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Particle stabilized water in water emulsions

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Abstract

Food products often contain mixtures of incompatible water soluble macromolecules such as proteins and polysaccharides. When two aqueous solutions of incompatible macromolecules are mixed they separate into two phases each enriched in one of the two macromolecules. Contrary to oil-water (O/W) emulsions, water/water (W/W) emulsions cannot be stabilized by addition of surfactants and in food applications macroscopic phase separation is avoided by gelling one or both phases. However, recently it was shown that W/W emulsions can be stabilized to varying extents by addition of particles. Such particle stabilized emulsions are also known as Pickering emulsions and have been studied extensively for O/W emulsions. Here the literature on particle stabilization of W/W emulsions is reviewed. The behavior of particle stabilized W/W emulsions is found to be quite different from that of O/W emulsions due to the much smaller interfacial tension and the much larger length scale at which the interface expresses itself. Besides the particle size, interaction of the particles with the macromolecules in the mixture and with each other at the interface appears to play a decisive role for stabilization.

1. Introduction

Water-in-water (W/W) emulsions are formed when aqueous solutions of two incompatible macromolecules are mixed (Frith, 2010) . Contrary to emulsions formed by mixing oil and water (O/W),

W/W emulsions cannot be stabilized by adding molecular surfactants, because the interface is expressed only on length scales larger than the correlation length of the macromolecule solutions, see fig.1. Nevertheless, stable W/W emulsions are present in a variety of food products, because macroscopic phase separation is inhibited by gelation of the continuous phase. In 2008 Poortinga (2008) reported that microparticles added to W/W emulsions formed by mixtures of incompatible polysaccharides accumulated at the interface. He noted that this led to an increase of the stability of the liquid W/W emulsions. Shortly after, Firoozmand, Murray and Dickinson (2009) confirmed that that accumulation of particles at the interface slowed down coarsening of W/W emulsions formed by mixtures of starch and gelatin.

Accumulation of particles at the interface and their stabilizing effect has been known for O/W emulsions for over a century and is regularly reviewed (Aveyard, Binks and Clint, 2003; Binks and Horozov, 2006; Chevalier and Bolzinger, 2013; Dickinson, 2012) . This type of emulsion is often called Pickering emulsion after one of the discoverers of this phenomenon (Pickering, 1907) . The mechanism that maintains the particles at the interface is the increase of the free energy (ΔG) when the interfacial area between phase A and phase B is increased by the departure of the particle. The surface of the particle at the interface is exposed to both phases, which means that ΔG is reduced only if the difference of the interfacial tension of the particles with phase A (γ_{PA}) and phase B (γ_{PB}) is smaller than that between the two phases (γ_{AB}). For spherical particles with radius R the area that is occupied is equal to $\pi R^2.(1-\cos(\theta))^2$, where θ is the contact angle of the particles with the interface, see figure 1. ΔG is equal to the reduction of the interfacial area multiplied with the interfacial tension:

$$\Delta G = -\pi R^2 \gamma_{AB}(1 - |cos(\theta)|)^2 \qquad 1$$

The contact angle is determined by the difference of the interfacial tension of the particles with phase A (γ_{PA}) or phase B (γ_{PB}):

$$\cos(\theta)=(\gamma_{PA}-\gamma_{PB})/\gamma_{AB}$$

It is assumed that gravitational forces can be neglected.

For nanoparticles in O/W emulsions ΔG is orders of magnitude larger than the kinetic energy (kT) as long as $(\gamma_{PA}-\gamma_{PB}) < \gamma_{AB}$, which is usually the case. However, the effect is much smaller for W/W emulsions, because the interfacial tension between two aqueous macromolecule solutions is orders of magnitude lower than that of the O/W interface and goes to zero at the critical point. For this reason Firoozmand et al. (2009) suggested that the origin of the accumulation of particles at the W/W interface was depletion of the particles from the polymer solutions towards the more solvent rich

interface. However, taking realistic values of the parameters in eq. 1 it follows that even for W/W emulsions ΔG can be much larger than kT as long as the particles are not too small and the mixtures are not too close to the critical point, see below.

In the last 4 years, particle stabilized W/W emulsions have attracted increasing attention and it has become apparent that the properties of these emulsions are significantly different from particle stabilized O/W emulsions. Much of the research so far was done for non-food grade model systems, but a number of food grade emulsions have also been investigated. In the following we will review first the work on model systems and then discuss the results obtained on systems that are potentially relevant to food. We will compare the properties of W/W emulsions with those of O/W emulsion and speculate on the potential of the former in food applications.

2. Model systems

Phase separation of mixtures of dextran and poly(ethylene oxide) (PEO) in aqueous solution has been studied in quite some detail in the past (Cesi, Katzbauer, Narodoslawsky and Moser, 1996; Ding et al., 2002; Forciniti, Hall and Kula, 1990; Kang and Sandler, 1987; Ryden and Albertsson, 1971) and for large molar masses phase separation is practically complete at relatively low polymers concentrations. This property, together with the fact that these polymers are neutral and that the viscosity of the solutions can be tuned easily by varying the molar mass, renders this system a good model to study W/W emulsions. As was mentioned in the introduction, a crucial parameter for the adsorption of particles at the interface is the interfacial tension. An interesting property of W/W emulsions is that γ_{AB} can be varied continuously without significantly changing the phase volumes by varying the total polymer concentrations while keeping the ratio fixed. It was found that γ_{AB} has a power law dependence on the tie line length (TLL): $\gamma \propto TLL^{\alpha}$. The TLL characterizes the difference in the composition of each phase: $TTL=[(w_{1A}-w_{2A})^2+(w_{1B}-w_{2B})^2\ ]^{0.5}$, with w the weight fraction of polymer 1 or 2 in phase A or B. Accurate measurements of very low interfacial tensions between two aqueous phases is not straightforward, but can be done either by analyzing the shape of the macroscopic interface near a vertical wall (Vis et al., 2015) or by measuring the shape relaxation of a dispersed droplet after shear deformation (Balakrishnan, Nicolai, Benyahia and Durand, 2012) .

Balakrishnan et al. (2012) studied mixtures of PEO and dextran with weight average molar masses $M_w = 5x10^5$ g/mol and 2×10^5 g/mol, respectively, in the presence of spherical latex particles. Often it is not possible to determine the contact angle, because the particles are too small or not perfectly spherical or because the particles and the interface cannot be clearly visualized. However,

Balakrishnan et al. managed to visualize the position of fluorescently labeled latex particles with R=1μm at the interface by using fluorescently labeled dextran. They found that the contact angle of the particles with the dextran rich phase was 145°. This means that the particles preferred to be wetted by the PEO rich phase, which was confirmed by the observation that excess particles were mostly situated in the PEO phase. Latex particles with R=0.1 μm were trapped at the interface for emulsions with PEO and dextran concentrations down to 1.7 wt% and 2.0 wt%, respectively. Using the measured interfacial tension at these conditions, $\gamma_{AB} \approx 10^{-6}$ N.m^{-1}, they found that $\Delta G \approx 7$ kT, which is largely enough to pin the particles at the interface.

Nevertheless, coalescence between particle covered droplets of the PEO phase was observed leading eventually to macroscopic phase separation. A closer inspection of the coalescence process showed that two droplets could stay in contact for periods of time exceeding 30 min until at a random time a narrow channel was formed between the PEO phases of the two droplets. Subsequently, the naked contact area between the droplets increased within less than a second until a single ellipsoidal droplet with a monotonic surface was formed that relaxed more slowly to a spherical shape. Particle tracking experiments showed that the latex particles diffused freely on the droplet surface. It was suggested that this allowed the appearance of a channel between adjacent droplets and that subsequently particles were ejected from the surface by the shear forces during the coalescence process. It was reported that the structure of particle stabilized W/W emulsions is the same after hand shaking as after more energetic mixing methods (Balakrishnan et al., 2012; de Freitas, Nicolai, Chassenieux and Benyahia, 2016; Nguyen, Nicolai and Benyahia, 2013; Nguyen, Wang, Saunders, Benyahia and Nicolai, 2015) . In addition, for the rather viscous mixture of amylose and xyloglucan in the presence of protein microgels (de Freitas et al., 2016) it was observed by microscopy that after shaking initially naked droplets of the dispersed phase are formed that coalesce and are progressively covered with particles. These results suggest that small shear forces are sufficient to drive the particles from the interface.

Subsequently, it was found that other types of spherical particles were much more effective to arrest coalescence of dispersed droplets in W/W emulsions. Ngyen et al. (Nguyen et al., 2013) used protein microgels that are spontaneously formed by heating solutions of the whey protein β-lactoglobulin in the presence of $CaCl_2$. They observed that microgels adsorbed spontaneously at the interface and inhibited coalescence, whereas as native proteins with a radius of about 2nm had no noticeable effect on the emulsion stability. The stability against coalescence depended on the concentration and the size of the microgels and on the interfacial tension. Emulsions of PEO droplets in the continuous dextran phase (P/D emulsions) with γ_{AB}>30 μN.m^{-1} did not show a visible PEO layer for at least one week after preparation if at least 0.1 wt% microgels were added with a hydrodynamic

radius R_h=150nm. The droplet size was found to decrease with increasing microgel concentration, decreasing volume fraction of the dispersed phase and decreasing size of the microgels. These observations can be qualitatively understood by the increase of the number of particles per droplet. However, there was no straightforward quantitative relationship between the surface area that could be covered by the microgels and the droplet size. In fact, for all emulsions a significant fraction of the microgels were not adsorbed at the interface.

Creaming of PEO droplets under the influence of gravity was observed, because the PEO phase was less dense than the dextran phase. The creaming velocity (v) was found to be largely determined by the size of the droplets, the density difference between the two phases ($\Delta\rho$) and the viscosity (η) of the continuous phase: $v \approx g.\Delta\rho.2.R^2/(9\eta)$. For a given droplet size, creaming is much slower for W/W emulsions than for O/W emulsions, because $\Delta\rho$ is much smaller and η generally much larger. Creaming stopped when a dense layer of close-packed stable PEO droplets was formed.

When the volume fraction of the dextran phase was larger than approximately 40% dextran, droplets in a continuous PEO phase (D/P emulsions) were formed. The microgels also formed a layer on the surface of the dextran droplets, but curiously this did not inhibit coalescence of the droplets. The different stability of P/D and D/P emulsions is perhaps related to the fact that the microgels prefer the dextran phase and therefore form a less effective barrier between adjacent dextran droplets. Asymmetry, between the behaviour of D/P and P/D emulsions was also observed with other particles, see below.

Effect of the particle morphology

Disk-like or rod-like particles can also be used as efficient stabilizers of emulsions (Binks et al., 2006) . These particles lie flat at the interface and can cover a larger area than spherical particles with the same volume fraction. Vis et al. (2015) reported that clay (gibbsite) platelets with a radius of about 85 nm and a thickness of about 7 nm were localized at the interface of W/W emulsions formed by mixtures of gelatin and dextran. Thin disks cover an area πR^2 independent of the contact angle. However, the contact angle still enters in the calculation of ΔG as it accounts for the interfacial tension of the particles with the polymer solutions:

$$\Delta G = -\pi R^2 \gamma_{AB}(1 - |cos(\theta)|) \qquad 3$$

Addition of 0.4 w% clay particles led to stable emulsions that showed no sedimentation of gelatin droplets in the continuous dextran phase even after three weeks. The latter was explained by the formation of a weak gel of droplets connected by aggregated clay platelets.

Peddireddy, Nicolai, Benyahia and Capron (2016) observed that P/D emulsions were stable when as little as 0.05 wt% cellulose nanocrystals (CNC) were added that have a highly anisotropic rectangular parallelepiped structure with average dimensions 160 nm × 6 nm × 6 nm. For such particles ΔG depends on the length (l) and the width (b) of the particles:

$$\Delta G = -lb\gamma_{AB}(1 - |cos(\theta)|) \qquad 4$$

The fraction of CNC at the interface and the droplet size decreased with increasing CNC concentration, but the coverage of the surface remained approximately constant at about 50%. The high coverage means that these charged rod-like particles at the interface were most likely oriented parallel to each other. Creaming of the PEO droplets was observed until a dense droplet layer was formed that remained stable for weeks, see fig. 2a. However, when 50 mM NaCl was added a weak gel was formed of droplets connected by aggregated CNC, see fig. 2b . CNC did not stabilize D/P emulsions even though they initially formed a layer at the surface of the dextran droplets, similarly to what was observed in the presence of microgels. As was the case for the microgels, CNC partitioned strongly to the dextran phase, and may therefore have formed a less effective barrier between the dextran solutions of adjacent droplets.

Gonzalez-Jordan, Benyahia and Nicolai,(2016) studied the effect of the particle morphology by exploiting the fact that protein particles with three different morphologies can be prepared by heating β-lactoglobulin solutions at different conditions (Nicolai, Britten and Schmitt, 2011) . In this manner, spherical microgels, fractal aggregates and rod-like fibrils were prepared and their effect on the structure and stability of P/D and D/P emulsions was investigated. Microgels and fractals are both spherical particles, but the latter have a much lower density and should therefore be able to cover a large area at the same protein concentration. All three protein particles formed interfacial layers, but the fractals were found to be much less effective stabilizers of P/D emulsions than the microgels or the fibrils even though the initial droplet size was approximately equal (R=5-10μm). D/P emulsions could not be stabilized by any of the particles in agreement with what was already described above for the microgels.

The investigations of the dextran/PEO mixtures discussed so far were done at neutral pH where proteins are negatively charged and partition preferentially to the dextran phase. However, Gonzalez-Jordan et al. (2016) noted that β-lactoglobulin partitioned to the PEO phase at pH 3.0 where they are positively charged. This had a marked effect on the structure and stability of the emulsions. At pH 3.0, P/D emulsions destabilized rapidly in the presence of either microgels or fibrils similarly to D/P emulsions at pH 7.0. This observation supports the idea that the interfacial protein particle layer forms an effective barrier only if it is wetted more strongly by the continuous phase. However, at pH

3.0 fractals were very effective stabilizer of both P/D and D/P emulsions even at concentrations as low as 0.05%. Significantly smaller droplets (R<3μm) were formed in the presence of fractals that did not cream or sediment significantly after one week standing.

pH-induced stabilization and destabilization

Nguyen and al. (2015) studied the effect of the pH on the emulsions formed by mixtures of dextran and PEO in the presence of pH-sensitive microgels consisting of covalently cross-linked poly(ethyl acrylate-co-methacrylic acid-co-1,4-butanediol diacrylate). The hydrodynamic radius of these microgels increased strongly in a narrow pH range from 60 nm for pH <6.5 to 220 nm for pH >7.5 due to an increase of the charge on the polymers. Curiously, the partition of the microgels between the two phases as a function of the pH was non-monotonic. The microgels partitioned towards the dextran phase between pH 7.2 and pH 7.8, but they preferred the PEO phase at lower and higher pH. The structure and the stability of the emulsions were found to be very sensitive to the pH between pH 6.5 and pH 8.0. Addition of 0.05% microgels rendered P/D and D/P emulsions stable between pH 7.0 and pH 7.5 without significant creaming for at least one week. However, at pH≥7.8 the emulsions were just as unstable as in the absence of microgels. For pH≤6.8 the emulsions became less stable, in particular the P/D emulsions. The effect of the pH on the stability was mirrored by the effect on the initial droplet size, which showed a minimum at pH 7.2. When the pH of a stable suspension at pH 7.2 was increased to pH 8.0, by addition of a small amount of HCl, the suspensions immediately completely destabilized. After returning the pH to 7.2 by addition of NaOH a stable emulsions was again formed after mixing.

The stability of the emulsions was also found to be very sensitive to addition of salt, in particular D/P emulsions. Stable D/P emulsions at pH 7.2, completely phase separated within one day if 10mM NaCl was added, but D/P emulsions at pH6.5 and 6.8 that were instable in pure water, became more stable when salt was added. Addition of salt also influenced the partition of the microgels between the phases without large changes in their size. It is clear that the behavior of these pH-sensitive microgels cannot be understood by considering only the size change. The authors suggested that the effects of the pH and salt on the structure and stabilization of the emulsions are related to changing interactions between the microgels. However, no detailed description of the mechanism could be given.

Block copolymers

Buzza, Fletcher, Georgiou and Ghasdian, (2013) showed that tri- and diblock copolymers can be used to stabilize D/P and P/D emulsions at neutral pH. Phase inversion was observed at

approximately the same volume fraction as in presence of protein microgels. The triblock polymers consisted of two different end blocks that preferred each a different phase, connected by a central hydrophobic block. They tested the stability of the emulsions in the presence of copolymers with a large range of different compositions. The suspensions were most stable when the size of the central block and the block favoring the dextran phase were large. The size of the relatively small block favoring the PEO phase was not important and stable suspensions were formed even in the absence of this block. The authors suggested that the block copolymers formed a layer at the interface with each of the two end blocks directed to the phase it favors, i.e. they considered the dispersed droplets as large polymersomes. However, this model does not explain why the diblocks were more effective than most of the triblocks. In addition, the emulsions were not stable upon dilution contrary to conventional polymersomes.

The authors did not investigate the structure of the polymers in aqueous solutions, but most likely polymeric micelles were formed by the diblocks with a hydrophic core and a hydrophilic corona. Considering the relatively small size of one of the blocks it is likely that the triblocks also formed polymeric micelles. Therefore, we suggest an alternative mechanism for the observed stabilisation, viz. that the block copolymers formed polymeric micelles that adsorbed to the interface and stabilized the emulsions by the same mechanism as other types of particles. This would explain why diblocks were effective and why the effectiveness increased with the size of the copolymers and did not depend much on their composition. Of course, the question remains whether it is possible in principle to stabilize the W/W emulsions with diblock copolymers consisting of two hydrophilic blocks that prefer different water phases. This has not yet been attempted and probably requires highly selective blocks that are significantly larger than the correlation length of the two phases.

Microreactors

Dewey, Strulson, Cacace, Bevilacqua and Keating, (2014) studied D/P emulsions in the presence of spherical lyposomes with R≈65 nm formed by self-assembly of egg phosphatidyl glycerol. The lyposomes formed a layer around the dextran droplets that inhibited coalescence. It was suggested that the stability was due to electrostatic repulsion rather than steric hindrance, because droplet coalescence was observed when salt was added. Mixing droplets covered with lyposomes with different fluorescence labeling showed that if intact droplets were induced to coalesce the lyposomes of the two droplets did not mix on the surface. However, when the emulsions were vigorously mixed the lyposomes where randomly distributed indicating that the droplets were completely disrupted during mixing. The layer of lyposomes prevented other lyposomes to enter the droplets, but did not

prevent entering of small DNA molecules with R_h<4 nm. The authors exploited the strong partitioning of proteins and nucleotides to the dextran phase in order to use the dextran droplets as microreactors for enzymatic reactions. Enzymes accumulated within the particle stabilized dextran droplets and catalyzed the transformation of small reactants that could penetrate the droplets through the layer of liposomes.

In a follow-up investigation the same authors used the same system to produce $CaCO_3$ within the dextran droplets (Cacace, Rowland, Stapleton, Dewey and Keating, 2015) . They exploited the fact that the enzyme urease partitioned strongly within the dextran phase where it reacted with urea in the medium that could penetrate through the liposome layer. The reaction product CO_3^{2-} reacted in turn with Ca^{2+} to form solid $CaCO_3$ within the droplets. Because the liposomes were not stable when free Ca^{2+} was present, Ca^{2+} was chelated with small molecule chelators that could penetrate the liposome layer. With these two examples it has been clearly demonstrated that W/W emulsions can be used for localized chemical reactions if at least one of the reactants strongly partitions to the dispersed phase and the reactants cannot escape.

3. Food grade W/W emulsions

W/W emulsions in foods have effectively been studied for many years, but not necessary consciously thinking of them as emulsions and certainly not consciously thinking of using particles to stabilize them. Biopolymer solutions that undergo segregative phase separation often result in droplets of one biopolymer-rich phase in another biopolymer phase as part of the phase separation process and this transient state can be arrested by gelling one or both of the phases. The high viscosities and similar densities the different phases generally aids this process cf. faster phase separation in oil-water systems. Indeed, by applying shear and jellification to such systems whilst undergoing phase separation, novel structures and textures can be formed – for a recent review see Norton, Espinosa, Watson, Spyropoulos and Norton (2015) . However, this last type of 'stabilization' must be distinguished from that discussed in the rest of this review – where interfacially active particles control the size and shape (stability) of the phase separating structures.

As has already been remarked at the outset, there have been many studies of O/W and even W/O systems stabilized by particles. Many of these studies have involved food materials, but a minority have used food-grade particles, particularly for W/O systems. There are even fewer examples of W/W

systems stabilized by particles, as also indicated above, and even less that have utilized food-grade particles, which is the focus of this last section of the review. The reason is obvious: if it is hard to think of water-insoluble food grade particles for W/O Pickering emulsions, it is even more difficult to find water-insoluble particles suitable for W/W Pickering emulsions. Some of these issues have been covered in recent reviews by Dickinson (2016) and Norton et al. (2015) .

The study by Poortinga (2008) was apparently the first to identify particles as affecting W/W phase separation of food systems, although the particles and systems were rather crude and impure. This was shortly followed by publication of the work of Firoozmand et al. (2009), who had been studying phase separation of a gelatin + starch (amylopectin) system in more detail: typically 7 wt.% gelatin + 4 wt.% starch. They demonstrated that 1 wt.% or less of selected synthetic polymer latex particles could significantly arrest spinodal type phase separation of the system (above the gelation temperature of gelatin). Firoozmand also showed that highly stable emulsion droplets could significantly affect the time-course of the phase separation, as illustrated in Figure 3. Phase separation was very significantly slowed with the droplets, though not as much as with the latex particles. This may have been because the mean size (ca. 400 nm) of the droplets was larger than that of the latices (ca. 250 nm), so that the 1 to 5 wt % of droplets added was insufficient to cover the W/W interface. However, the morphology of the phase-separating structures was even more significantly affected by the droplets than the latices. This work was apparently the first to promote the idea of fluid oil droplets as water-insoluble 'particles' that could be interfacially active at the W-W interface. In principle, this might be applicable to many other food systems. Oil droplets require a stabilizing layer – in this case sodium caseinate was used – which means that adding the emulsion to the gelatin-starch system also added in a small amount of this third biopolymer – the excess sodium caseinate in the aqueous phase of the emulsion. However, this amount was very minor compared to concentrations of gelatin and starch in the system.

Hanazawa and Murray (2012; 2013) followed up this work with an additional study of the phase separation of a xanthan gum + sodium caseinate system as influenced by the addition of oil droplets stabilized by the same caseinate. (The emulsion was prepared separately then added to the mixed biopolymer system). Phase separation is a little more complicated in this system, because it also depends on concentration of calcium ions [Ca^{2+}] and the pH, but essentially the same sort of findings were obtained as with the gelatin-starch system. In other words, addition of the droplets significantly slowed down phase separation, as long as the pH and [Ca^{2+}] were not such so as to cause excessive precipitation of the caseinate and flocculation of the droplets. Furthermore, Hanazawa et al. (2013) went on to show that a significant increase in interfacial viscosity at the W/W interface could be detected in a macroscopically phase separating system with added droplets under conditions where microscopic phase separation was inhibited. In addition, this effect was enhanced by a higher solid fat

content of the droplets, indicating that particle aggregation or network formation (e.g., by partial coalescence) at the W/W interface is an important re-enforcing mechanism. There is a direct analogy here with the Pickering and partial coalescence mechanisms of stabilization of W/O droplets in fatty in high fat systems (see Ghosh and Rousseau, (2011)).

An additional complication in studying any W-W system that contains protein is that proteins adsorb to most surfaces and so they will also adsorb to the surface of any particles added, whether they are initially stabilized by protein or not. This must change the wetting characteristics of the particles, plus their tendency to aggregate. It is therefore perhaps easier to understand particle-control of W/W emulsions where neither of the biopolymers is surface active, like the dextran-PEO systems extensively studied by Nguyen et al. (2015). In this case there is also the possibility of introducing protein particles (insoluble solid or microgel particles) as an alternative and distinct stabilizing entity, as these workers have done.

Returning to mixtures of food biopolymers, using protein microgel particles is likewise what Murray and Phisarnchananan, (2016) reported recently with guar gum + starch (amylopectin) or locust bean gum (LBG) + starch systems. They were able to demonstrate significant arrest of the phase separation of these systems by including a relatively low volume faction (but at least 5 vol.%) whey protein isolate (WPI) microgel particles (size 150 nm), much in the same way as Nguyen et al. (2013) and Gonzalez-Jordan et al. (2016) have done for their dextran-PEO systems. Phase separation was more significantly slowed at pH values closer to the isoelectric pH (pI) of the WPI. Separate experiments showed that aggregation of the microgel particles was enhanced towards the pI, as would be expected due to reduced electrostatic repulsion between the protein molecules. Previous work by Murray and Phisarnchananan (2014) with non food-grade silica nanoparticles had showed even stronger effects on W/W emulsion structure and stabilization but attempts with larger, food-grade silica particles and the same caseinate-stabilized oil droplets used by Hanazawa & Murray (2012; 2013) produced weaker stabilization effects. Again, this was believed to be largely due to the larger particles size in these latter systems, which cannot cover enough W/W interface fast enough. In addition, microgel particles are deformable and may spread at the W/W interface.

One advantage of protein microgel particles is that 'bottom-up' synthesis methods, where one grows protein aggregates to a certain size, allows preparation of food-grade particles of much smaller size than traditional top-down approaches of producing emulsions, etc. In an interesting extension of this idea, de Freitas et al. (2016) have recently produced food-compatible protein (β-lactoglobulin) microgel particles coated in polysaccharide to control the stability of xyloglucan + starch (amylopectin)

W/W emulsions. An additional potential advantage of this approach is that by changing the nature of the adsorbed polysaccharide layer one might be able to exert another level of control over the particle wetting and partitioning characteristics.

An even more exotic particle idea is to use edible single celled organisms (bacteria, yeasts, etc.) as the stabilizing particles. Such cells can be in the correct size range and come with a ready-made coating of mucopolysaccharide material that may or may not ensure their effective anchoring at the W/W interface of a phase separating system. Firoozmand and Rousseau (2014) have recently applied this idea to the classic W/W system gelatin + maltodextrin. Up to 2 wt.% of negatively charged cells could control the microstructure and rheology, stabilizing bijel-type bicontinuous structures.

A common observation in all the above systems has been the preponderance of the stabilizing particles to end up much more concentrated in one aqueous phase than the other, even when there is a significant layer of particles at the W/W to affect phase separation. In other cases, particles end up totally in one or the other phase and have little apparent influence on the phase separation kinetics. Figure 4 shows an example from the starch + gelatin system studied by Firoozmand et al. (2009) already mentioned, where different types of particles distribute very differently. Thus far the reasons for this are not clear – it does not always seem to depend strongly on particle charge or hydrophobicity, although there is evidence that if the particles have more tendency to aggregate or 'cross-link' at the interface then they may be more likely to be held there. In the recent study by de Freitas et al. (2016) on polysaccharide-coated protein microgel particles, the pH *was* shown to control which of the phases excess particles partitioned into. In the gelatin-starch-emulsion system studied by Firoozmand excess emulsion droplets as particles tended to accumulate in the protein-rich phase, even though protein adsorbed to their surfaces had the same charge as the protein (gelatin) in the protein-rich phase, which might be expected to increase repulsion from the this protein phase. On the other hand, preferential partitioning into the starch phase was observed by (Murray et al. (2014; 2016) for the gum + starch systems by the same emulsion droplets, silica or protein microgel particles. In a study by Moschakis, Murray and Biliaderis (2010) on coacervate formation in chitosan + gum Arabic mixtures, O/W emulsions were seen to affect the structure and texture of the coacervates formed.

Clearly particle size is important in determining stabilization – if particles are too large they will simply not be able to cover enough W/W interfacial area (as indicated in the examples given above), as well as adsorbing too slowly or sedimenting or creaming out too quickly. On the other hand, the basic equation controlling the particle attachment energy (eq 1) indicates that larger particles will detach less easily. This may be another reason why accumulation and aggregation into larger particles

at the W/W interface may be the key to understanding why some particles are better than others, as discussed by Murray et al. (2014).

Finally, if successful stabilization of W/W emulsions is a race between the growth of some water domains and transport of particles to their interface, computer simulation Ettelaie and Murray (2015) may provide a window for predicting the combinations of particle size and diffusion kinetics that are most likely to result in stabilization via the Pickering mechanism. Certainly the field of particle-stabilized W/W emulsions is predicted to be a fertile area for study in the future and hopefully will lead food scientists to new methods of structuring complex food systems for novel or improved organoleptic and health properties.

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Figure 1

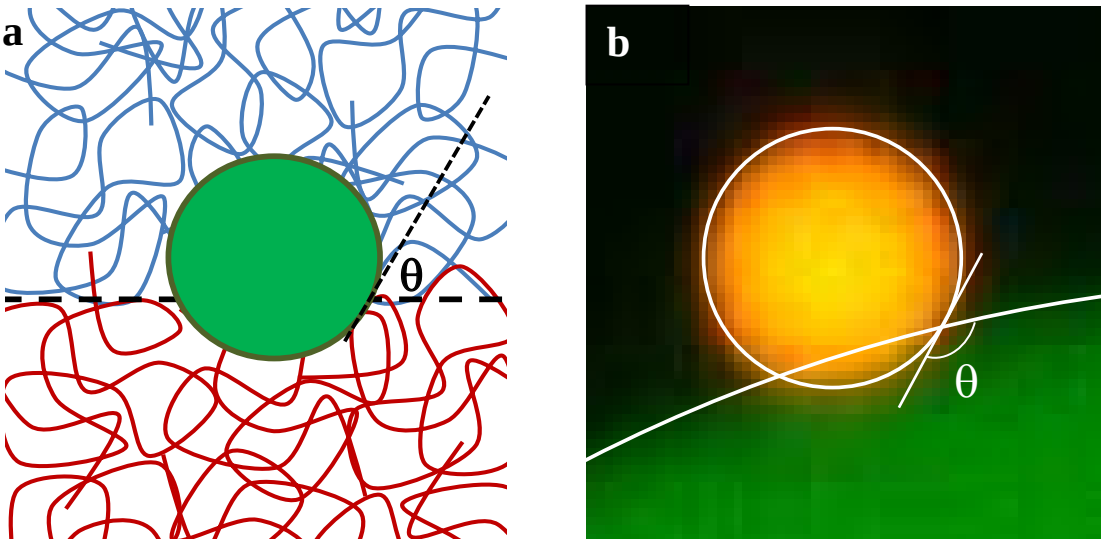

Figure 1a. Schematic drawing of a particle at the interface between two incompatible polymer solutions. θ is the contact angle of the particle with the interface.

Figure 1b Latex particle (R = 1μm) at the surface of a large dextran drop in a continuous PEO phase. The dextran phase. (Balakrishnan

Figure 2

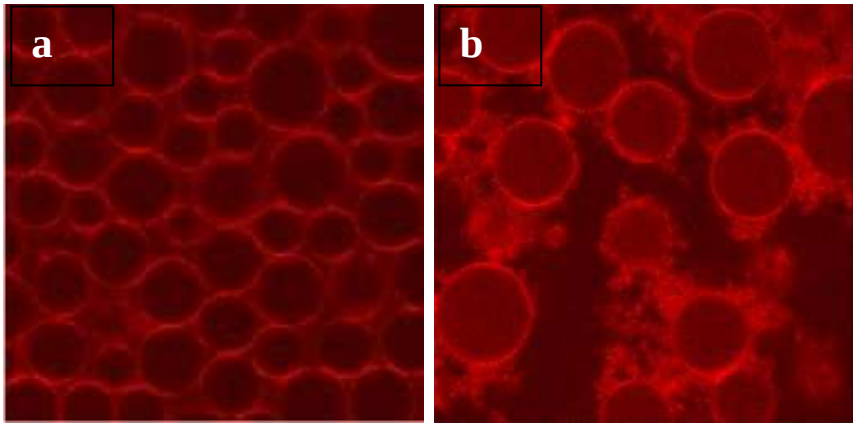

Figure 2. CSLM images (160 µm x 160 µm) of droplets of a dextran rich phase stabilized by CNC in a continuous PEO rich phase. Figure 2a shows the creamed layer of droplets formed in the absence of salt and figure 2b shows the network of droplets bridged by CNC aggregates formed in 50mM NaCl. (adapted from Peddireddy et al.(2016))

Figure 3

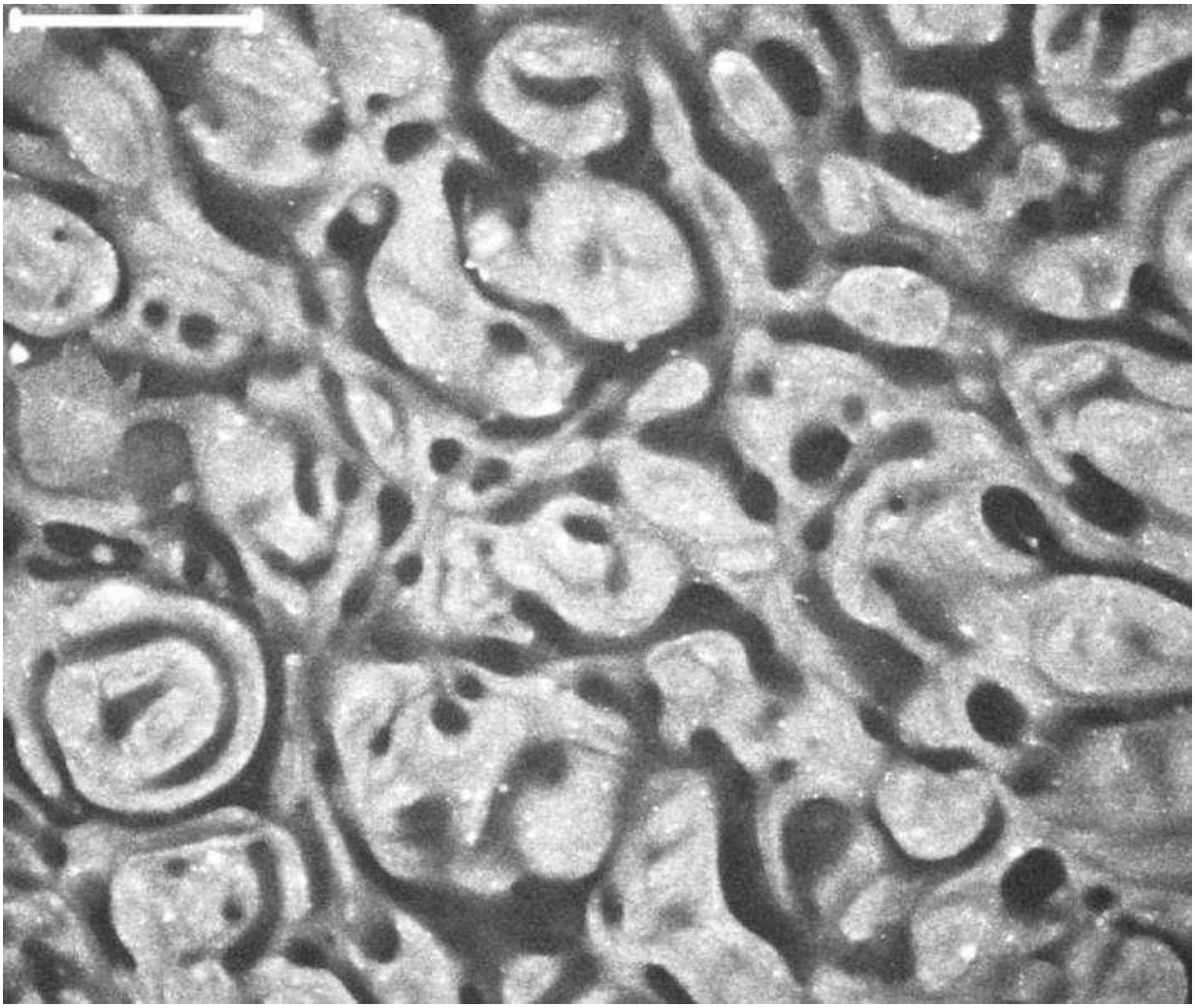

Figure 3. CLSM image illustrating effect of 5 vol.% caseinate-stabilized *n*-tetradecane droplets (0.4 µm diameter) on inhibition of the phase separation of 8 wt.% starch + 9 wt.% gelatin at 40°C (after 24 h). Dark regions = starch-rich phase, lighter regions = gelatin-rich phase also enriched by the droplets, which appear as bright white spheres

Figure 4

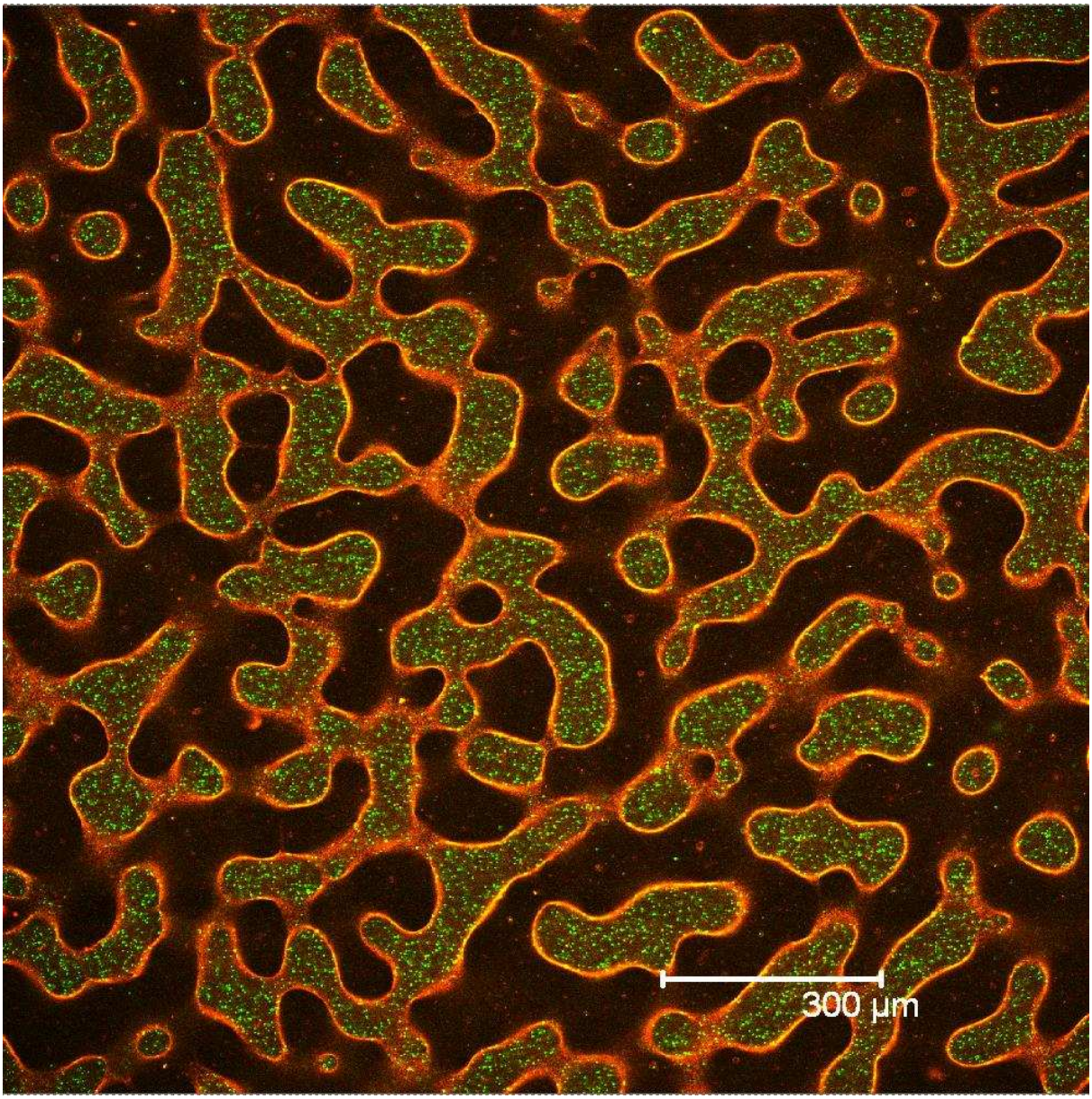

Figure 4. CLSM image illustrating self-selective partitioning of colloidal particles in a 24 h old phase-separating mixture of 8% starch + 9 % gelatin at 40°C. Dark regions = starch-rich phase, lighter regions = gelatin-rich phase. Red particles = PEG coated polystyrene latex (0.32 µm diameter), green particles = negatively charged COOH coated polystyrene latex particles (0.2 µm (diameter). PEG coated particles preferentially locate at the W/W interface and inhibit the phase separation whereas COOH coated particles largely locate in the gelatin-rich phase. (Firoozmand et al.,2009))