particles. The results reported are averages of three measurements.

2.2.3. Preparation and characterization of emulsions containing zein particles

Water-in-water emulsions were prepared by dissolving fish gelatin and dextran at an initial ratio of 2/1 (total polymer concentration 12% w/w) in aqueous suspensions of particles of a concentration of 0.1–0.2% (w/w). Complete dissolution of polymers was achieved with magnetic stirring at 60 °C. No high shear treatment was necessary due to the ultra-low interfacial tension. The pH of emulsions was 5.6. Sodium azide (0.05% w/w) was added in the final emulsions in order to prevent the microbial growth. All

samples were stored in the refrigerator at 4 °C.

Emulsions with and without particles were assessed in terms of their macroscopic appearance and stability against coalescence, after they were left overnight at room temperature to allow the macroscopic separation into two phases. The process of the macroscopic separation or demixing of emulsions was recorded overnight using a USB digital microscope (Dino-Lite), which created time-lapse movies at a frame rate of one frame per 30 s. The interface of macroscopically separated emulsions was also studied in terms of its microscopic structure by using the horizontal Ultra View RS Confocal Imaging System; for this purpose, the emulsion was left in a cuvette until it was separated into two layers. The interface between the two phases was then observed in a horizontal direction. Rhodamine B was used as a fluorescent dye to stain the gelatin phase. Finally, optical microscopy was used to obtain images of the microscopic structure of fresh emulsions.

3. Results and discussion

The behavior of zein colloidal particles and their interactions at the water-water interface were studied using as a model system phase-separating aqueous mixtures of gelatin and dextran. The concentration of particles in our study was chosen to be low, 0.1–0.2% w/w, in order not to induce an increase of viscosity, even when all particles localize in the same phase. Besides, zein particles are prone to aggregation when concentrated, which did not allow their addition to emulsions in high concentrations.

Zein particles were synthesized by the anti-solvent precipitation method and dialyzed against acidic water of pH 3.7, in order to obtain aqueous dispersions of particles. Dialysis has proven to be a crucial step in this process, in order to obtain cleaned particles without any possible surface-active components which may have altered the behavior of particles. Particle solutions that were not subject to dialysis, but only to rotary evaporation (i.e. not cleaned), showed a slightly different behavior (data not shown). Zein particles in aqueous dispersions (without buffer) appeared to have a number-averaged radius of 80–100 nm and a spherical shape, according to cryo-TEM images (Fig. 1). However, a fraction of micron-sized particles was also present, resulting in a large polydispersity. Interestingly, these large-sized particles and their behavior at interfaces were directly observable using optical microscopy, as exemplified below in Fig. 3 and 4.

3.1. Interactions of particles with polymers

First, the interactions of particles with both gelatin and dextran were investigated by dynamic light scattering measurements. These interactions may be related to surface charge properties, which were also determined by measuring the zeta-potential of particles. Fig. 2a shows the zeta-potential of particle dispersions plotted as a function of pH. The isoelectric point of zein is in the range of pH 5.8–6.5. At that pH, the net particle charge is zero, which leads to the aggregation of the particle dispersions. By lowering the pH to 3, the particles become positively charged [$\zeta = +20$ mV], whereas above the isoelectric point, the particles appear negatively charged. Fig. 2b shows the hydrodynamic radii derived from scattering data of particles in buffer of pH 3.2, 7.1 and 8 without polymer and in 0.01% (w/w) gelatin and dextran solutions. It turns out that at pH 7 the apparent particle size is larger than that at higher or lower pH, most probably due to aggregation at neutral pH, close to the isoelectric point. However, in the presence of gelatin or dextran, the apparent particle size decreases (notice the faster decay in presence of polymer in the time correlation function graphs provided in the supplementary information). The scattering is dominated by the particles, which are much larger

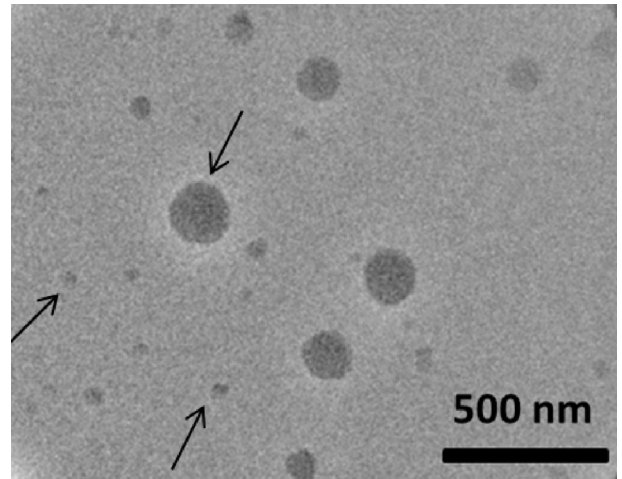


Fig. 1. Typical cryogenic transmission electron micrograph of zein nanoparticles (arrows).

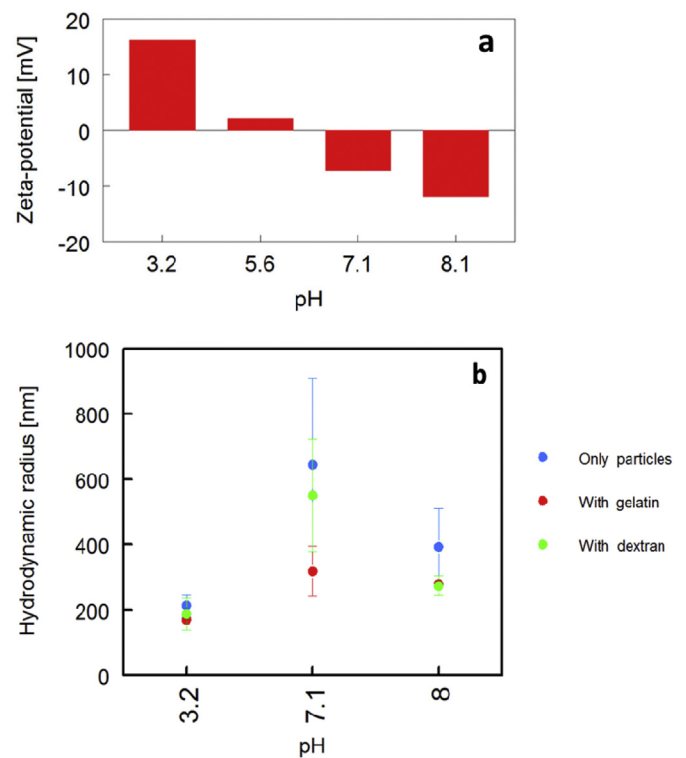


Fig. 2. (a) Zeta-potential of particle dispersions and (b) hydrodynamic radius of particles (0.003% w/w) in buffer and in gelatin/dextran solutions (0.01%, w/w) plotted as a function of pH (see the time correlation function graphs in the supplementary information).

than the polymer molecules. This observed decrease in hydrodynamic radius might be a consequence of adsorption of polymer on the particles. That will lead to an increase of the hydrophilicity and a disaggregation of the particles in smaller ones. It is remarkable that gelatin and dextran have similar effects, in spite of the fact that dextran is an uncharged polymer that generally does not interact with proteins. Interactions between protein and dextran, though, were reported earlier, and tentatively ascribed to the formation of hydrogen bonds (Antonov & Wolf, 2005). The adsorption of polymers may, therefore, be not only a charge-controlled phenomenon.

Apparently, the poor solubility or hydrophobicity of zein particles induces also the adsorption of uncharged polymers, such as dextran. The similar effects of dextran and gelatin on the hydrodynamic radius of zein particles in dilute polymer solutions are indicative of an energetically favorable interaction with both phases (relative to pure water) and this is in agreement with the fact that particles display an intermediate contact angle, as we will show below. These interactions between particles and polymers may also play a role in the dynamics of particle adsorption at the water-water interface.

3.2. Particle accumulation and aggregation at the water-water interface

When added to phase-separating aqueous mixtures of fish gelatin and dextran, particles showed a clear preference for the gelatin-rich phase. This can be understood from the hydrophobicity of zein and the relative hydrophobicity (in the gelatin-dextran pair) of gelatin. However, the particles were not fully wetted by the gelatin-rich phase. Fig. 3 shows the contact angle of approximately 65° of an exceptionally large particle, which is clearly partly in one phase, and partly in the other. The presence of particles of sizes of the order of $1\ \mu\text{m}$ or larger – the fraction visible by optical microscopy – at the interfaces is consistent with the adsorption energy of the order of $10^3 k_B T$ predicted by Young's law, provided that the contact angle is not close to 0° or 180° . Eventually, most of the particles ended up accumulated at the interface between the gelatin-rich and dextran-rich phases. The accumulation was accompanied by a degree of particle aggregation (Firoozmand et al., 2009), as is clearly seen in Fig. 4. Apparently, the adsorption of particles at the interface led to an increased local particle concentration which followed by aggregation of particles with each other. So, particle aggregation is probably driven by depletion attraction (Tuinier, Dhont, & de Kruif, 2000). The increase of particle concentration and ionic strength can be expected to lead to aggregation of particles, as the electrostatic repulsion is weakened. Some evidence of aggregation is also provided by the pH dependence of the hydrodynamic radius.

To sum up, we suggest a 2-d planar interface depletion. We form the hypothesis that particles first adsorb at the interface and form a contact angle, and then aggregate with each other. This aggregation

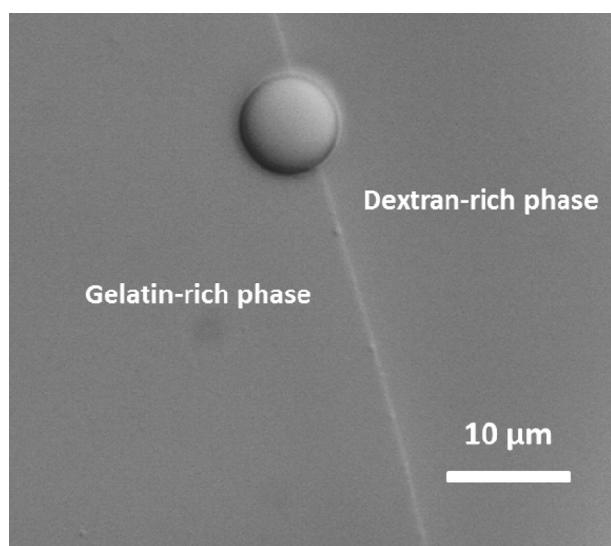


Fig. 3. Particle residing mostly in the gelatin-rich phase with a contact angle of 65° (measured from the dextran-rich phase).

occurs possibly due to the increased number of localized at the interface particles (depletion). This hypothesis is also supported by the fact that, when adding solutions of (already) aggregated particles to the emulsions, particles do not seem to adsorb at the droplet interface (data not shown). Aggregation of particles at interfaces is a known phenomenon (Sinn, Alishahi, & Hardt, 2015) and has been proposed as an alternative mechanism for the Pickering stabilization of oil-in-water emulsions in addition to the particle layer formation around the droplets. In this case, the steric particle-based barrier is not a simple monolayer or bilayer which is densely packed, but a region of a network of particles adsorbed at the interface with the whole aggregated structure held together by attractive inter-particle forces (Gautier et al., 2007; Tcholakova, Denkov, & Lips, 2008; Dickinson, 2010).

3.3. Interfacial particle-rich 'foam-like' layer

As it is mentioned above, zein particles accumulate and aggregate at the water-water interface. However, this microscopically observed particle accumulation at the interface did not lead to a stable emulsion. Nevertheless, particle accumulation did affect the coarsening kinetics and the final macroscopic morphology of emulsions at the late stage of the demixing process. Specifically, aggregates of particles were able to form and stabilize a macroscopically observable interfacial particle-rich zone in the late stage of the demixing process (Fig. 5). Fig. 5 shows stills of an overnight time-lapse movie of emulsions with and without particles where we can see the different stages of the macroscopic formation of the interfacial zone (see full movie of emulsions at pH 5.6 in supplementary information). This zone appeared to have the macroscopic morphology of a wet (non-drained) foam, as we can see in the movie of emulsion phase separation, and therefore, from now on, it will be referred as a 'foam-like' layer. It should be mentioned that the foam-like layer was always formed in presence of particles regardless the pH (see full movie of emulsions at pH 7 in supplementary information).

Supplementary video related to this article can be found at <http://dx.doi.org/10.1016/j.foodhyd.2016.10.036>.

In order to understand in greater detail the microstructure of the foam-like layer, horizontal confocal microscopy was used. Some results are shown in Fig. 6. According to those images, we can conclude that the foam-like layer consists of large dextran-rich globules (appearing black) surrounded by gelatin-rich phase (binded to the dye rhodamine), with interfaces occupied by particles in an irregular, cluster-like arrangement. Some of these interfaces are part of lamellae of gelatin-rich phase which are in-between dextran globules. These lamellae appeared to be stabilized by the particles, as concluded from an absence of development during several days. In other words, after the foam-like layer was formed, a meta-stable state of the emulsion was reached, in which the dispersed dextran droplets in the layer did not coarsen further. This can be explained by the fact that particles may provide some mechanical stability to the foam-like layer, and further coarsening is arrested due to the mechanical cohesion of the layer. The stability of the foam-like layer was outstanding even after centrifugation of the emulsion at 14,000 g for 2 h (Fig. 7).

From these results, we conclude that particle aggregation may have both negative and positive consequences for the ability of particles to stabilize water-in-water emulsions. On the one hand, particles involved in an aggregate are less available for adsorption at the interface and for lowering the interfacial tension than free particles. On the other hand, though, an interface lined by aggregates will obtain a degree of (visco) elasticity which may contribute to stabilization against coalescence. The latter is probably observed in the foam-like layer.

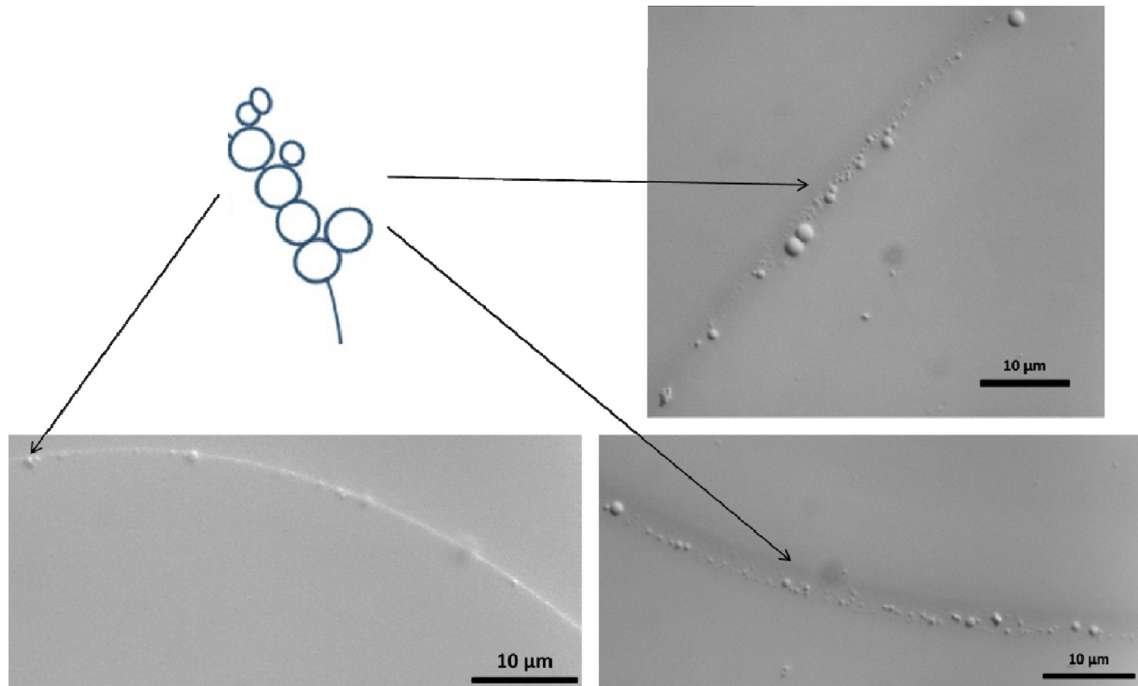


Fig. 4. Optical microscopy images of a water-in-water emulsion containing zein particles, which show the particle accumulation at the curved water-water interface accompanied by aggregation of particles.

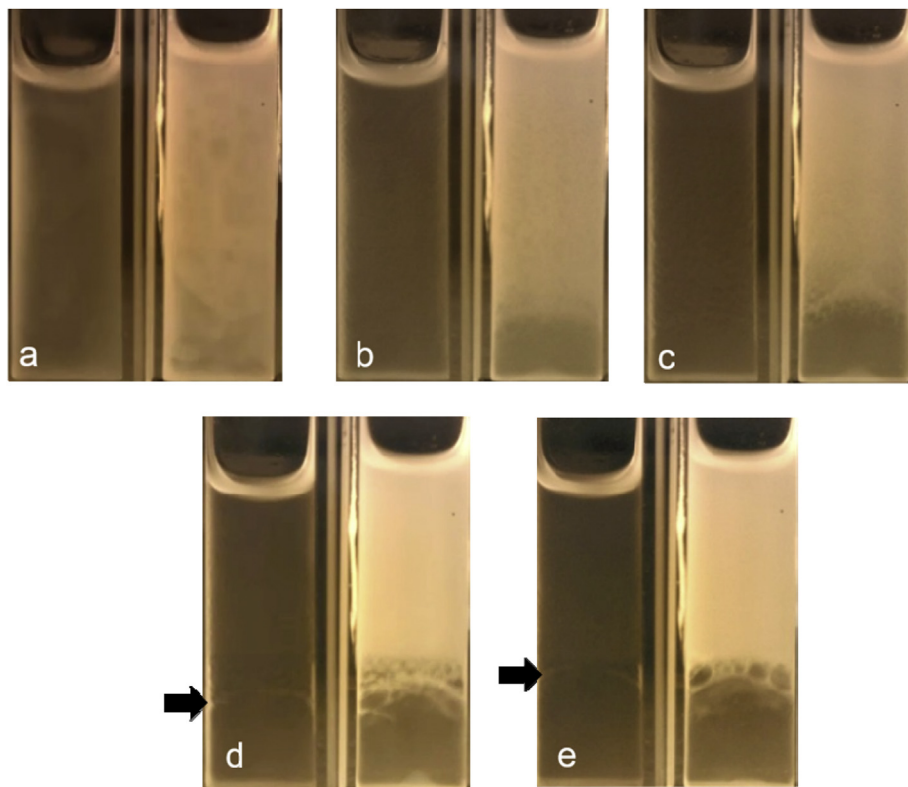


Fig. 5. Movie stills from different stages of the interfacial particle-rich zone's formation during the macroscopic phase separation of emulsions without (left) and with particles (right). (a) 0 h, (b) 1 h, (c) 1.5 h, (d) 2 h, (e) 3 h. The pH of emulsions was 5.6. The movie was recorded overnight at one frame per 30 s. The width of the cuvettes was 1 cm. The black arrows indicate the position of the interface in emulsions without particles. The full movie can be found in the [supplementary information](#).

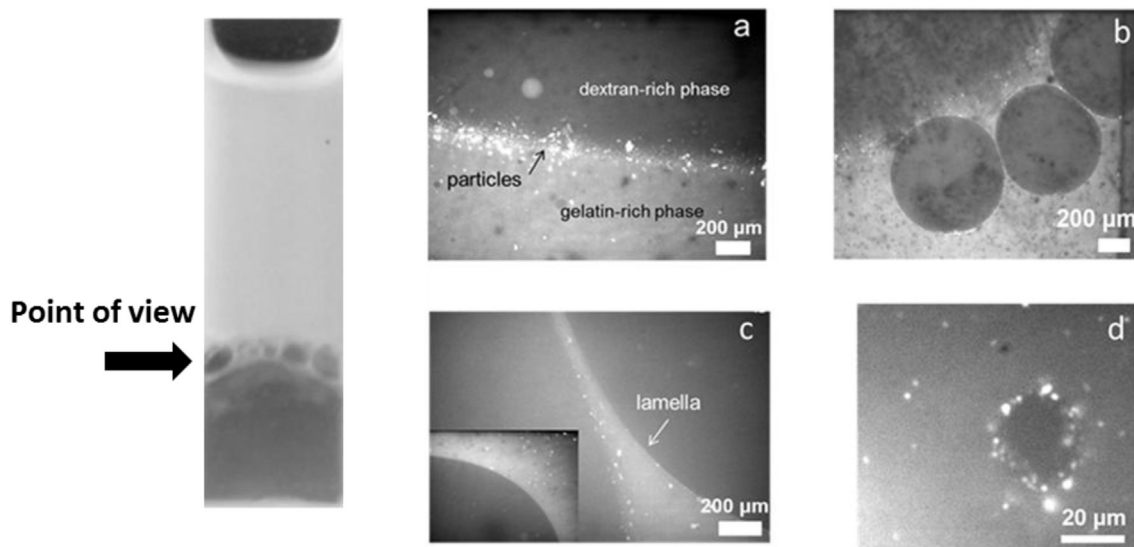


Fig. 6. Horizontal microscopy images of the interface of a macroscopically phase-separated emulsion containing particles. The emulsion was dyed with rhodamine, which binds to the gelatin-rich phase (dextran-rich droplets appear black). We can see (a) particle accumulation at the interface between the gelatin-rich and the dextran-rich phase, (b) dextran droplets trapped in the interfacial particle-rich zone nearly touching each other without coalescing, (c) the interfacial particle-rich film/lamella which consists of a gelatin wetting layer containing particles, (d) magnification of a dextran droplet covered by particles.

3.4. Surface coverage and sedimentation as limiting factors for emulsion stability

When added to phase-separated mixtures of gelatin and dextran, zein particles did not lead to the macroscopic stabilization of the demixed solutions, as we already mentioned. In fact, after mechanical mixing, the demixing process stayed almost unaffected by the presence of the colloidal particles. Nonetheless, we observed the expected stabilizing ability of particles at low particle concentration. Low particle concentration was chosen to avoid the

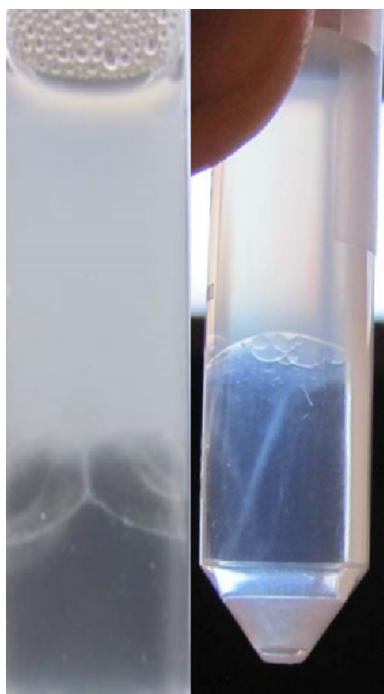


Fig. 7. Foam-like layer of a macroscopically phase-separated emulsion (left) remaining stable even after centrifugation of the emulsion at 14,000 g for 2 h (right).

increase in viscosity of emulsions, as well as the aggregation of particles in their initial aqueous dispersions.

Full emulsion stability against gravity-induced layer formation could not be expected due to the low surface coverage of emulsion droplets that the particles could provide. Assuming that all the particles were adsorbed at the droplet interface, we can estimate the needed amount of particles to provide full coverage (assuming a close packed surface fraction). For droplets of size R_{drop} , the full coverage is obtained when the mass concentration of particles c_{part} is equal to $4\phi\rho R_{part}/R_{drop}$, where ϕ is the volume fraction, ρ the density of droplets and R_{part} the particle radius. For droplets of radius of 25 μm , c_{part} is approximately 5 mg/mL, which is much higher than the particle concentrations we used (0.2%, w/w or 2 mg/mL). So, the chosen particle concentration is not sufficient to provide full coverage. At this particle concentration, the droplet size that will allow full particle coverage is 0.64 mm, which is reasonably close to the 0.7 mm diameter observed in experiments (Fig. 6b) and confirms a link between particle adsorption at interfaces and the observed structures. In the foam-like layer, dispersed droplets do not coalesce further, so this is the maximum 'equilibrium' size they reach.

Even in the case of full coverage, though, the stabilization may still not be reached due to the effect of gravity: fully covered droplets are made heavy by particle adsorption and they eventually sediment. This leads to poor stability of any emulsion, except if the mass density of colloidal particles closely matches that of the liquid. It is generally known that when the effect of gravity dominates over thermal motion, sedimentation or creaming will occur. We can determine the droplet size R_{drop} fully covered by particles that still resists the gravitational force and does not sediment, by equalizing the gravitational potential and the thermal energy:

$$R_{drop} = \left(\frac{3}{4\pi} \frac{k_B T}{g |\Delta h| \Delta \rho} \right)^{1/3} \quad (1)$$

where $\Delta\rho$ is the buoyant density of the particles and Δh is a distance of the same order of magnitude as of R_{drop} . R_{drop} is in the order of 10^{-7} m and is too small to be realistic.

The gravitational length L_{grav} , which is defined as the length over

which the gravitational energy of a droplet in a suspension equals the thermal energy, is given by: $L_{grav} = \frac{3}{4\pi} \frac{k_B T}{g|\Delta\rho|R^3}$ (Philipse & Koenderink, 2003). Here the equation refers to one droplet with radius R . However, when we have a droplet covered with particles, about $4(R_{drop}/R_{part})^2$ particles will fully cover the surface of the droplet. In that case we have to take into account the weight of the particles, which dominates the total weight of the droplet with particles. Therefore, neglecting the weight of the droplet, we end up to the following expression for the gravitational length:

$$L_{grav} = \frac{3}{16\pi} \frac{k_B T}{g|\Delta\rho|R_{drop}^2 R_{part}} \quad (2)$$

For the stability to be observed, L_{grav} should be much larger than the height of the container (typically of the order of 10^{-2} m), which is not the case. L_{grav} is in the order of $10 \mu\text{m}$, so gradients due to gravity are sharp, indicative of strong sedimentation. If we consider the sedimentation length L_{grav} to be 10 times larger than the height of the container for the stability to be observed, then, from equation (2), the buoyant density $\Delta\rho$ of the particles should be in the order of $10^{-5} \text{ kg m}^{-3}$. The actual buoyant density is in the order of 100 kg m^{-3} .

4. Conclusions

Colloidal zein particles are synthesized by the anti-solvent precipitation method and dialyzed to remove any possible surface-active components which can alter their behavior. When added to phase-separating aqueous mixtures of fish gelatin and dextran, particles show a contact angle of 65° . The non-zero contact angle is in accordance with the similar attractive interactions of particles with both polymers, as was investigated from dynamic light scattering measurements. Eventually, particles accumulate at the interface between the gelatin-rich and the dextran-rich phase, an accumulation that is accompanied by particle aggregation. Those aggregates are able to arrest the late stage of the demixing process of emulsions leading to the formation of an interfacial particle-rich zone, referred as a foam-like layer, due to its morphology similar to a wet foam. This layer remains stable even after centrifugation of the emulsion ($14,000 \text{ g}$ for 2 h) and contains dispersed dextran droplets surrounded by particle-stabilized gelatin layer. The dextran droplets do not coarsen further leading the emulsion to an apparent equilibrium state. In the present conditions the particles cannot be expected to provide full stabilization of emulsions, as sedimentation is expected to be relevant. Sedimentation can be avoided if the mass density of the colloids closely matches that of the liquid or if the emulsion droplets are sufficiently small not too sediment. However, such a small droplet size is not realistic; therefore interactions between particles and droplets that lead to a network formation are required for stabilization against gravity.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.foodhyd.2016.10.036>.

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