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Thèse de Doctorat

Alberto GONZÁLEZ JORDÁN

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New water/water emulsions stabilized by Pickering effect

JURY

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Résumé

Les émulsions eau /eau (W / W) ont récemment suscité un grand intérêt en raison de leur fort potentiel d'application dans différentes industries telles que l'agroalimentaire, les produits pharmaceutiques, les cosmétiques et les soins personnels. Le caractère particulier des émulsions W / W est leur stabilisation par ajout de particules. L'objectif de ce travail de thèse est de comprendre cet aspect en étudiant une émulsion modèle W / W à base de dextran et du poly (oxyde d'éthylène) stabilisée par des particules à base de protéines du lactosérum. Dans un premier temps, nous avons étudié l'effet de la morphologie des particules protéiques et leur partitionnement sur la stabilité des émulsions W / W. En particulier, la stabilité s'est révélée dépendre de la structure des particules quand ses derniers étaient sous forme de microgels, d'agrégats fractals ou de fibrilles. Il a été montré que la stabilité s'améliorait lorsque les particules se localiser préférentiellement dans la phase continue. Deuxièmement, nous avons étudié la gélification, des microgels et des agrégats fractals, induite en réduisant le pH entre 6,5 et 3,5 ou en ajoutant 0,3 M NaCl à pH 7,0 aussi bien quand l'excès des particules se situe dans la phase continue ou dispersée. Dans le premier cas, un réseau se formé dans la phase continue de dextran, permettant d'inhiber le crémage des gouttelettes de PEO, les agrégats fractals étant plus efficaces que les microgels. Dans le second cas, des particules protéiques denses pourraient être formées par gélification des gouttelettes de dextran dispersées. Troisièmement, nous avons exploré l'adsorption des protéines natives sur les particules de latex et leur capacité à stabiliser les émulsions W/W.

Abstract

Water/water (W/W) emulsions have attracted great interest recently due to their high potential for applications in different industries such as food and beverages, pharmaceutical, cosmetics and personal care. An important issue is the stabilization of W/W emulsions by adding particles. The aim of the research for this thesis was to shed light on this issue by studying a model W/W emulsion formed by mixing dextran and poly(ethylene oxide) with particles based on whey proteins. Firstly, we studied the effect of the morphology of protein particles and their partitioning on the stability of W/W emulsions. The stability was different when microgels, fractal aggregates or fibrils were added. We showed that stability improved when the particles partitioned to the continuous phase. Secondly, we investigated gelation of the fractal aggregates and microgels induced by reducing the pH between 6.5 and 3.5 or by adding 0.3M NaCl at pH 7.0 with excess particles either in the continuous or the dispersed phase. In the first case, a network was formed in the continuous dextran phase, making it possible to arrest creaming of PEO droplets, fractal aggregates being more effective than microgels. In the second case, dense protein particles could be formed by gelation of the dispersed dextran droplets. Thirdly, we explored the effect of adsorbing native proteins onto latex particles on their capacity to stabilize W/W emulsions.

Introduction

Mixing incompatible macromolecules in an aqueous solution can lead to separation into two phases, each of them being enriched in one or the other. Understanding the properties of these aqueous two-phase mixtures and studying new methodologies to stabilize them has attracted great interest from food, pharmaceutical, cosmetic and personal care industries due to their high potential for applications.

Foods are a good example of these systems as they often contain incompatible soluble macromolecules such as proteins and polysaccharides. The interaction between their components will determine their properties. Stable water-in-water (W/W) emulsions could be used as healthier substitutes of oil/water emulsions (O/W), keeping a similar mouth feeling but reducing the consumption of fat, which is one of the current challenges of the food industry. Contrary to O/W emulsions, W/W emulsions cannot be stabilized with surfactants, but it has recently been discovered that they can be stabilized by adding particles. This opens a door for the development of a large variety of new products.

The stability of W/W emulsions is essential as poor stability has been the main issue limiting the practical applications of W/W emulsions. As concluded by Esquena (2016) in the latest review on the subject, the stabilization of W/W emulsions is of the utmost technological importance, and finding methods for effective stabilization of W/W emulsions is an important challenge. Not all types of particles effectively stabilize W/W emulsions. The conditions that are required to obtain stabilization and the mechanism by which stabilization occurs are still unclear. The main aim of the work presented here was therefore to shed light on the mechanisms and factors affecting the stabilization of W/W emulsions using a model W/W emulsion formed by mixing dextran and poly(ethylene oxide) and particles based on whey proteins.

In chapter 3 we focus on the effect of the morphology of the protein particles and their partitioning between the two phases on the stability. In chapter 4, we explore the effect of so-called cold gelation of the protein particles induced by adding salt or reducing the pH as a mechanism to inhibit creaming. We also used this process to form micron size dense protein particles. Finally, in chapter 5 we investigate the capacity of native whey proteins to modulate the efficacy of latex particles to stabilize the W/W emulsions.

Chapter 1 Literature overview

Potential applications of W/W emulsions

Mixtures of proteins and polysaccharides are common in a great variety of food products. The digestion kinetics of the proteins may be altered when combined with certain polysaccharides, for instance by self-assembly causing gelation in the stomach at low pH conditions. An example is the combination of whey protein and alginate, which slowed down the digestion maximizing the uptake of nutrients and delayed gastric emptying promoting satiety (Norton et al. 2015). Moreover, W/W emulsions can be used for extraction and purification of biomolecules such as proteins in certain mixtures of proteins and polysaccharides, or to separate cells as there is no use of organic solvents.

Another application is the protection of specific micronutrients and the modulation of their release. The use of encapsulation to protect, deliver and control the release of bioactives to specific locations in the gastrointestinal tract is widely used in the pharmaceutical industry and is now being translated to the food industry (Norton et al. 2015). W/W emulsions can be used as a vehicle to convey water soluble substances, such as pigments, flavors, minerals, probiotics and vitamins and to targeted release of different active compounds to specific parts of the digestive system.

Different shells with specific properties can be formed at the W/W interfaces. Polymersomes and colloidosomes that vary their permeability or stability depending on the environment have good potential for controlled release. Shells of biological materials such as cells could be made by a wide variety of biological interactions, being the colloidosomes responsive to specific environmental triggers (Poortinga 2008). Buzza et al. (2013) highlighted the main differences between conventional polymersomes and polymersome structures based on W/W emulsions: the latter are formed with simple operations (mixing or homogenizing and without the addition of organic solvents); are normally larger (5–100 μm vs sub μm); the encapsulant solutes “selfload” into either the dispersed or the continuous phase with relatively rapid mass transfer across the interfaces; and the collapse of the structure can be triggered by dilution with water.

Moreover, W/W emulsions can also be used to deliver lipophilic constituents by encapsulating oil droplets within hydrogel particles. Matalanis et al. (2010) used segregative followed by aggregative phase separation of pectin/caseinate mixture to encapsulate casein-coated lipid droplets. The oil partitioned to the caseinate-rich dispersed phase at pH 7. Upon acidification to pH 5 interactions between the two phases promoted stabilization of the biopolymer particles.

Another application of W/W emulsion is their use as microreactors. Dewey et al. (2014) used dextran droplets in PEO stabilized with liposomes for enzymatic reactions. The strong partition of nucleotides and proteins to the dextran drops and the ability of the liposomes interface for access into/out of substrates and products allowed using the system for ribosome cleavage reaction. They also applied same mixture for enzymatic synthesis of CaCO_3 nanoparticles. Urea entered the dextran drop reacting with urease, which partitioned strongly to the dextran phase, forming CO_3^{2-} . CO_3^{2-} reacted with Ca^{2+} producing solid CaCO_3 . Small molecule chelators with intermediate binding affinity were employed to control Ca^{2+} availability during CaCO_3 mineralization, as liposomes were unstable in the presence of free Ca^{2+} (Cacace et al. 2015).

In addition, microgels and anisotropic particles can be formed using W/W emulsions. When one of the polymers is able to form a gel, it arrests the phase separation process at a specific point and allows studying the structures obtained; the relation between phase separation and gel formation will determine the morphologies, which in turn modifies the rheological and sensorial properties (Turgeon et al. 2003). A new range of texturing agents can be formed, including new nanoparticles, which may be more digestive and bioaccessible compared to microstructures (Norton et al. 2015). Gelation under shear flow can lead to different shapes, from spherical to anisotropic, influencing the textural properties (Wolf et al. 2000; Wolf et al. 2001). In addition to the possibility to vary the shape other advantages of using W/W emulsions to form microgels are the absence of surfactant and oil phase (Shewan & Stokes 2013). The main systems employed are gellan/carrageenan (Wolf et al. 2000; Wolf et al. 2001); gelatine/guar (Wolf et al. 2000) and gelatine/maltodextrin mixtures (Aleviopoulos et al. 1996; Stokes et al. 2001; Butler & Heppenstall-Butler 2003). The properties of the gelatin, with a viscosity that depends greatly on the temperature gelifying at about 35°C makes it easy to control the size and morphology (Stokes et al. 2001; Matalanis et al. 2011; Shewan & Stokes 2013).

The food microstructure has a clear impact in the sensory and textural perception of the product. Thus, W/W emulsions can lead to the development of new products in categories such as dairy, sauces, beverages, ready meals, nutraceuticals and so on.

Due to the extensive attention for this topic over the last few years two reviews have been recently published on water-in-water emulsions and on particle stabilized W/W emulsions (Esquena 2016; Nicolai & Murray 2017).

Pickering emulsions

Pickering emulsions were first described by Ramsden and Pickering many years ago (Ramsden 1903; Pickering 1907). They refer to emulsions where solid particles are located at the surface of the dispersed droplets inhibiting coalescence. The particles must have certain affinity for both liquid phases, being exposed to both. The particles replace the less favorable liquid/liquid interface by a solid/liquid interface. Once the particle is located at the interface of an oil/water interface, a great amount of energy is required to remove it. If the number of particles is enough to cover the drops, it forms a barrier that promotes stability.

The contact angle of the particle with the interface (θ) will depend on the affinity of the particles with each of the polymer-rich phases (A and B):

$$\cos(\theta) = (\gamma_{PA} - \gamma_{PB}) / \gamma_{AB} \quad (\text{Eq 1})$$

Values above and below 90° indicate stronger affinity for the dispersed and continuous phase, respectively. The free energy to detach a particle from the interface (which increases the interfacial area between A and B) is given by:

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

R is the radius of the particle and γ_{AB} is the interfacial tension between the two liquid phases. ΔG is the result of the interfacial tension multiplied by the reduction of the interfacial area. It will be reduced when the interfacial tension of the particle with A and with B is smaller than γ_{AB} . It is also clear that the radius of the particle has a great impact in the value. It should be noted that gravitational forces are neglected.

Two recent papers have reviewed the fundamentals and physical chemistry of O/W Pickering emulsions (Chevalier & Bolzinger 2013) and food-grade O/W Pickering systems (Dickinson 2012).

W/W emulsions

Differences between W/W and O/W emulsions

One of the greatest differences between W/W emulsion and O/W or W/O emulsions are the lower values of interfacial tension, which are orders of magnitude lower for W/W emulsions; values of dozens of $\mu\text{N/m}$ for the former versus values of dozens mN/m for the latter (Vis, Peters, et al. 2015b; Vis, Peters, et al. 2015a; Liu et al. 2012; Balakrishnan et al. 2012; Scholten et al. 2004; Ding et al. 2002; Forciniti et al. 1990; Schürch et al. 1981; Buzza et al. 2013). The interfacial tension of W/W tends to zero at the critical point (Antonov et al. 2004). To measure it, different methods can be applied: analysis of the shape of the macroscopic interface near a vertical wall (Vis, Opdam, et al. 2015); analysis of the shape relaxation of individual droplets after cessation of shear (Balakrishnan et al. 2012; Ding et al. 2002); using a spinning drop tensiometer (Scholten et al. 2004); or by a rheo-optical methodology, based on flow small-angle light scattering experiments used by Antonov et al. (2004).

Electrostatic charges in the case of polyelectrolytes and neutral polymers will reduce the interfacial tension, as electrostatic potential difference emerge at the interface (Vis, Peters, et al. 2015a; Vis, Peters, et al. 2015b)

Another peculiarity of W/W emulsions is that the interface length scale was comparable to the correlation length of the polymer solutions (Nguyen et al. 2015; Balakrishnan et al. 2012), which is larger than molecular surfactants size. Contrary to o/w emulsions, amphiphilic molecules or surfactants cannot stabilize W/W emulsions.

W/W emulsions are generally highly unstable and coalescence is fast as there are no repulsion forces between droplets. The common method to avoid the macroscopic phase separation, i.e., maintaining the droplets in the continuous phase, for W/W emulsions has usually been to gel one or both of the phases, preventing therefore coalesce (Norton & Frith 2001).

The stability of the W/W emulsions has an important role in the characteristics and the stability of the foodstuffs that contain these systems. The emulsion will be considered destabilized when a monolayer is formed by coalesce of the dispersed phase (See: Figure 1 & Figure 2). Creaming or sedimentation of the dispersed phase will naturally occur and the velocity of a drop to cream or sediment under gravity depends on the viscosity of the continuous phase (η), the density difference between the two phases ($\Delta\rho$), and the radius of the droplet:

$$v = g\Delta\rho 2R^2 / (9\eta) \quad (\text{Eq 3})$$

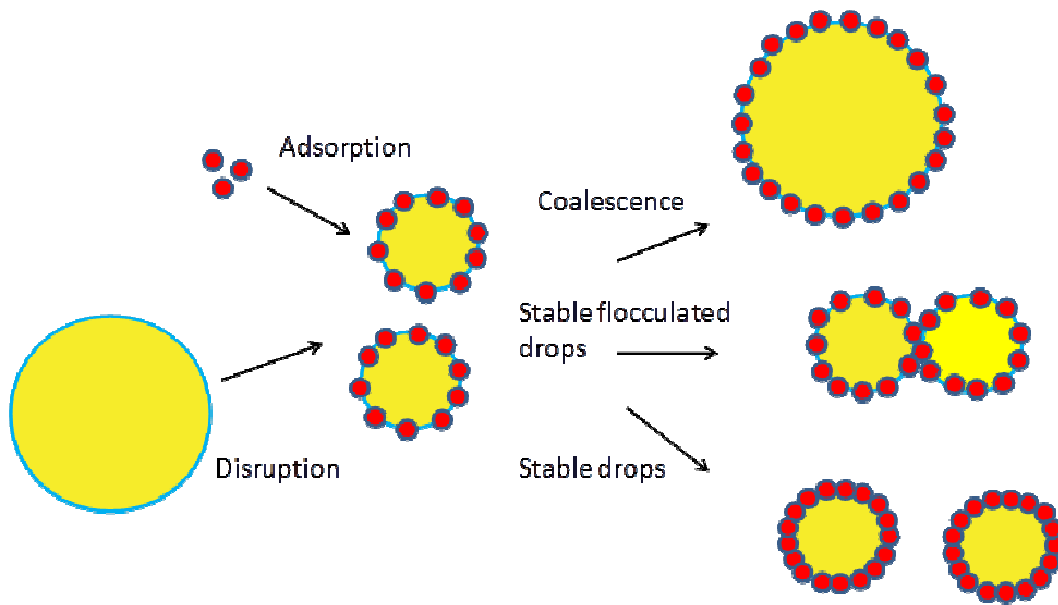


Figure 1 Scheme of Pickering emulsions formation and evolution

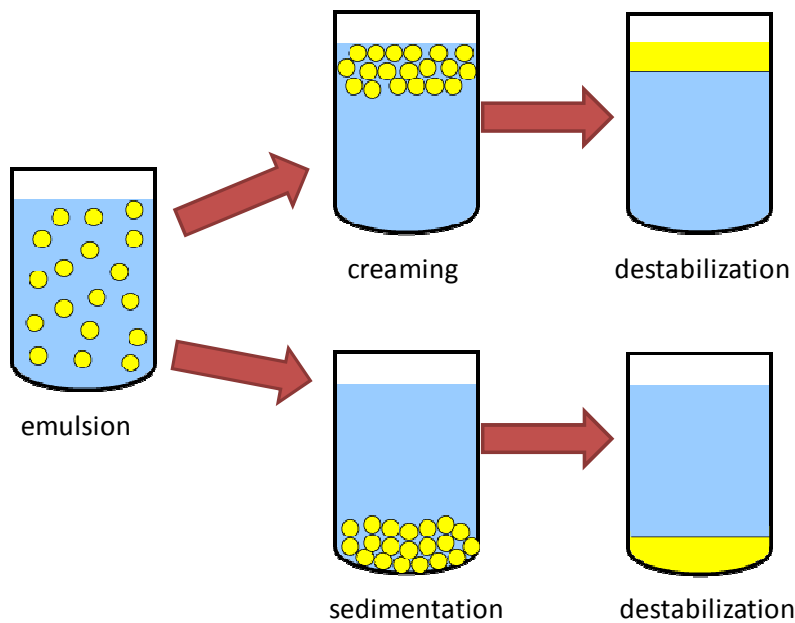


Figure 2 Scheme of creaming, sedimentation and destabilization of an emulsion

Definition and fundamentals of W/W emulsions

According to Esquena (2016) the first scientific paper on W/W emulsions was made by Beijerinck in 1896 when he observed by serendipity phase separation mixing gelatin and water-soluble starch, dyed with iodine. He was able to invert the emulsion and modify the drops sizes by varying the ratios of the polymers and the agitation intensity. He studied a second system consisting in agar and gelatin (Beijerinck 1910).

As suggested by Esquena (2016) the term W/W emulsions is preferable to aqueous-two phase mixtures as it is self-defining, non-ambiguous and most common in recent works. Also, this term excludes other water-in-water systems such as colloidal dispersions of liquid crystals.

W/W emulsions are formed by a mixture of two aqueous macromolecule solutions that are thermodynamically not compatible and therefore separate in two phases. One of the aqueous phases will form droplets dispersed into the other aqueous phase. Eventually drops will coalesce until the two phases are macroscopically separated.

Hydrophilic polymers phase separate by segregative or aggregative mechanisms determined by the hydration of the polymers and the attraction and repulsion between them (Piculell & Lindman 1992). Associative phase separation occurs normally when mixing two polymers of opposite charge and form a complex that precipitates (coacervate) and a supernatant with negligible concentration of the polymers.

On the other hand, segregative phase separation occurs when the polymers prefer contact with its own type (See: Figure 3). Phase separation is opposed by mixing entropy so that at low concentrations a single phase is still obtained and only at higher concentrations two aqueous phases are obtained. Temperature, salt, pH, and molecular weight may have a strong effect on this concentration (Vis et al. 2016). The binodal represents the limit between one or two phases. Figure 4 shows an example of the phase diagram for mixtures of PEO and dextran reported by Nguyen et al. (2013). The phase with higher volume fraction will generally be the continuous phase while the phase with smaller will be the dispersed phase. Phase inversion normally takes place near the 50/50 volume fraction ratio. Also, regions of both types of emulsions can be formed near this point. Increasing polymer concentrations above the binodal results in the formation of different tie-lines. A tie-line represents compositions leading to phases with equal composition but differing in the volume fraction of each phase. The interfacial tension between the two phases is constant along the tie-line. The increase of the concentration of both polymers results in a different tie-line. The interfacial tension increases with the tie-line length (TLL) following a power-law (Balakrishnan et al. 2012). The tie-lines converge at the critical point, the value below which only one phase is formed. It will normally represent the 50/50 volume fraction in the binodal line.

The phase diagrams are usually elaborated by empirical methods since modeling the phase behavior of the polymers mixtures presents several difficulties: lack of information about polymer-polymer and polymer-solvent interactions, polydispersity and not well defined molar weight (Esquena 2016).

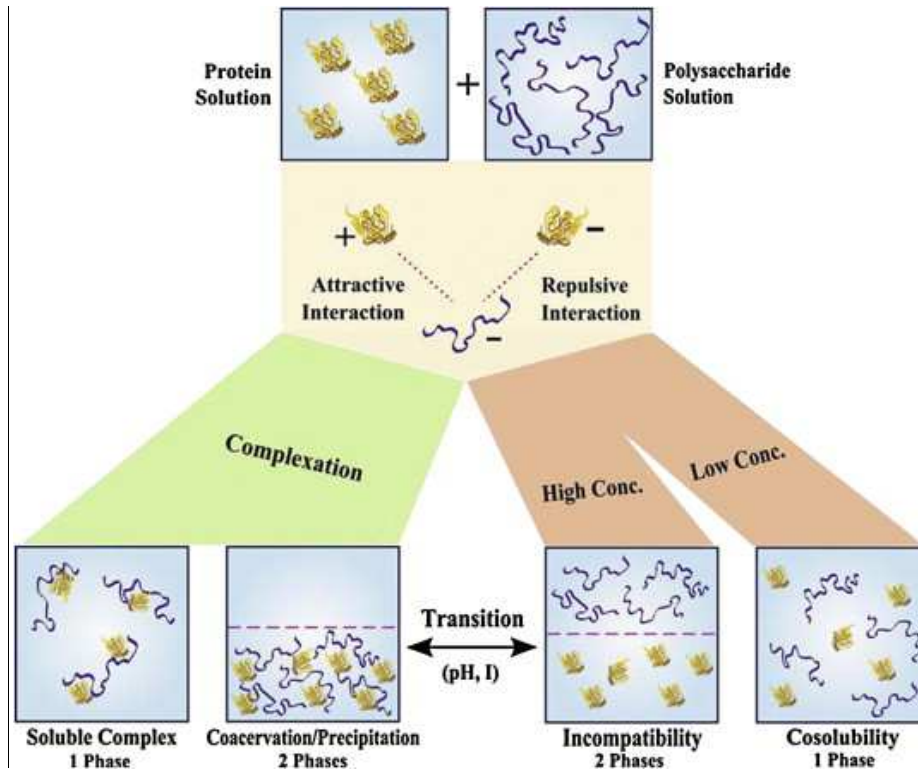


Figure 3 Scheme of segregation and aggregation phase separation. From: Matalanis et al. (2011)

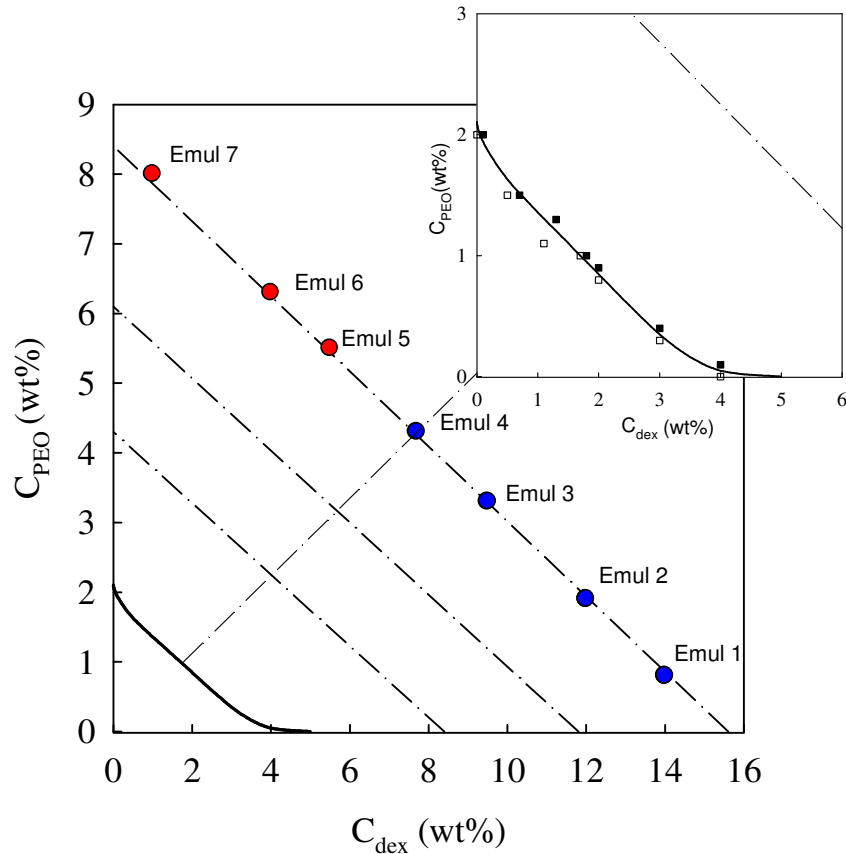


Figure 4 Phase diagram for aqueous mixtures of PEO ($M_w = 2 \cdot 10^5$ g/mol) and dextran ($M_w = 5 \times 10^5$ g/mol). The solid line represents the binodal. The dashed dotted lines represent different tie-lines. The dashed line indicates an equal volume fraction for the two phases. The circles represent different emulsions in the same tie-line (blue for dextran as continuous phase and red for PEO as continuous phase). Adapted from: Nguyen et al. (2013)

Nguyen et al. (2015) observed the influence of interactions between pH-sensitive microgels at the interface and its curvature of PEO/Dex mixtures. These particles swelled when pH was increased from 6.5 to 7.5 ($R_h = 60\text{-}220\text{nm}$), but that itself could not explain why the emulsions were stable for a week at pH between 7.0 and 7.5 and destabilized at higher and lower pH, especially in dextran in PEO (D/P) emulsions. They attributed this to interactions between the microgels (hydrophobic interactions and repulsion due to electrostatic interaction that could inhibit contact of bare interfaces between two droplets). The droplets had the smallest sizes at pH 7.2 and increased in size at higher and lower pH. They also observed that increasing the pH to 8.0 and subsequently decreasing it to 7.2 (pH-cycling) allowed creating stable emulsions even after the rapid destabilization at high pH.

The interaction between polymers can often be modulated by temperature, pH and ionic strength, therefore causing changes in the phase behavior. The hydration capability of the electrolytes can also be important. Alves et al. (1999) stated that in gelatin/locust bean gum

mixtures at moderately high concentrations the conformational entropy constraints are the main factor determining phase separation. The systems comprising degraded, dissociated, associated or colloidal dispersed gelatin molecules phase separated only at definite values of pH and ionic strength; the compatibility was minimal at low ionic strength and pH close to its isoelectric point.

Phase separation may be driven by conformational ordering resulting in a decreased entropy penalty for the formation of two phases. A reduction in temperature will reduce the entropy of mixing and may also change the various interaction parameters between the components of the mixture, leading to an increased incompatibility of the system (Norton & Frith 2001).

To create an emulsion, energy is required to form droplets of one phase suspended in another immiscible phase with a large surface area between the two phases. The energy required (free energy change) to increase the interface surface area by an amount ΔA is: $\Delta G = \gamma \Delta A$, where γ is the interfacial tension (Dickinson 2009).

However, there is a huge discrepancy between this estimation from thermodynamics and energies that are needed in practice as small droplets have highly curved interfaces, and breaking of larger droplets into smaller ones requires rapid application of a disruptive force to overcome the interfacial forces holding the larger droplet together (Dickinson 2009). This force is described by the Laplace pressure which is the difference in pressure at the concave side of a curved phase boundary and that at the convex side and depends on drop radius and interfacial tension (Shewan & Stokes 2013).

Due to the low interfacial tension it can be possible to modulate the drop size by varying the shear rate. Just by modifying the shear rate and gap of two parallel plates from 100 s^{-1} and $100 \text{ }\mu\text{m}$ to 10 s^{-1} and $500 \text{ }\mu\text{m}$ the drop size of maltodextrin/gelatin system varied from $7 \text{ }\mu\text{m}$ to $30 \text{ }\mu\text{m}$ (Stokes et al. 2001).

If shear is applied while gelling the size and shape can be controlled, creating non-spherical samples, such as anisotropic elongated microgel particles (Wolf et al. 2000; Wolf et al. 2001). They showed a clear influence of shear stress intensity (0.1 to 10 Pa) while cooling on the microstructures of the gelatin/guar and gellan/k-carrageenan mixtures, forming spherical particles, long extended particles, and irregularly shaped increasing the shear stress. The comparison of the deformation measured for gelled particles to values predicted for the liquid state showed that the droplet shape retracts during gelation. They attributed this to an increase of the interfacial tension at gelation or that changes in drop rheology induce the shape relaxation.

By crosslinking of the dispersed phase the non-spherical shapes can be kinetically stabilized and their morphology depends on temperature, molecular ordering and the relative phase volume

of the equilibrium phases (Shewan & Stokes 2013; Butler & Heppenstall-Butler 2003). Thus, tuning parameters such as flow stress, temperature under shear and time, kinetically arresting phase separation can lead to a great variety of shapes.

In order to form droplets with a controlled size and relatively high monodispersity (coefficient of variation $\leq 10\%$) microfluidic techniques can be used (Moon et al. 2016; Cheung Shum et al. 2012).

Food grade systems

Grinberg & Tolstoguzov (1997) reviewed about a 100 protein-polysaccharide aqueous systems stating that under certain conditions (dependent on specific structural and compositional features, molecular weight, conformation of the biopolymers etc.) any protein/polysaccharide system is spontaneously demixed into two liquid phases with separation of the protein and the polysaccharide. Later on, Doublier et al. (2000) and Turgeon et al. (2003) tried to shed light on the kinetics, thermodynamic and structural aspects of these mixtures.

The first w/w Pickering emulsions in food systems were described by Poortinga (2008) (maltodextrin/methylcellulose and whey protein/methylcellulose) and confirmed a year later by Firoozmand et al. (2009) in a more detailed study where adding polystyrene latex particles to gelatin/oxidized starch mixtures (above the gelatin gelation temperature) slowed down the spinodal phase separation.

Later on, Hanazawa & Murray (2013; 2014) used O/W droplets to stabilize edible W/W mixtures. The droplets were located at the interface of sodium caseinate and xanthan mixtures that phase separated when Ca^{2+} was added. More than 20mM induced phase separation at pH 6.4 and 5.9 while only 5mM was needed for pH 5.4. They observed that sodium caseinate particle size increased with increasing Ca^{2+} and lowering the pH, which induces the phase separation. The oil droplets aggregated to some extent at the interface due to added Ca^{2+} and/or lower pH, strengthening the droplets network. At too high calcium concentration (32 mM) or too low pH the systems were unstable. They attributed this to either a stronger phase separation driving force (as the molecular weight of the sodium caseinate increased) and/or excessive aggregation of droplets that could not effectively cover the interface. When comparing three types of oils (liquid, solid and a 50/50 mixture), they observed that the mixture was more efficient to stabilize the systems (with 22mM Ca^{2+}) due to accumulation and enhanced partial coalesce of partially solid droplets at the interface.

Murray & Phisarnchananan (2014) were able to slow down the phase separation of mixtures of gelatinized waxy corn starch and locust bean gum/guar gum by addition of non-edible silica nanoparticles (20nm diameter). The particles showed a strong preference for the starch

domains and tended to aggregate as concentration and hydrophobicity (by surface modification) increased. They suggested that particles flocculation in the gum phase and accumulation at the interface was possibly caused by a depletion mechanism. In a subsequent work, the authors (Murray & Phisarnchananan 2016) stabilized the same system by adding WPI microgels (size 150nm). The stability increased with increasing microgel concentration and it was also greater at pH 4 compared to pH 7. Microgels showed strong partition to the starch phase and extensive aggregation at pH 4.

In a recent work, de Freitas et al. (2016) used β -lactoglobulin (β -lg) microgels to stabilize xyloglucan (XG) and amylopectin (AMP) mixtures. The dispersed amylopectin-rich in continuous xyloglucan-rich system was stable at $\text{pH} \leq 5.0$. Above this point the (β -lg) microgels were located AMP drops and not entering the interface, while below they partitioned preferentially to the XG phase. They hypothesized that particles should partition at least to some extent into both phases in order to enter the interface. Moreover, they observed that XG adsorbed the microgels surface at pH below 5.5, being the reason for the particles to increase the affinity for the XG phase.

Another type of edible particles were used by Firoozmand & Rousseau (2014). They employed nonviable, edible single-celled microorganisms (*Lactobacillus bulgaricus*, *Saccharomyces cerevisiae*, *spirulina* and *chlorella*) as micron-sized particles to control the microstructure and rheology of gelatin/maltodextrin systems. The cells altered the microstructure of the system, especially after the addition of 1 wt % NaCl.

PEO/Dex as model system

In order to gain understanding on the physical-chemistry fundamentals of W/W emulsions, non edible model systems have been used. The mixture of PEO and dextran is a system in which both polymers are neutral and changes of their molecular masses modify the solutions viscosities to a large extent. Besides, phase separation occurs at relatively low concentrations for high molecular masses. That makes it one of the most studied systems (Kang & Sandler 1987; Bamberger et al. 1984; Ryden & Albertsson 1971; Brooks et al. 1984; Forciniti et al. 1990; Schürch et al. 1981; Cesi et al. 1996). Moreover, the PEO/Dextran system has also been used for studying the partition of proteins or other molecules in w/w emulsions (Diamond & Hsu 1990; Johansson 1976; Johansson 1970b; Johansson 1970a; Westrin et al. 1976; Tubio et al. 2004); fabricate of fibrillosomes (Song et al. 2016).

In addition, this system has already been previously studied in the research unit PCI where the present investigation was conducted (Peddireddy et al. 2016; Nguyen et al. 2015; Nguyen et al. 2013; Balakrishnan et al. 2012). In previous research, fluorescently labeled latex particles with

radius $R=1 \mu\text{m}$ were successfully trapped at the interface of PEO/dextran mixtures (Balakrishnan et al. 2012). They showed that these particles diffused freely on the droplet surface. This allowed a channel to be formed between two droplets in contact that induced coalescence. The shear forces during this coalesce process were sufficient to eject particles from the interface. It was also shown by Nguyen et al. (2013) that contrary to native β -lactoglobulin (β -lg) proteins microgels of β -lg were located at the interface and the inhibition of coalescence to an extent that depended on the concentration and size of the microgels. Droplet size appeared to also be related to these parameters as well as to the volume fraction of dispersed phase. Moreover, stability seemed also to be related with the particles affinity for PEO or dextran. Confirmation of this phenomenon by modulating the partition will be one of the objectives of this work.

Another interesting subject is the effect of particle morphology on the stability of W/W emulsions. Vis et al. (2015) used gibbsite (synthetic clay) nanoplates to stabilize gelatin/dextran mixtures. They found that they lied parallel to the interface minimizing so as to occupy maximum surface area. The energy of adsorption of the nanoplates depends linearly on $(1 - |\cos \theta|)$ while it does quadratically for spheres:

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

It was found that gibbsite aggregated and was able to form weak gels connecting droplets inhibiting creaming of droplets for weeks.

Peddireddy et al. (2016) used hydrophilic nanorods (cellulose nanocrystals) to stabilize PEO/Dex mixtures and determined the adsorption free energy after as:

$$\Delta G = -lb\gamma (1 - |\cos \theta|) \quad (\text{Eq 5})$$

where l is the length and b is the width of the rods. When increasing the nanorod concentration the interface coverage was almost constant about 50% while the droplet size and fraction at the interface decreased. They assumed the particles to be oriented parallel to each other. Moreover, the addition of 50mM NaCl induced formation of a weak gel that allowed inhibiting creaming of the droplets. As previously mentioned, the strong affinity to one of the phases, dextran, could be the reason of these particles to be capable of stabilizing the emulsions only when PEO was the dispersed phase.

Diblock and triblock copolymers have also been used to stabilize w/w emulsions. Buzza et al. (2013) used diblocks and triblocks with a hydrophobic central block and two ends with different preferences for the PEO and dextran phases at neutral pH. Triblocks did not yield a noticeable

improvement of the stability comparing with diblocks. The most stable systems were found for larger central blocks and larger blocks with affinity for dextran. They proposed that a monolayer was formed on the droplets where each of the two ends will direct to their preferential phase. However, this model cannot explain why some diblocks provided better stability than triblocks. They suggested that the diblocks and triblocks formed polymeric micelles that adsorbed to the interface by the same mechanism as other types of particles. This would also explain better why stability increased with copolymer size and was independent of the chain length ratios.

Liposomes were also found to stabilize PEO and dextran emulsions. Dewey et al. (2014) used negatively charged liposomes with $R \approx 65$ nm to form artificial microreactors. The samples were stable against coalesce due to electrostatic repulsion instead of steric hindrance. Electrostatic jamming caused the liposomes to maintain their structure and to remain immobile at the interface. The addition of 10mM of NaCl resulted in fast drop coalescence as the charges were screened reducing electrostatic repulsion between the droplets.

Partition in W/W emulsions

As previously mentioned particles in W/W mixtures may be located at the interface, almost exclusively in one of the phases or have a partition between the two phases. Thus, silica nanoparticles and WPI microgels showed preference for the starch domains in the starch and locust bean gum/guar gum mixtures (Murray & Phisarnchananan 2014; Murray & Phisarnchananan 2016). Moreover, in the gelatin/oxidize starch system studied by Firoozmand et al. (2009) the latex particles had preference for the gelatin phase. They assumed gelatin to be adsorbed to the surface of the latex particles and therefore they will be thermodynamically compatible with the gelatin-rich domains. The pH can modify the partition of different types of particles in W/W systems. Coating xyloglucan to β -lg microgels by reducing the pH switched their partition from amylopectin to xyloglucan phase (de Freitas et al. 2016). Nguyen et al. (2015) showed that excess microgel particles (covalently cross-linked poly (ethyl acrylate-co-methacrylic acid-co-1,4-butanediol diacrylate) partition in a non-monotonic manner in PEO/Dex mixtures as a function of the pH or the ionic strength. They partitioned to the dextran phase between pH 7.2 and 7.8 and to PEO at lower and higher pH. They also found that 1mM NaCl was enough to invert the partition from PEO towards dextran.

The partition depends not only on the protein type and its hydrophobicity (Diamond & Hsu 1990; Tubio et al. 2004; Asenjo & Andrews 2011), but also on surface charge, protein and polymer concentrations and molecular weights, pH and ionic strength (Asenjo & Andrews 2011).

β -lactoglobulin

β -lactoglobulin (β -lg) is the most abundant whey protein and it is widely used as ingredient in the food industry. It is a globular protein with a radius of about 2nm. Its iso-ionic point (IIP) = 5.0 (Kharlamova et al. 2016).

When heated in solution, the structure unfolds partially and irreversible aggregation occurs. The size and the structure of the aggregates depend on protein concentration, heating protocol, pH and type and concentration of salt. They can form fractal aggregates, fibrils or microgels. Above a critical protein concentration gels will be formed.

Nicolai et al. (2011) described the β -lg heat aggregation process for pH >5.7 as follows: 1) the initial equilibrium of monomers and dimers shifts towards the former; 2) the proteins structure becomes more mobile allowing the hidden hydrophobic groups and the thiol groups to interact; 3) primary aggregates consisting of curved strands (hydrodynamic radii of $R_h = 15-25$ nm) are formed at pH > 6.1 and spherical microgels (radii 100-300nm) are formed at pH<6.1; 4) at higher concentrations the primary aggregates associate into polydisperse self-similar aggregates that grow larger with increasing concentration; 5) above a critical gel concentration the system forms a system spanning network.

The molar mass of self similar aggregates increases with their radius as:

$$M \propto R^{df} \quad (\text{Eq 6})$$

with df the fractal dimension [$df \approx 1.7$] (Mahmoudi et al. 2007). The density (ρ) of fractal aggregates increases with increasing radius:

$$\rho \propto R^{(3-df)} \quad (\text{Eq 7})$$

At pH 2 the proteins hydrolyze and a fraction of the residual peptides assemble forming fibrils with a cross section of few nanometers and a length of 1 to 20 microns (Jung & Mezzenga 2010). No covalent bonds are formed at this pH and the individual protein filaments associated laterally forming twisted ribbons with an helical structure (Adamcik et al. 2010). The rodlike fibrils used for this work have a persistence length between 0.5 and 1 μm , a cross section of 5nm and lengths of 1-20 μm .

Microgels can also be formed by heating β -lg solutions at neutral pH adding of CaCl_2 . They are approximately spherical, have a hydrodynamic radius ranging from 100 to 300 nm and their internal protein concentration is 0.2-0.45 g/mL (Phan-Xuan et al. 2014).

Cold gelation

The term cold gelation refers to the aggregation and gelation of protein aggregates by reducing electrostatic repulsion. After the formation of aggregates by heat denaturation of native proteins gelation can be induced by adding salt or varying the pH near to the isoelectric point (pI), thus decreasing the repulsion between the aggregates. It was shown that besides physical bonds disulfide bridging also occurs during cold gelation (Alting et al. 2003). This process can take place at room temperature, but increasing the temperature increases the rate of gelation (Bryant & McClements 1998; Ako et al. 2010). Increasing the amount of salt also increases the speed of gelation as it screens electrostatic repulsion, which facilitates binding (Ako et al. 2010). Much higher amount of monovalent than divalent salt is necessary to induce cold gelation, as divalent ions can bind specifically (Ako et al. 2010; Nicolai et al. 2011).

Ako et al. (2010) determined the activation energy for gels formed with β -lg aggregates with $R_g = 65$ nm ($C = 50$ g/L) and 0.3 M NaCl to be $E_a = 70$ kJ/mol. They also stated that the structure of the cold-set gels is more homogeneous than that of heat-set gels formed with native proteins at the same salt concentration and pH, as the aggregates at the start of the cold gelation process were formed at different conditions.

The structure of large aggregates formed by association of small ones after addition of salt are similar to those formed by heating native proteins at concentrations close to the critical gel concentration ($C_g \approx 95$ g/L).

Gels made from aggregates of different size at same pH (7.0) presented no difference in their structure (Ako et al. 2010). However, when not only the size but also the pH at which the aggregates were formed, a difference was found, with gels from microgels being more heterogeneous than gels from fractal aggregates (Donato et al. 2011).

The critical gel concentration to form cold-set gels by microgels is higher than for fractal aggregates and for a given protein concentration gels formed by microgels are less stiff. A possible reason is that microgels are much denser than fractal aggregates and therefore the volume fraction of the latter is much higher for a given protein concentration.

Fibrils, fractals and microgels precipitate between pH 6 and pH 4. Nevertheless, when the pH of the microgels and fractals is reduced rapidly from 7.0 to 3.0 the aggregates remain in suspension without significant modification. When the pH of a fibrillar aggregate suspension formed at pH 2 is increased rapidly increased to pH 7.0 they remain in suspension, but the average length of the fibrils is reduced (Veerman et al. 2003; Loveday et al. 2011).

Chapter 2 Materials and methods

Formation and characterization of protein particles

The β -lg (Biopure, lot JE 001-8-415) was purchased from Davisco Foods International, Inc. (Le Sueur, MN, USA) and consisted of approximately equal quantities of variants A and B. The powder was dissolved in pure water (Millipore) containing 200 ppm NaN_3 to protect against bacterial growth. The solutions were filtered through 0.2 μm pore size filters (Anatope), and the pH was set by addition of 0.1 M HCl or 0.1 M NaOH under vigorous stirring. Fractals and microgels were prepared by heating the β -lg solutions at pH 7.0 overnight at 80 °C. After the heat treatment at C = 95 g/L more than 95% of the proteins formed fractal aggregates (Mehalebi et al. 2008). After heat treatment at C = 40 g/L in the presence of 4.4 mM of CaCl_2 more than 80% formed microgels. The fraction of microgels was determined as the precipitated fraction after centrifugation at room temperature for 1h at 5×10^4 g (Allegra 64R centrifuge, Beckman Coulter, USA). The remaining proteins consisted principally of small strands (Phan-Xuan et al. 2014). Fibrils were prepared following the method described in (Jung & Mezzenga 2010). Solutions of C = 20 g/L were heated during 5h at 90 °C and pH 2.0 under stirring. The fibrils had a cross section of 5 nm and lengths of between 1 and 20 μm as confirmed by transmission electron microscopy images (Jung & Mezzenga 2010). Sedimentation at pH 4.6 indicated that 75% of the proteins were aggregated. However, the fraction of proteins that formed fibrils may be smaller. The remaining proteins were unassociated peptides and residual native. Increasing rapidly and while stirring the pH of the fibril solution from pH 2.0 to pH 7.0 adding the required amount of NaOH could avoid aggregation. It was possible however that the average length of the fibrils was reduced. It should also be noticed that at same concentration of proteins the fraction of protein in the form of aggregates is lower for fibrils than for microgels and fractals.

The protein concentration was determined by measuring the adsorption of UV light with wavelength 280 nm (Varian Cary-50 Bio, Les Ulis, France) using an extinction coefficient of $0.96 \text{ L g}^{-1} \text{ cm}^{-1}$. The z-average hydrodynamic radius (R_h) was determined for highly diluted solutions using dynamic light scattering (DLS). The measurements were done in a cross-correlation dynamic light scattering instrument (LS instrument, Fribourg). The wavelength of the incident light was $\lambda=632$ nm. Samples were placed in a thermostatic bath and measurements were done varying the scattering angle (θ) from 13 to 150°. The hydrodynamic radius of the fractals and the microgels was found to be 150 nm. However, neither the microgels nor the fractals are monodisperse in size, and the fractals in particular have a broad size distribution. Reducing the pH of the particle solutions between 6 and 4 caused aggregation. However, the pH could be decreased from 7.0 to 3.0 without aggregation of the particles.

Preparation of the emulsions

Dextran and PEO were purchased from Sigma-Aldrich. The weight-average molar mass was $M_w = 1.6 \times 10^5$ g/mol for dextran and $M_w = 2 \times 10^5$ g/mol for PEO. The powders were dissolved under stirring in ultra pure water. The PEO powder contained about 1% silica particles, which were removed by centrifugation of the PEO solutions at 5×10^4 g for 4 h. The emulsions were prepared by mixing aqueous solutions of PEO, dextran, proteins and/or latex in the required amounts using a vortex mixer. The pH was adjusted by addition of 0.1 M HCl or 0.1 M NaOH under stirring. Neither the order of the addition of the components nor the stirring speed or duration had a significant influence on the structure or behavior of the emulsions. The binodal did not depend on the pH.

Purified whey protein isolate (WPI) powder was purchased from Lactalis (Laval, France). It contained 92% protein of which 70% β -lg and 20% α -lac as determined by size exclusion chromatography. The powder was dissolved in pure water (Millipore) containing 200 ppm NaN_3 to protect against bacterial growth.

Polystyrene-based spheres (Fluoresbrite® Polychromatic Red Microspheres, diameter of $0.5\mu\text{m}$ (batch 690690) were purchased from PolySciences (Hirschberg an der Bergstrasse, Germany). The particles are internally dyed which frees the surface of the beads for protein adsorption. Suspensions were sonicated to disrupt disperse aggregates of latex particles. Suspensions of native WPI and the latex particles were added to the PEO and dextran emulsions. No difference in the latex behavior was found for the same pH by dissolving the latex particles and the WPI in the dextran, the PEO or in the emulsion or by varying the contact time and the stirring speed.

Latex surface area coverage by WPI

The number of latex particles per ml was: $3.64 \cdot 10^{11}$. The area of a particle of radio $0.25 \mu\text{m}$ is: $0,785 \mu\text{m}^2$. The total area was equal to the number of particles multiply by the area of the particle:

$$A_{\text{latex}} (\mu\text{m}^2) = (V_{\text{latex}} (\text{ml}) \cdot 3.64 \times 10^{11}) \cdot (4\pi r^2) \quad (\text{Eq 8})$$

The area that can be covered by native WPI was:

$$A_{\text{WPI}} (\mu\text{m}^2) = (V_{\text{WPI}} (\text{ml}) \cdot [\text{WPI}]/\rho)/V A_c \cdot (0.9) \quad (\text{Eq 9})$$

V is the volume and A_c the average cross section area of a globular WPI protein; $\rho = 1,3$ g/ml is the WPI density. The radius of a native WPI protein is about 2 nm. Therefore, $A_c \approx 5.0 \cdot 10^{-5} \mu\text{m}^2$ and $V \approx 4.19 \cdot 10^{-21}$ ml. The fraction of latex surface that can be covered by WPI is $A_{\text{WPI}} / A_{\text{latex}}$.

Confocal Microscopy

Two different confocal laser scanning microscopes (CLSM) were used for this work. The images of the first two chapters were acquired with a Leica TCS-SP2 (Leica Microsystems Heidelberg, Germany) with two different water immersion objectives: HC×PL APO 63X and HC×PL APO 20X. The images of the third chapter were obtained with a Zeiss LSM800 (Carl Zeiss Microscopy GmbH, Germany) with two water immersion objectives: 63X and 25X. In both cases images of 512 pixels × 512 pixels were taken. The solutions were inserted between a concave slide and a cover slip and hermetically sealed. Fluorescein isothiocyanate (FITC) dextran was added to visualize the dextran and 5 ppm of rhodamine B to visualize the proteins. The incident light was emitted by a laser beam at 543 nm and/or at 488 nm. The fluorescence intensity was recorded between 560 and 700 nm. It was verified that the use of labeled dextran and proteins had no influence on the emulsions.

Rheology

The dynamic moduli were measured on three different stress imposed rheometers (AR2000, ARG2 and DHR3, all from TA Instruments, Guyancourt-France) equipped with a cone/plate geometry (diameter 40 mm, angle 2°). The samples were poured right after preparation between the cone and the plate and covered with mineral oil to avoid evaporation. The temperature was controlled at $20 \text{ °C} \pm 0.1$. The measurements were done in the linear regime at a fixed strain of 1% and a frequency of 0.1 Hz.

Turbidity

The turbidity of the top phase of the emulsions containing latex particles after complete phase separation was measured using a UV-visible spectrometer Varian Cary-50 Bio (Les Ulis, France) and 10mm length cuvette. A small amount from the top of the tube was carefully taken and diluted appropriately in order to avoid saturation. The values represent the absorbance at 280nm (maximum absorbance value for the fluorescent latex particles) after normalizing by the dilution factor. They are a comparison of the relative absorbance.

Chapter 3 Results and discussion (1)

Influence of protein particle morphology and partitioning on the behavior of W/W emulsions

Particles with a different morphology than homogeneous spheres can also stabilize W/W emulsions. Peddireddy et al. (2016) used hydrophilic nanorods (cellulose nanocrystals) and Vis et al. (2015) used gibbsite (synthetic clay) nanoplates. Here we compare the stability against coalescence of PEO and dextran mixtures with particles of the same nature but different morphology. To this end we have exploited the fact that protein particles in the form of fibrils, microgels and fractal aggregates can be formed from β -lactoglobulin (β -lg) by heating at different conditions as described in Chapter 2. β -lg microgels have already been shown to be able to stabilize PEO in dextran emulsions at neutral pH, but not dextran in PEO emulsions (Nguyen et al. 2013). The effect of protein fibrils and fractals has not yet been investigated. We also investigated the effect of the protein charge by studying the emulsions at two different pH, 7.0 and 3.0, where the proteins have opposite charge.

Emulsions at pH 7

Emulsions containing 1.9 wt% PEO and 12 wt% dextran were prepared at pH 7 (Emulsion 2 in Figure 4 of Chapter 1). The volume fraction (Φ) of the PEO rich dispersed phase was 25%, with a PEO concentration of 8.2 wt% and negligible dextran concentration. The concentration of dextran in the continuous phase was 15.8 wt% with negligible PEO concentration. The interfacial tension between the two phases was $75 \mu\text{N}/\text{m}^2$ (Balakrishnan et al. (2012)).

Different protein concentrations ($C = 0.05 - 0.75 \text{ wt}\%$) were added to the PEO/Dex mixture. Pictures of the emulsions at different times after mixing are shown in Figure 1. PEO drops creamed to the top and in samples with added fractal aggregates and microgels a clear layer appeared indicating that coalescence occurred. The amount of destabilized PEO droplets decreased with increasing microgel concentration. Destabilization was negligible at least for a week for $C \geq 0.5\%$. At $C \geq 0.5\%$ coalesce of PEO droplets was arrested but not their creaming

under gravity. An increase of the turbidity of the dextran phase is observed with increasing C as the excess microgels partition to this phase at pH 7.0.

In the emulsions to which fractal aggregates were added the clear PEO top layer appeared sooner than for microgels. The layer was visible after a week for all the protein concentrations studied. However, it was surprising that this layer was smaller for C=0.1%. A smaller layer indicates less coalesce of PEO droplets and therefore higher stability. We confirmed the repeatability of the experiment and prepared two emulsions with 0.075% and 0.15% which were also significantly more stable than 0.05% and 0.2%. However, we have no explanation for this peculiar behavior.

When fibrils were added, the stability of the emulsions was higher than for microgels and fractal aggregates. No clear PEO layer was observed after a week even for the lowest protein concentration. Also, the rate of creaming was considerably reduced. The effectiveness is even higher if we take into account that the fraction of protein in the form of aggregates is lower for fibrils than for microgels and fractals (See: Chapter 2). It should be noted that fibrils have a persistence length of about 1 μm which is smaller than the radii of the droplets and therefore there is no enthalpic penalty from bending the fibrils at the interface.

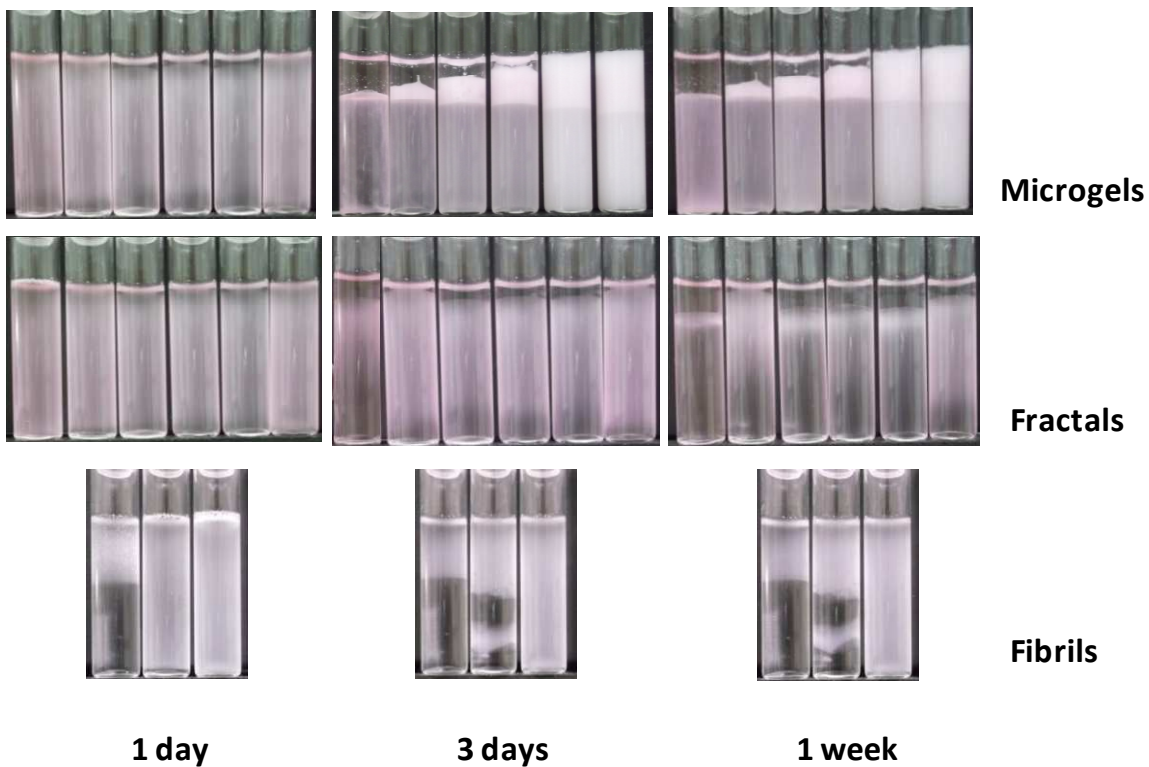


Figure 1 Photographs at different times after mixing PEO into dextran emulsions at pH 7.0 containing different amounts of microgels or fractals (0.05, 0.1, 0.2, 0.3, 0.5, 0.75% (left to right)) or fibrils (0.05, 0.1, 0.3% (left to right))

Figure 2 shows CLSM images of PEO in dextran (P/D) emulsions at pH 7.0 with different concentrations of microgels, fractals, or fibrils. Rhodamine B was added to visualize the proteins. Proteins partitioned to the dextran phase at this pH which make this phase more fluorescent at high concentrations. However, as residual free rhodamine B was preferentially located in the PEO phase, at low protein concentrations the PEO phase presented higher fluorescence. In the presence of microgels a ring of proteins can be clearly seen around the PEO droplets. Also, larger clusters of microgels can be seen, maybe due to depletion interactions between the microgels and the dextran chains. In presence of fractals or fibrils the ring is not visible although a layer of proteins must have been formed at the interface as the stability was increased. Only at the two lowest fractal concentrations (0.05 and 0.1%) the ring is barely visible. The reason for the low fluorescence is that fractals are much less dense than microgels and fibrils are most probably oriented parallel to the interface forming a thinner layer.

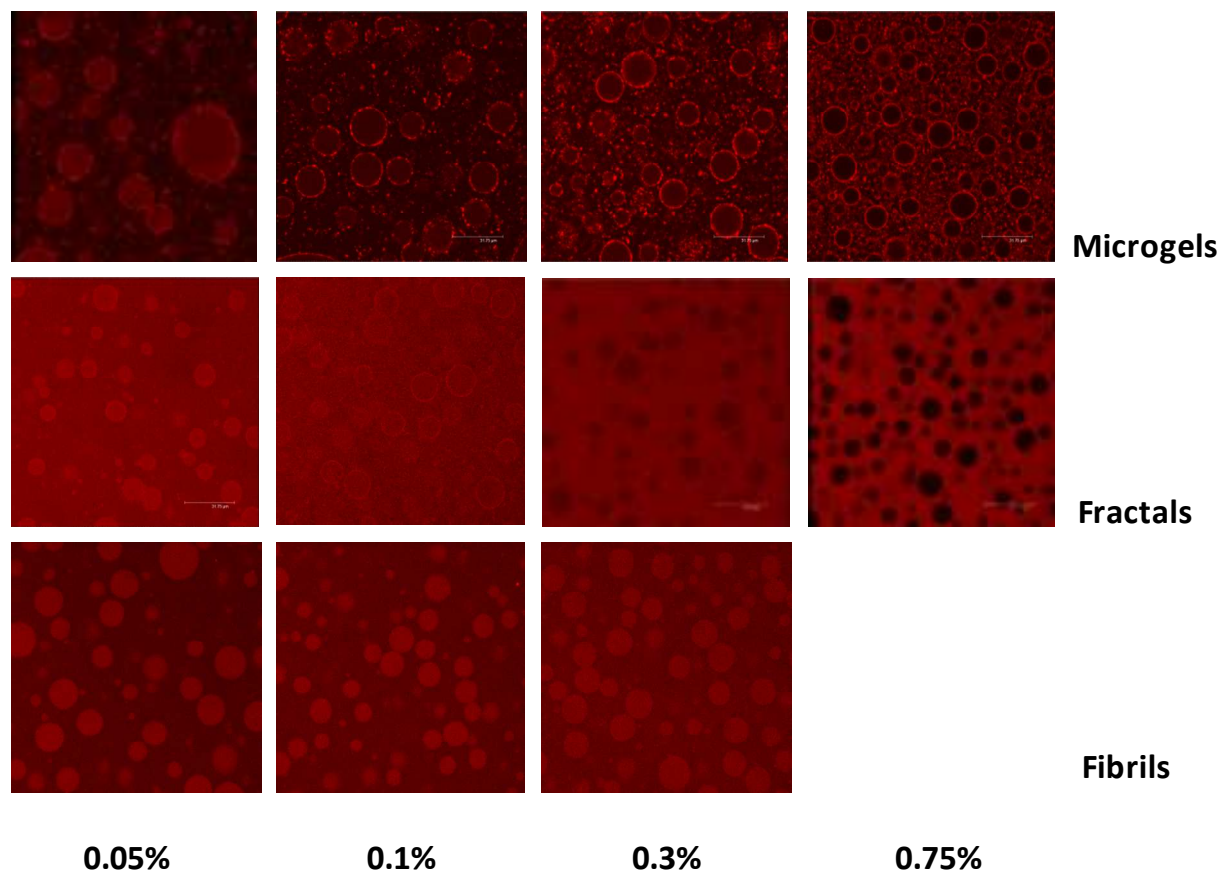


Figure 2 . CLSM images ($160 \times 160 \mu\text{m}^2$) of PEO in dextran emulsions at pH 7.0 in the presence of different concentrations of microgels, fractals, or fibrils. Proteins are labeled with rhodamine B, but residual unbound rhodamine B is preferentially situated in the PEO phase.

The number-average radius of the droplets (R) decreased weakly with increasing protein concentration: from $10 \pm 7 \mu\text{m}$ at 0.05% to $6 \pm 4 \mu\text{m}$ at 0.75% for microgels; from $8 \pm 5 \mu\text{m}$ at 0.05% to $5 \pm 3 \mu\text{m}$ at 0.75% for fractals; and from $7 \pm 4 \mu\text{m}$ at 0.05% to $6 \pm 4 \mu\text{m}$ at 0.3% for fibrils. The rate of creaming depended on the size of the droplets, the difference in density between the two phases, and the viscosity of the continuous phase (Nguyen et al. 2013). It is unlikely that the protein concentration in the continuous dextran phase would have a significant influence on the viscosity as it was very small and we did not appreciate any difference in the flow of tilted emulsions at different protein concentrations. The morphology of the protein particles did not have a pronounced effect on the initial droplets size. Thus the rate of creaming was expected to be similar. The slower creaming exhibited by samples containing fibrils is most likely caused by a reduction of the drops coalescence during creaming. Also, it may be possible that the high asymmetry of the fibrils increased the effective viscosity felt by the droplets even though it could not be appreciated when tilting the samples.

Emulsions with a volume fraction of 25% dispersed dextran phase in the continuous PEO phase were prepared by mixing 6.3 wt% PEO and 4.0 wt% dextran (Emulsion 6 in Figure 4 Chapter 1). The interfacial tension of these dextran in PEO (D/P) emulsions was the same as for the P/D emulsions discussed above. Microgels, fractals and fibrils were added but the emulsions were not stable so that dextran droplets coalesced and precipitated forming a homogeneous layer at the bottom. It was already reported previously that microgels were not able to stabilize D/P emulsions (Nguyen et al. 2013). Figure 3 shows confocal images of a D/P emulsion containing 0.3% microgels at different times. The layer covering the dextran drops is visible, however, after only 3 hours the droplets coalesced forming big dextran domains.

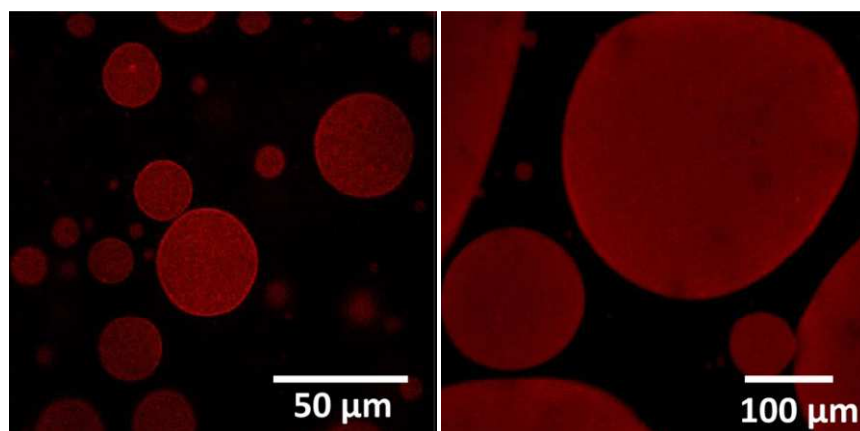


Figure 3 CSLM images of droplets of the dextran phase taken at the bottom of a dextran in PEO emulsion in the presence of 0.3% microgels at pH 7.0 immediately after mixing (left) and after 3 h (right). Notice that the scales of the images are different

Emulsions at pH 3

P/D and D/P emulsions containing different concentration of microgels, fractals and fibrils were prepared at pH 3.0. Notice that the interfacial tension does not depend on the pH. Figure 4 shows pictures of the emulsions at different times after preparation.

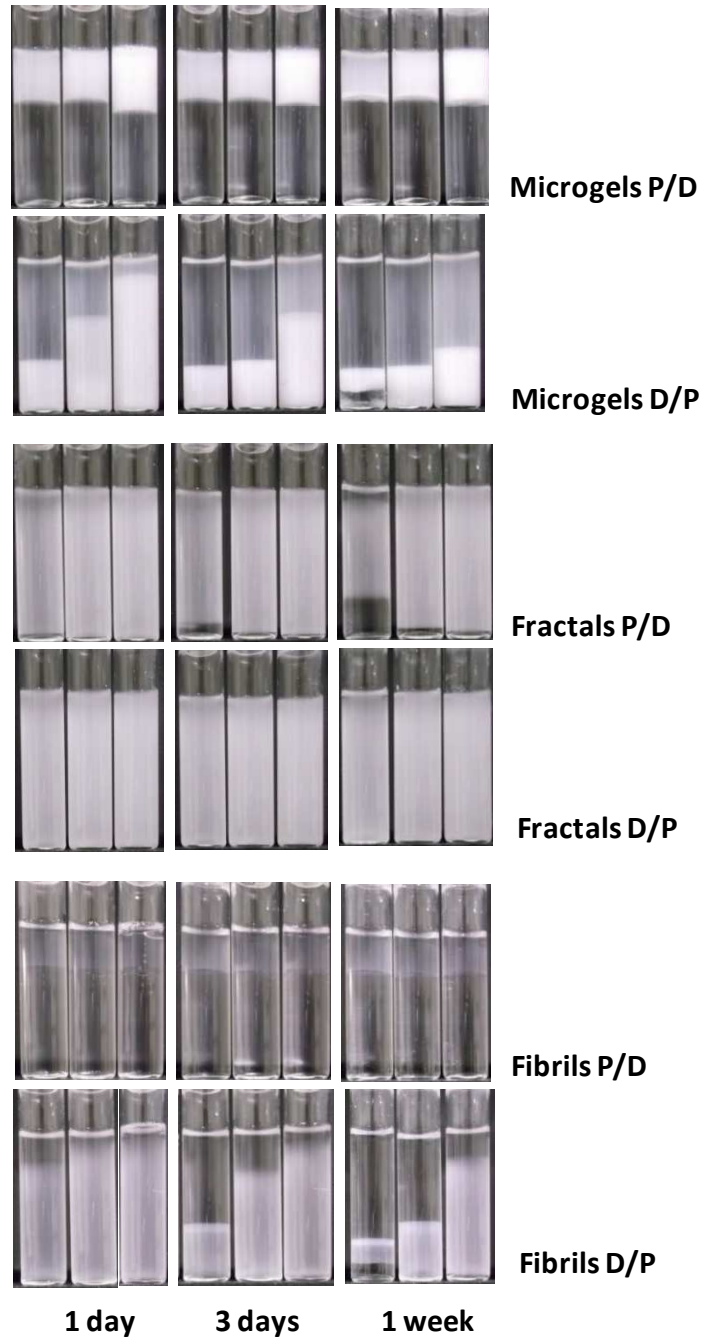


Figure 4 Photographs at different times after mixing PEO in dextran (P/D) emulsions and dextran in PEO (D/P) emulsions at pH 3.0 with different amounts of microgels, fractals, and fibrils: 0.05, 0.1, and 0.3% (left to right).

Contrary to pH 7.0 the P/D emulsions at pH 3 containing fibrils showed macroscopic phase separation after a few hours, even at the highest protein concentrations. On the other hand, microgels and fractals could stabilize P/D emulsions at pH 3. The rate of creaming was, however, very different than at pH 7. In the presence of microgels PEO droplets creamed much faster than in the presence of fractals at the same concentration. This was caused by flocculation of drops forming clusters that creamed very fast. It is also remarkable that in the presence of microgels the dextran phase remained completely transparent. This indicates that the partition of the excess microgels switched from dextran at pH 7.0 to PEO at pH 3.0.

Stable D/P emulsions could be prepared at pH 3.0 with the three different particle morphologies. The rate of precipitation of dextran droplets was different, however. In samples containing microgels sedimentation was found to be the fastest. Samples with fibrils sedimented slower than the ones with microgels. No macroscopic sedimentation was observed for fractals after a week at the three protein concentrations.

Figure 5 shows confocal images of the emulsions right after mixing. The average radii of the PEO droplets stabilized by microgels or fibrils was similar for the three protein concentrations investigated ($R=7 \pm 5 \mu\text{m}$). In the presence of fractals the size did depend on the protein concentration and was significantly smaller than for the other two morphologies ($R=5 \pm 3 \mu\text{m}$ at $C=0.05\%$ and $R=2.5 \pm 1.5 \mu\text{m}$ at $C=0.3\%$). As in the P/D mixtures, in D/P samples, the size did not vary greatly with protein concentration for microgels ($R=4.5 \pm 2.5 \mu\text{m}$) or fibrils ($R=3.5 \pm 3 \mu\text{m}$), but it varied considerably in the presence of fractals ($R=2.5 \pm 1.5 \mu\text{m}$ at $C=0.05\%$ and $R=1 \pm 0.5 \mu\text{m}$ at $C=0.3\%$). Interestingly, in all cases the dextran droplets in D/P emulsions were smaller than the PEO droplets in P/D emulsions. The smaller droplet sizes formed with fractal aggregates explain why both creaming and precipitation was much slower.

The droplets size did not vary considerably with time after mixing for P/D and D/P systems with fractals at $C= 0.3 \text{ wt}\%$. The same behavior was also observed with fibrils for D/P mixtures, but dispersed PEO droplets in the continuous dextran phase coalesced and formed a homogeneous layer on the top within a few hours. In the case of microgels, the dextran droplet size remained constant while PEO droplets coalesced creating larger droplets, but did not form a homogeneous layer. Confocal images of aged P/D and D/P emulsions containing microgels taken near the top and the bottom of the samples respectively are shown in Figure 6.

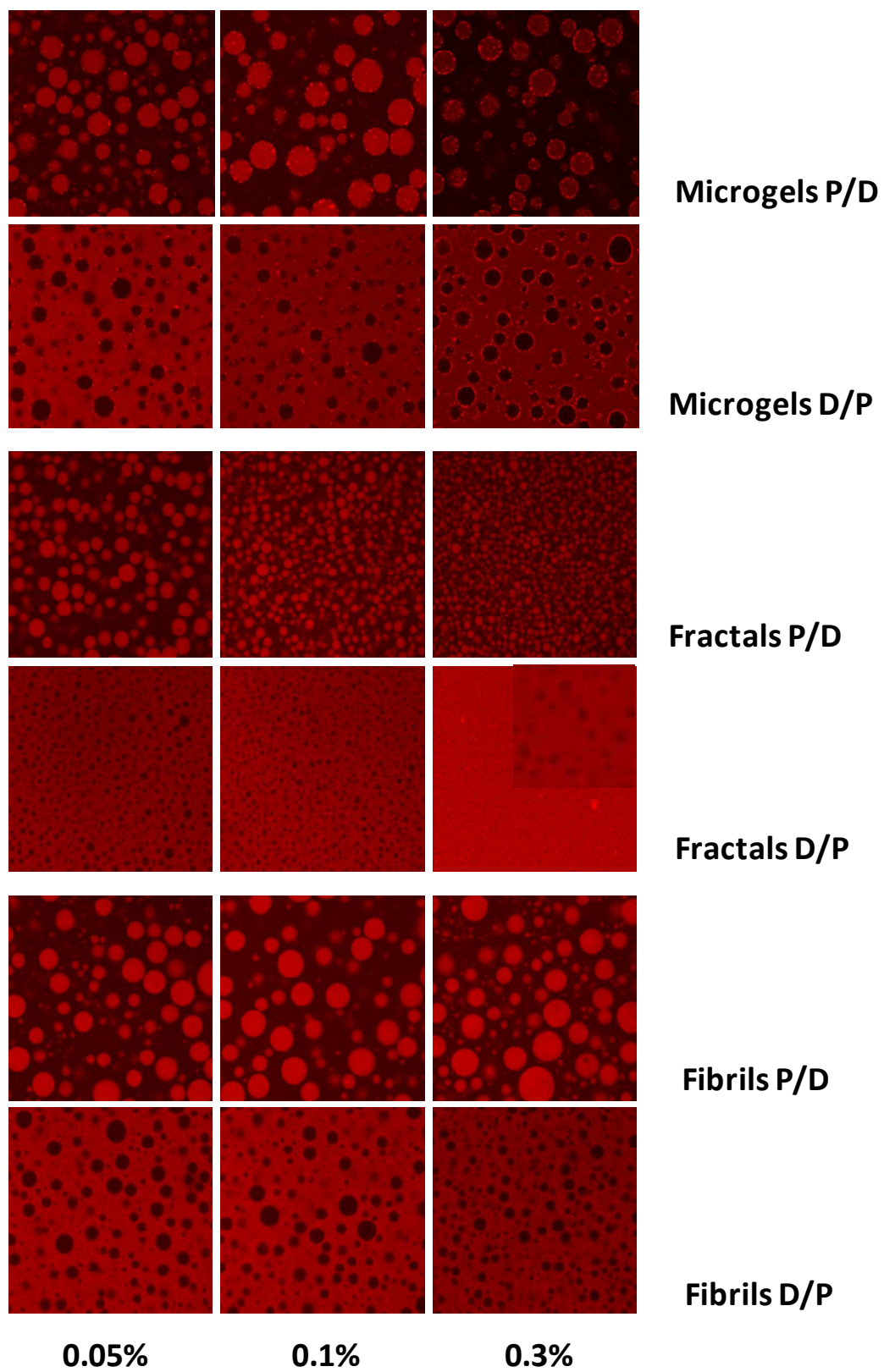


Figure 5 CLSM images ($160 \times 160 \mu\text{m}^2$) of PEO in dextran and dextran in PEO emulsions at pH 3.0 in the presence of different concentrations of microgels, fractals, or fibrils. The inset in one of the images is a magnified view ($20 \times 20 \mu\text{m}^2$)

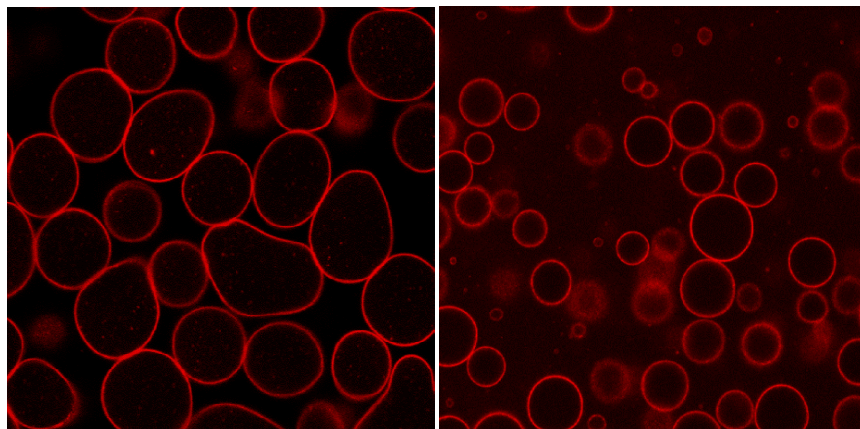


Figure 6 CSLM images ($160 \times 160 \mu\text{m}^2$) of creamed droplets of the PEO phase taken at the top of a P/D emulsion (left) and of sedimented droplets of the dextran phase taken at the bottom of a D/P emulsion (right). The emulsions were at pH 3.0 and contained 0.3 wt% microgels. The images were taken 2 days after preparation.

Partitioning of proteins between the two phases

As previously discussed in Chapter 1, the partition of the proteins between the two aqueous phases depends on the interaction between the proteins and the polymers in each phase, which in turn may depend on the hydrophobicity and surface charge of the proteins. We have found here that excess microgels partition preferentially to the dextran phase at pH=7.0 and to the PEO phase at pH=3.0. Figure 7 compares confocal images of PEO droplets in P/D emulsions containing 0.3% microgels at pH 7.0 and pH 3.0. It can be seen that aggregates of particles at the interface were clearly oriented towards the dextran at pH=7.0 and towards the PEO at pH=3.0. The surface exposed by the PEO droplets in P/D at pH 7.0 is comparable to the dextran droplets in D/P at pH 3.0. The contact angle will therefore be very different at the different pH values. Unfortunately, we were unable to measure the contact angle due to the small size of the protein particles.

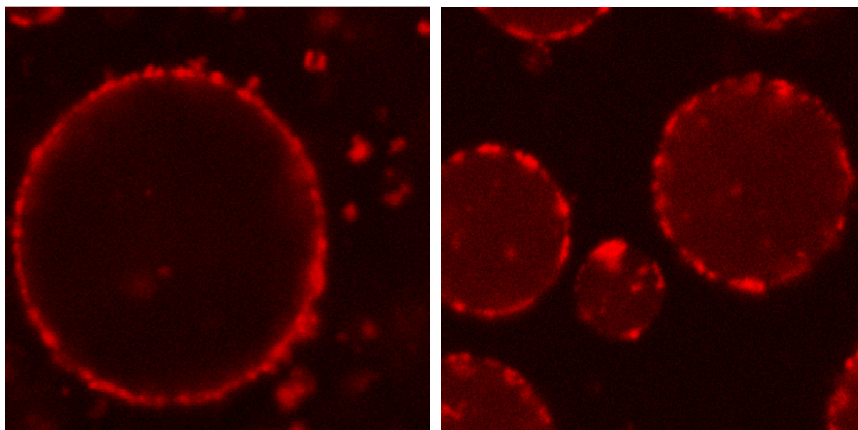


Figure 7 CLSM images ($30 \times 30 \mu\text{m}^2$) of PEO in dextran emulsions in the presence of microgels (0.3%) at pH 7 (left) and pH 3 (right).

In order to have more information about the partitioning of β -lg between the two phases we studied native proteins in PEO/dextran mixtures on the same tie line, but with approximately equal phase volumes at different pH (Figure 8). Contrary to aggregates, native β -lg is stable at all pH values between 9 and 3. Moreover native β -lg is too small to stabilize the dispersed droplets and therefore macroscopic phase separation is reached rapidly. The partition coefficient K was defined as the ratio of the protein concentration in the PEO phase to that in the dextran phase after phase separation. K varied with the pH and it was minimum at pH 5.0, i.e. near the isoelectric point of β -lg (Kharlamova et al. 2016). K also decreased when 0.1M NaCl was added at pH 7.0. The graph also shows that $K > 1$ at $\text{pH} < 4.0$ showing inversion of the partition of β -lg towards the PEO phase. This inversion is consistent with the different preference of the β -lg particles for the two phases at pH 7 and pH 3. Since neither dextran nor PEO are charged it one cannot attribute the inversion to different electrostatic interactions. It is possible that more hydrophobic aminoacids are exposed to the surface at low pH which would favor contact with the PEO.

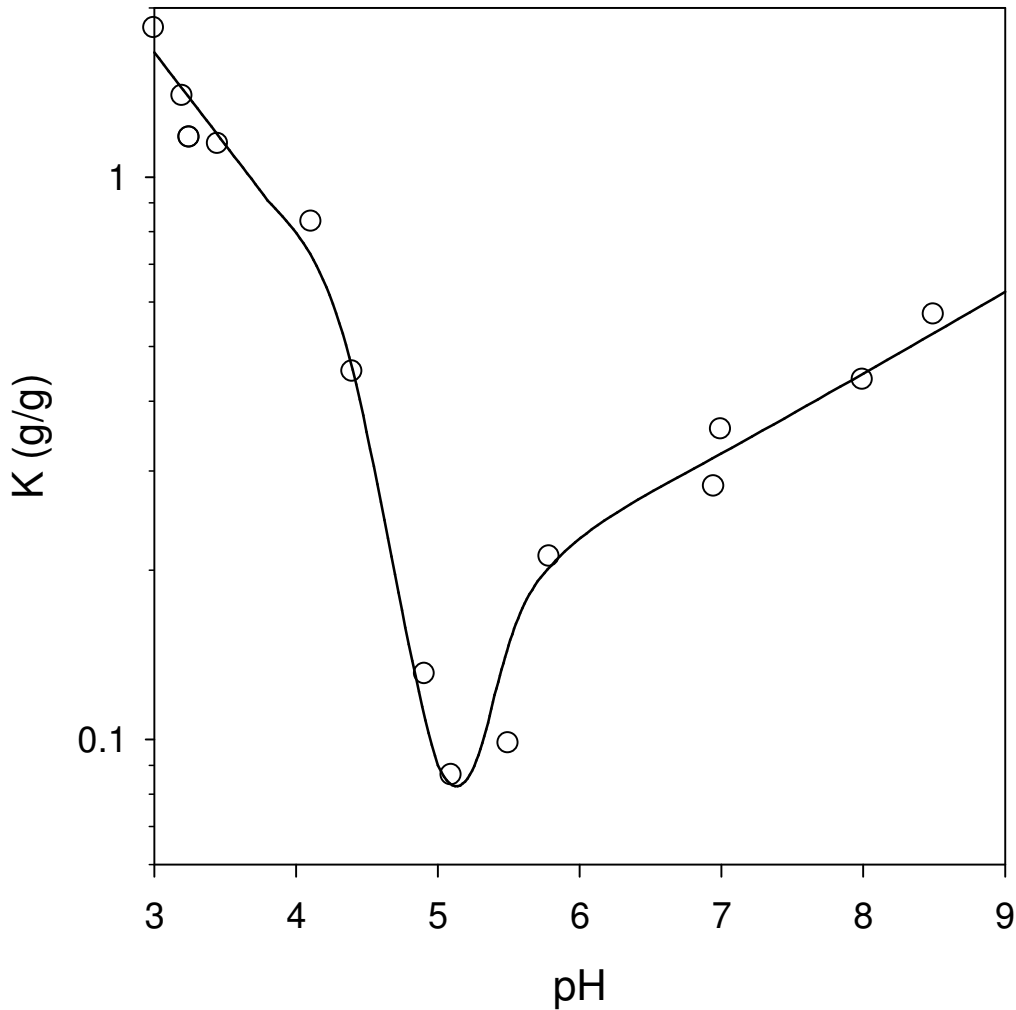


Figure 8 Partition coefficient of β -lactoglobulin in PEO and dextran mixtures as a function of pH

Conclusion

The difference in contact angle of the particles at the interface and the switch of protein partitioning may explain the different behavior of the P/D and D/P emulsions at different pH. It seems that more stable emulsions are formed when particles have a preference for the continuous phase. However, the behavior at pH 7.0 and 3.0 was not totally opposite. The stability is also affected by interactions between the proteins at the interface which depends on particle morphology and pH.

At same concentration, the number aggregates is higher for fractals than for the dense microgels particles. This should make fractal aggregates more efficient to stabilize the emulsions. On the other hand, the spatial conformation of the fibrils could explain the inhibition of creaming of P/D emulsions at pH 7.0. It is possible that they increased the effective viscosity even though samples could flow when tilting. It is expected that fibrils form a very thin layer at the interface being parallel oriented to it.

However, there are still some unanswered questions such the intriguing optimal concentration of fractals to stabilize P/D emulsions at pH 7.0 and the fact that they were the most efficient aggregates at pH 3.0 whereas fibrils were most effective for P/D at pH 7.0.

The three different morphologies of protein particles, spheres, fractal aggregates and rodlike fibrils were able to stabilize PEO and dextran mixtures too different extents depending on the conditions. At neutral pH fibrils stabilized P/D emulsions against coalescence better than microgels, whereas fractals were the less efficient. However, at pH 3.0 the same emulsions could not be stabilized by fibrils, but it was very stable when fractals were added. Contrary to neutral pH, where phase separation occurred very quickly for the three types of particles, stable D/P emulsions could be formed at pH 3.0. Significantly smaller drops were formed with fractals than with microgels and fibrils.

In conclusion, the stability of W/W emulsions formed by mixing dextran and PEO depends on the concentration of protein particles, their morphology, the preference of the proteins for each phase and their interaction with each other at the interface. It appears that when particles have a preference for the continuous phase, the emulsions are more stable.

Chapter 4 Results and discussion (2)

Cold gelation of W/W emulsions stabilized by protein particles

In the previous chapter we showed that β -lg microgels, rod-like fibrils and fractal aggregates were able to form stable W/W emulsions. Their stability and their structure depended on particle morphology, concentration and the partition of the particles between the phases, which varied with the pH. However, creaming or sedimentation of dispersed droplets occurs even if the droplets do not coalesce. Here we exploit the possibility to inhibit creaming by inducing excess protein particles to form a weak network in the continuous phase. Protein aggregates gel when the electrostatic repulsion between them is reduced either by reducing their net charge density by changing the pH or by screening electrostatic interactions by adding NaCl. This process of protein gelation is known as cold gelation and is discussed in Chapter 1.

Fractal aggregates and microgels have shown different efficiency to inhibit coalescence of dispersed droplets in PEO/Dextran mixtures. Here we will compare how cold gelation of excess protein particles with these two morphologies in the continuous dextran phase influences the stability and microstructure of PEO in Dextran emulsions. We will also show that cold gelation of excess protein aggregates in dispersed dextran droplets of dextran in PEO emulsions can be used to make micron size dense protein particles.

The same PEO and dextran mixture as was used in the study reported in Chapter 3 was used for this investigation. It contained 1.9 wt% PEO and 12 wt% dextran (Emulsion 2 in Figure 4 of Chapter 1). The dispersed PEO phase represented a volume fraction (Φ) of 25% at $C_{\text{PEO}} = 8.2$ wt% in a continuous dextran phase at $C_{\text{Dex}} = 15.8$ wt%. The interfacial tension between the two phases was $75 \mu\text{N/m}^2$ (Balakrishnan et al. (2012)). 0.5% of β -lg microgels or fractal aggregates were added to the mixture. As discussed in Chapter 3, the β -lg protein particles partition to the dextran phase when $\text{pH} \geq 4.0$. That means that the concentration in the dextran phase is up to 25% higher than 0.5%.

Effect of the pH

The net charge of β -lg aggregates is zero at the iso-ionic point (pH 5.0) (Kharlamova et al. 2016). Therefore the electrostatic repulsion between the aggregates is reduced when the pH is adjusted closer to pH 5.0. In order to observe the behavior of microgels and fractals by themselves they were added to pure water and to a dextran solution with same concentration as the continuous phase of the emulsions ($C_{\text{Dex}} = 15.8 \text{ wt\%}$) at different pH. The concentration of proteins was 0.67%, which represents the maximum concentration in the dextran phase of the emulsion if all the proteins partition to it. CLSM images of the fractals and microgels in the dextran solution are shown in Figure 1. At pH 6.5 and 3.5 the distribution of particles was homogeneous on length scales accessible to CLSM ($>0.1 \mu\text{m}$). Individual microgels are visible while fractal aggregates, due to their lower density, are not visible as individual particles. At pH between 6.0 and 4.0 both types of particles aggregated and formed dense clusters. Closer to the iso-ionic point, i.e., between 5.5 and 4.0 large protein flocs were formed. The same results were found in pure water (not shown) indicating that dextran had no impact on the behavior of the protein particles.

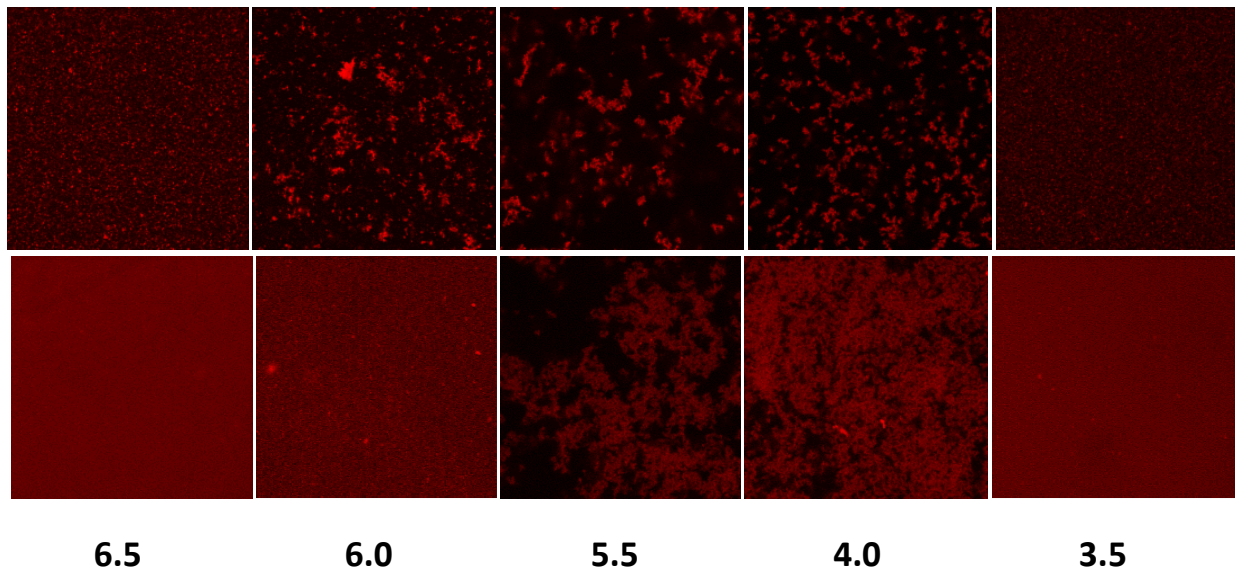


Figure 1 CLSM images ($160 \times 160 \mu\text{m}^2$) of microgels (top) or fractals (bottom) at $C_{\text{prot}} = 0.67\%$ in dextran solutions at different pH indicated in the figure ($C_{\text{dex}} = 16\%$)

Figure 2 shows emulsions of microgels and fractals at different pH 1 day and two weeks after mixing. There were clear differences between neutral pH and $\text{pH} \leq 6.0$ for fractals and microgels. In the case of fractals, a clear destabilized PEO layer was formed at pH 7.0. This layer was not present in the other samples indicating that at lower pH droplet coalescence was

prevented. No creaming was observed after two weeks between pH 5.0 and pH 4.0. Samples at pH 3.5, 5.5 and 6.0 showed slower creaming than at neutral pH. In the case of microgels, no destabilization of PEO was found for any of the samples after two weeks. The creaming speed and the volume of the final creamed layer was however different. At $\text{pH} \leq 6.0$ creaming was faster than at pH 7.0 and a creamed layer was visible within 24 hours. The layer had a volume fraction of 25% at neutral pH and larger at lower pH (especially at $\text{pH}=4.0$) where it reached steady state after a few days. It follows that the creamed PEO droplets were not close-packed at $\text{pH} \leq 6.0$. Another effect of the pH was that the bottom dextran layer in emulsions with microgels was turbid at pH 7.0 whilst it was clear for $\text{pH} \leq 6.0$, implying that very few microgels particles remained in this phase at $\text{pH} \leq 6.0$.

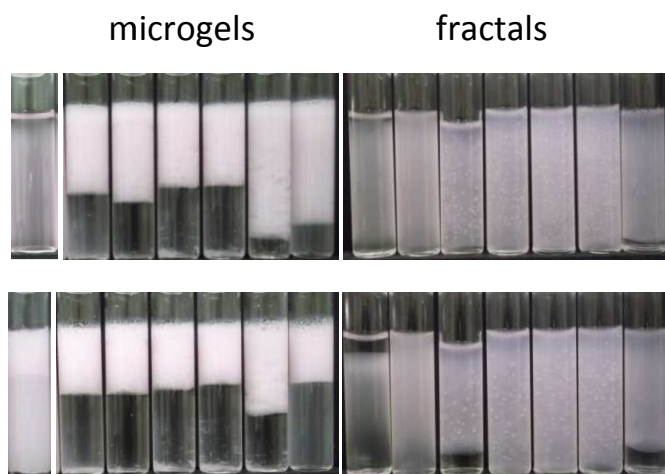


Figure 2 Visual appearance of PEO in dextran emulsions with microgels or fractals 1 day (top) and 2 weeks (bottom) after mixing. The pH of the emulsion was from left to right: 7.0, 6.0, 5.5, 5.0, 4.5, 4.0 or 3.5

The samples were turned upside down after two weeks and the emulsions with microgels did not flow when the pH was less than 6.0. Samples with fractal aggregates did not flow for a pH between 5.5 and 4.0. The gel that was formed by cold gelation in the continuous phase of these samples could support its own weight. However, the same amount of protein aggregates in a corresponding dextran solution did not form self supporting gels. It follows that the PEO droplets covered by a layer of aggregates was incorporated in the network and thereby reinforced the gel so that could support its own weight.

CLSM images of the emulsions taken right after mixing are shown in Figure 3. Droplets of PEO showed similar diameters between pH 6.0 and 4.0 ($13 \pm 3 \mu\text{m}$). Smaller drops were formed at

pH 7.0 ($6 \pm 1 \mu\text{m}$) and pH 3.5 ($10 \pm 2 \mu\text{m}$). A layer of microgels was clearly visible around the drops for all samples. The density of proteins at the interfacial layer increased with decreasing pH as the amount of excess microgels decreased. Some drops, however, showed incomplete coverage at pH 5.0 and 4.5. At pH 4.0 some of the drops were not spherical most likely because the interfacial layer gelled before the drops could relax. The excess microgels were found in the dextran phase for pH > 4.0 and in both phases for pH 3.5 confirming the change in the partition of $\beta\text{-Ig}$ that was shown in Chapter 3.

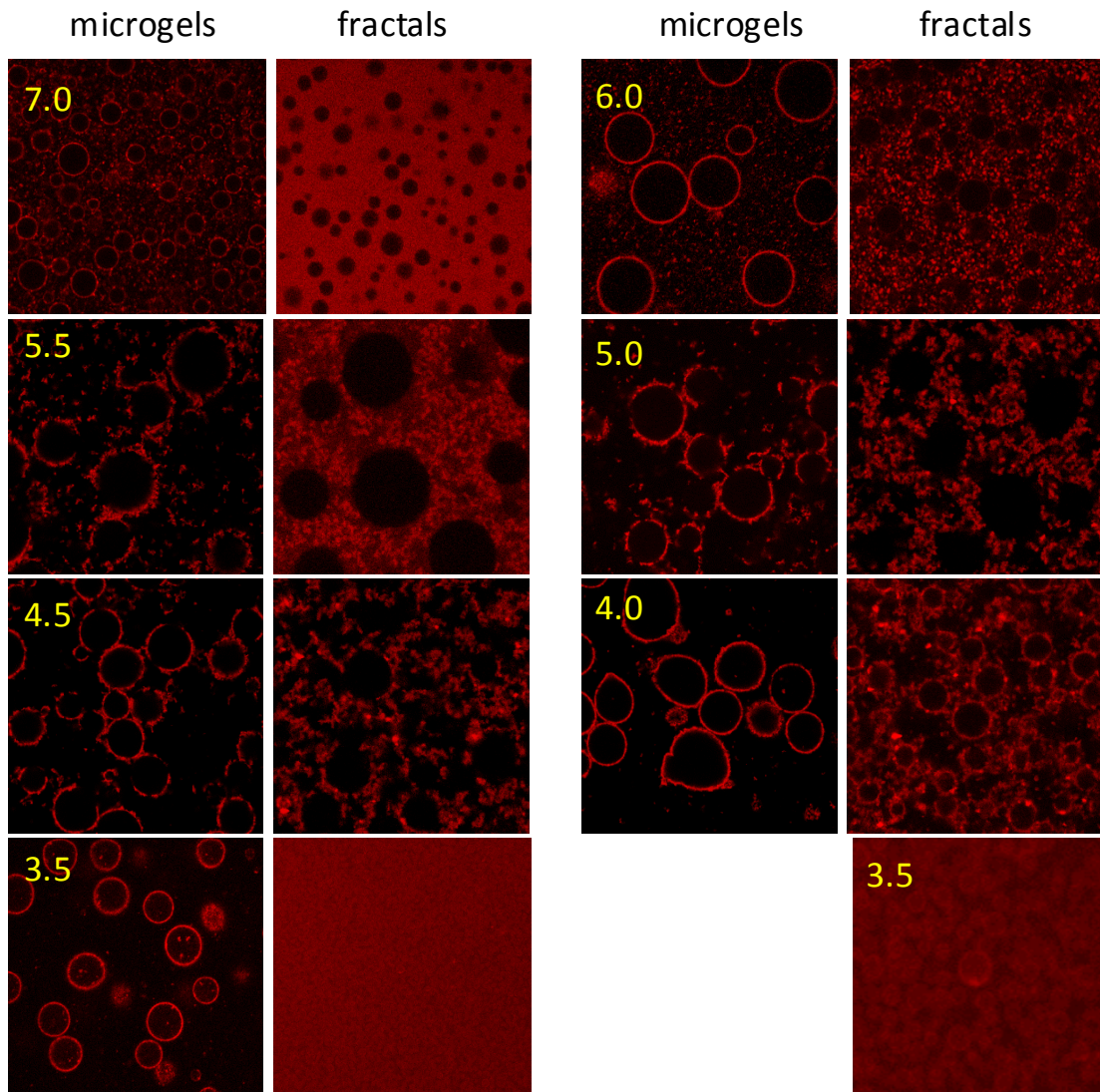


Figure 3 CLSM images ($160 \times 160 \mu\text{m}^2$) of PEO in dextran emulsions with microgels or fractals at different pH as indicated in the figure. The image on the bottom right represents a zoom of the system containing fractals at pH 3.5 ($40 \times 40 \mu\text{m}^2$)

It can be seen that microgels aggregated at pH between 5.5 and 4.5, which caused flocculation of the droplets. It was not that clearly visible for pH 6.0, 4.0 and 3.5, but the faster creaming of the droplets compared with that at pH 7.0 suggests that flocculation also occurred. Creaming finished once the flocculated droplets formed a percolating network, resisting the buoyancy force. Aggregation of excess microgels was also responsible for the difference in the turbidity of the dextran layer between pH 7.0 and $\text{pH} \leq 6$. At $\text{pH} \leq 6$ practically all the microgels creamed together with the PEO drops while at pH 7.0 they remained in the dextran bottom phase.

Between pH 5.5 and pH 4.5 aggregation of microgels is faster as their charge density is smaller. This is likely the reason for the presence of droplets that were not fully covered. The particles probably did not have enough time to move to the interface before forming clusters. The clusters avoided that the uncovered parts reached each other and thereby inhibited coalescence of the droplets.

In the samples where fractal aggregates were added, the interfacial layer at the droplets was not visible for pH 7.0 and 3.5. The lower contrast is consequence of lower fluorescence because their density is low and because the concentration of excess fractals in the continuous phase is higher.

The average droplet diameter was maximum at pH 5.5 and decreased at higher and lower pH: $4.5 \pm 1 \mu\text{m}$, $6.5 \pm 1 \mu\text{m}$, $19 \pm 4 \mu\text{m}$, $14 \pm 3 \mu\text{m}$, $12 \pm 2 \mu\text{m}$, $7.5 \pm 1 \mu\text{m}$ and $1.4 \pm 0.2 \mu\text{m}$ for pH 7.0, 6.5, 5.5, 5.0, 4.5, 4.0 and 3.5, respectively.

Clusters of fractals at the interface can be seen between pH 6.0 and pH 4.0. A system spanning network was formed in the dextran phase by the excess fractals, which inhibited creaming of the dispersed droplets. This network that incorporated the protein covered droplets was strong enough to support its own weight when tilting the samples.

At pH 3.5 cold gelation did not occur and the droplets were not aggregated in clusters as was the case for pH 4.0. However, the droplets size was considerably smaller than at pH 7.0 which most likely related to changes of the β -lg partition associated with the pH.

Effect of the salt concentration

A second manner to induce gelation of protein aggregates is to screen interactions by adding NaCl at pH 7. In order to observe the behavior of the aggregates by themselves we have added 0.1M and 0.3M NaCl a mixtures of microgels and fractals ($C_{\text{PROT}} = 0.67\%$) in dextran solutions at $C_{\text{dex}} = 15.8\%$. CLSM images show that the suspensions remained homogeneous with 0.1M, but protein flocs were formed after adding 0.3M NaCl, see Figure 4.

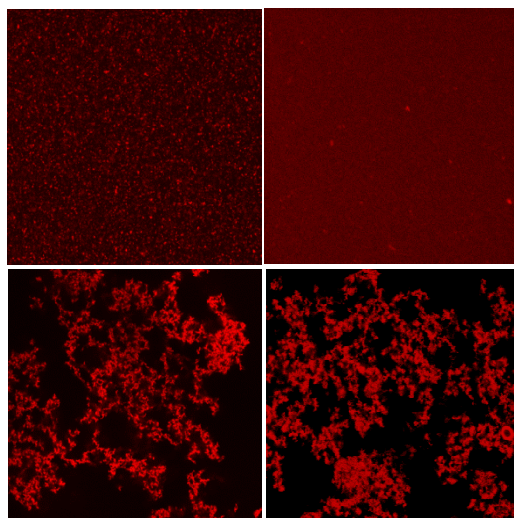


Figure 4 CLSM images ($160 \times 160 \mu\text{m}^2$) of microgels (left) or fractals (right) at $C_{\text{prot}} = 0.67\%$ in dextran solutions at 0.1 M (top) or 0.3 M (bottom) NaCl ($C_{\text{dex}} = 16\%$, pH 7.0)

Figure 5 shows the same PEO in dextran emulsions that were used to study the effect of the pH 1 day and two weeks after mixing. The systems with 0.1M NaCl were similar to the samples without salt. However, there were clear differences for emulsions with 0.3M NaCl. No destabilized layer of the PEO phase was formed and creaming was significantly slower for fractal aggregates. No creaming was observed for microgels. The samples were tilted after two weeks showing that the emulsions with 0.1 M NaCl flowed while those at 0.3M did not. However, application of a small mechanical stress was enough to break the gel.

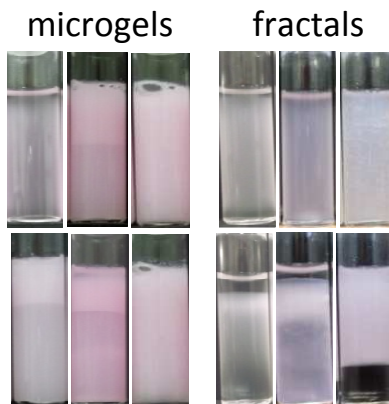


Figure 5 Visual appearance of PEO in dextran emulsions at pH 7.0 with microgels or fractals 1 day (top) and 2 weeks (bottom) after mixing. The emulsion contained from left to right: no salt, 0.1 M or 0.3 M NaCl.

CLSM images of the emulsions with 0.1M and 0.3M NaCl right after mixing are shown in Figure 6. The droplet size did not vary in the presence of microgels ($7.5 \mu\text{m} \pm 3 \mu\text{m}$), but increased in the presence of fractals ($5.5 \mu\text{m} \pm 2 \mu\text{m}$ at 0.1 M and $9 \mu\text{m} \pm 3 \mu\text{m}$ at 0.3 M). Proteins aggregation is clearly visible at 0.3M NaCl. A system spanning network was rapidly formed by the microgels which prevented creaming of the PEO droplets. The PEO droplets creamed in the presence of fractals until the excess proteins formed a network that resisted the buoyancy force.

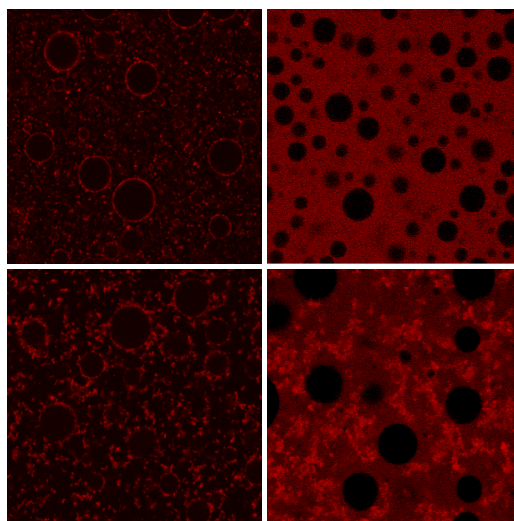


Figure 6 CLSM images ($160 \times 160 \mu\text{m}^2$) of PEO in dextran emulsions at pH 7.0 with microgels (left) or fractals (right) at 0.1 M (top) or 0.3 M (bottom) NaCl

A detailed study on cold gelation of fractals aggregates at neutral pH and 0.3 M NaCl was reported by (Ako et al. 2010). In Figure 7a we compare the evolution of G' at 0.1 Hz as function of time for 0.3M NaCl and 0.67 % fractals in water and in dextran ($C_{\text{Dex}} = 15.8 \text{ wt}\%$) with the emulsion containing 0.5 % fractals. Very weak gels were formed in all cases. The elastic modulus increased slowly with time at 20 °C. At same time, G' was higher in dextran than in pure water perhaps due to depletion interactions. The emulsion gelled quite rapidly even though the elastic modulus was also very low. Steady state was not reached within 100 min. Figure 7b shows a comparison of the emulsion containing 5, 10 and 20 g/L fractal aggregates. Increasing the protein concentration resulted in stronger gels. Frequency dependent measurements at the end of the experiment showed in all cases that G' depended weakly on the frequency and was larger than G'' (except fractals in dextran for frequency $> 10 \text{ Hz}$) confirming that gels were formed (Figure 8).

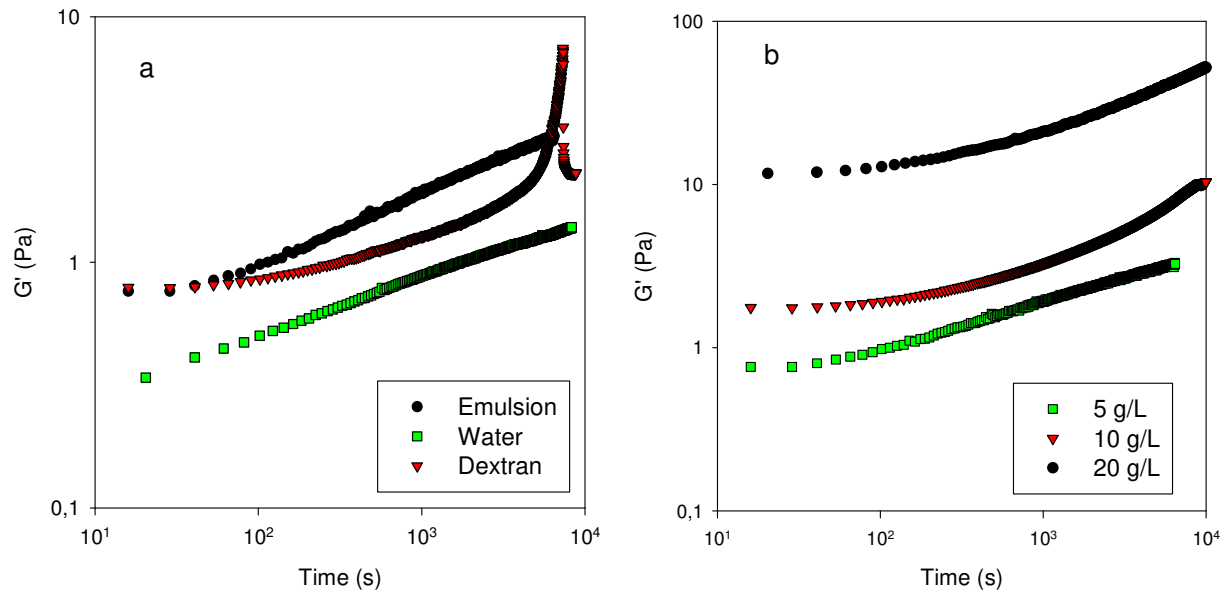


Figure 7 Storage shear modulus at 0.1 Hz as a function of time at 20°C. Fig. 7a compares the evolution of a PEO in dextran emulsion with that in a dextran solution at the same concentration as in the dextran phase of the emulsion and with that in water. The fractal protein aggregate concentration was 0.5 g/L in the emulsion and 0.67 g/L in water and the dextran solution. Fig 7b shows the evolution of G' for emulsions with three different protein concentration.

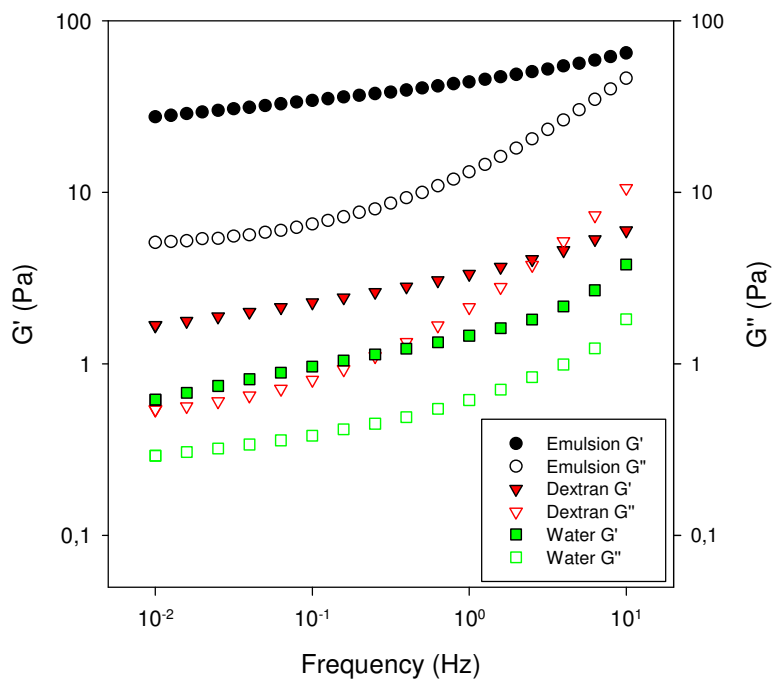


Figure 8 Shear storage and shear loss moduli as function of the frequency (Hz) at 20°C. The fractal protein aggregate concentration was 0.5 g/L in the emulsion and 0.67 g/L in water and the dextran solution

Cold gelation greatly influences the behavior and microstructure of the emulsions. The kinetics of this process is key for the evolution of the sample. Creaming can be prevented if a gel that is strong enough to resist buoyancy is formed relatively fast. If the gelation is too fast, some proteins have no time to reach the interface and drops are not completely covered. Nevertheless, gelation of the continuous phase also stabilizes these emulsions, but droplets are easily deformed by the elastic forces of the surrounding gel.

As was discussed in Chapter 3, the morphology of the proteins plays a role in the formation and evolution of the emulsions. Therefore it is not surprising that emulsions with fractals and with microgels behaved differently for the same concentrations and conditions. Creaming was completely avoided with fractal aggregates at pH between 5.5 and 4.0 but not with microgels. On the other hand, addition of 0.3M NaCl prevented completely creaming with microgels but not with fractals.

The speed of cold gelation depends on the salt concentration, pH, the size, morphology and concentration of protein particles. The structure and evolution of the emulsion can therefore be tuned by varying the amount of salt or the pH as well as by using protein particles with different morphologies.

Cold gelation of the dispersed phase

An emulsion with the same PEO and dextran concentrations in the two phases and therefore the interfacial tension, but a volume fraction of dextran of 25% (Emulsion 6 in Figure 4 of Chapter 1) was used to study cold gelation of the dispersed phase. Figure 9 shows CLSM images taken after mixing of the emulsion with fractals and microgels at different pH. For all pH values except 3.5 rapid flocculation and sedimentation of dextran droplets occurred. The excess proteins were located at the dextran phase for $\text{pH} > 4$ and in both phases at $\text{pH} 3.5$. A ring of microgels around spherical dextran drops was clearly observed at $\text{pH} 7.0$ and 3.5 . Less spherical drops filled with clusters of microgels are seen for $\text{pH} 6.0$ and 5.5 . At $\text{pH} 5.0$ and 4.5 irregular domains with sizes of tens of microns of dextran containing flocs of microgels were formed. At $\text{pH} 4.0$ a dense layer of microgels at the interface can be seen. Some drops had spherical shapes while others seemed to be the result of the coalescence of smaller drops that could not relax to the spherical shape.

Emulsions containing fractal aggregates formed quite spherical dextran droplets at all pH except 5.0 and 4.5. The non-spherical drops likely resulted from the rapid formation of a protein network within the droplet that was strong enough to withstand the interfacial tension.

At pH 7.0, 6.0 and 3.5 the droplets size did not vary significantly and was similar in the presence of fractals and microgels ($6 \mu\text{m} \pm 2 \mu\text{m}$).

Rapid flocculation and sedimentation of dextran droplets was also observed in the presence of 0.1 M and 0.3 M NaCl at pH 7.0. CLSM images of the emulsions are shown in Figure 10. No major effect of adding 0.1M NaCl was observed, but at 0.3M irregular protein flocs were formed in the presence of microgels while dense flocs of fractals were located inside the dextran drops. It is likely that the microgels aggregation was so fast that spherical dextran domains could not be formed.

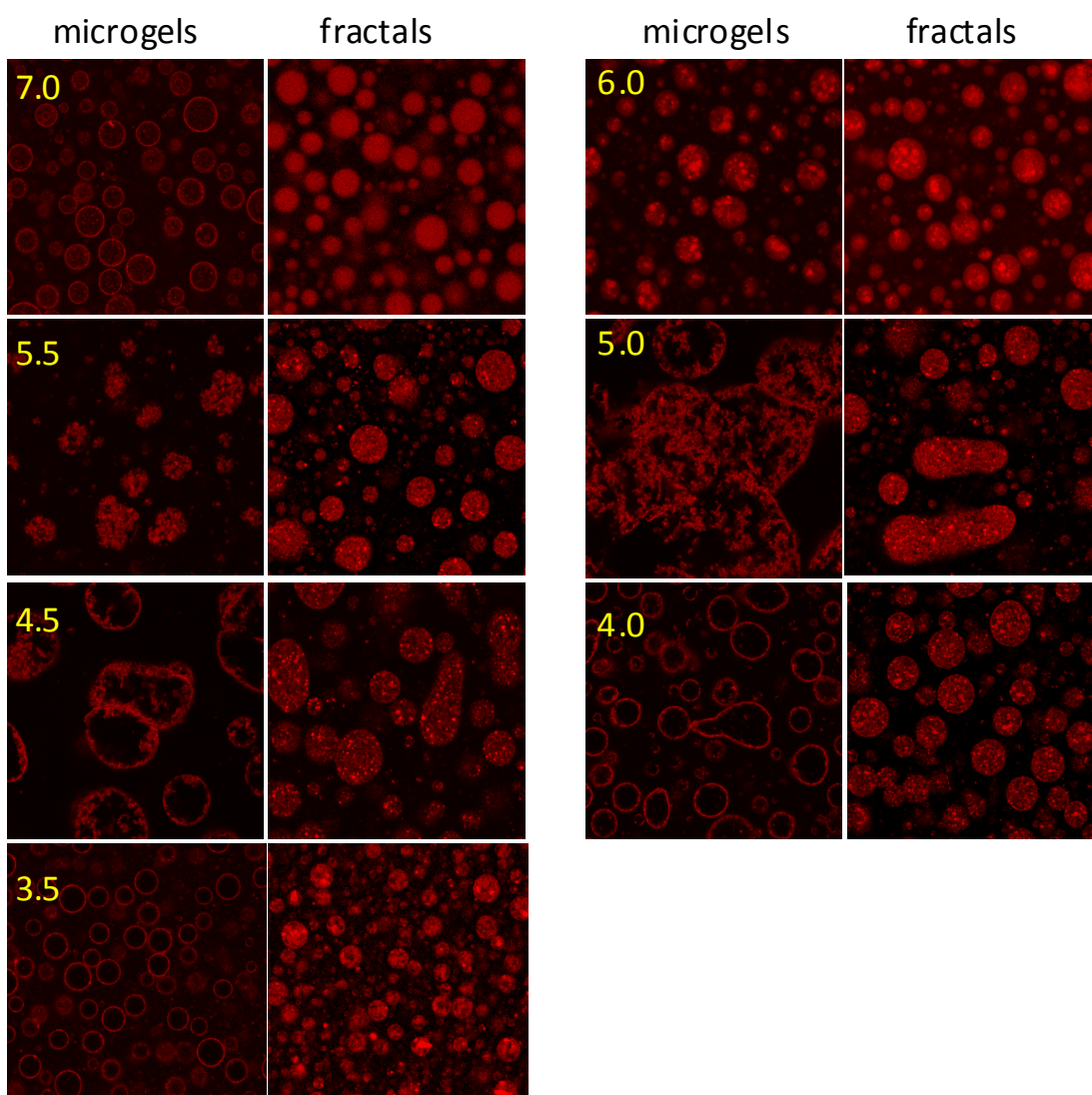


Figure 9 CLSM images ($160 \times 160 \mu\text{m}^2$) of dextran in PEO with microgels or fractals at different pH as indicated in the figure.

It appears that aggregation of proteins within the dispersed phase of a W/W emulsion can be used to form dense protein microparticles without the inconvenience of having to remove the oil phase if a water in oil emulsion is used (Sağlam et al. 2011; Zhang & Zhong 2010).

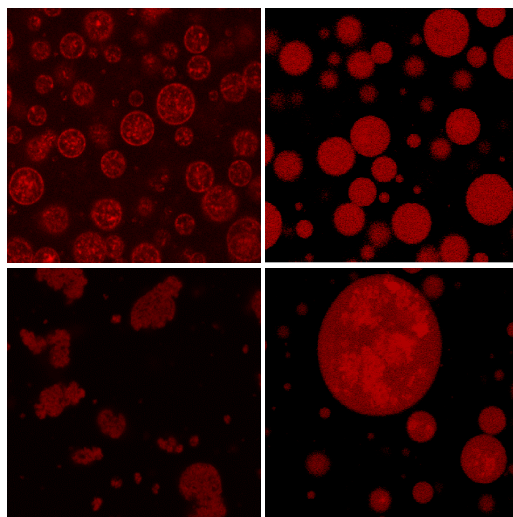


Figure 10 CLSM images ($160 \times 160 \mu\text{m}^2$) of dextran in PEO emulsions at pH 7.0 with microgels (left) or fractals (right) at 0.1 M (top) or 0.3 M (bottom) NaCl

Emulsions with a low volume fraction of dextran (2.5%) were used in order to form small droplets containing 0.5% fractals at pH 7.0 (Figure 11). Gelation of the fractals was induced by adding a divalent salt (CaCl_2) instead of NaCl, which induces gelation at much lower salt concentrations. Different molar ratios of calcium to protein (R) and different aggregation times were employed. Subsequently, the samples were diluted 10 times to below the binodal so that dextran and PEO no longer phase separated.

Protein microparticles formed by aggregation of fractal aggregates could be seen. Only 15 min were needed to form particles $R \geq 2$. These particles were however less dense than particles formed after 1h 30 min and fell apart upon dilution (Figure 12). We verified that after dilution the particles were free of dextran by using fluorescently labeled dextran (data not shown). The structure of the particles depended on the R and time before dilution. In order to obtain particles with the most spherical shape possible and a size of few microns it was important to finely tune these parameters.

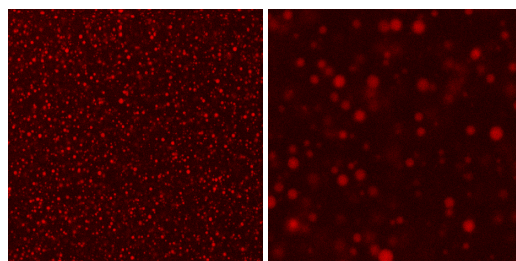


Figure 11 CLSM images (500x500 μm^2 left; 160x160 μm^2 right) of dextran in PEO emulsions (PEO/Dex (ϕ): 97.5/2.5) with 0.5% of fractal aggregates

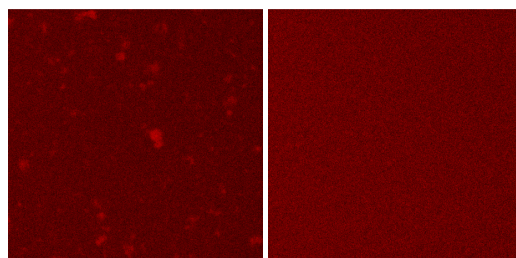


Figure 12 CLSM images (160x160 μm^2 right) of dextran in PEO emulsions (PEO/Dex (ϕ): 97.5/2.5) with 0.5% of fractal aggregates, R2 and aggregation time of 15min. Left: before dilution; right: after dilution in pure water (1/10)

Particles that were formed during 1h30min and 4h were centrifuged during 30 min at 12.000 rpm after dilution by a factor 10. The residual fraction of proteins in the supernatant was determined by UV adsorption. Table 1 shows the fraction of particles that precipitated. After 1h 30 min a ratio of $R = 1.5$ was enough to form stable particles. Nevertheless the fraction of particles that precipitated was very low (14%). At $R = 3$, 60% of the fractals formed stable particles. Larger fractions of protein in the form of stable particles were obtained at the same R after 4 h (79% at $R = 3$).

CLSM images of the samples summarized in Table 1 after redispersion of the sedimented particles in pure water are shown in Figure 13. The particles were not the same in terms of size and sphericity. We found the best compromise between size, spherical shape and amount of particles was for particles formed after 1h 30 min and $R = 3$.

Table 1 Fraction of particles formed as function of calcium R and time

Aggregation time	R	Fraction of particles
1h 30min	1,5	0.14
1h 30min	3	0.60
4h	1	0.17
4h	1,5	0.26
4h	2	0.54
4h	3	0.79

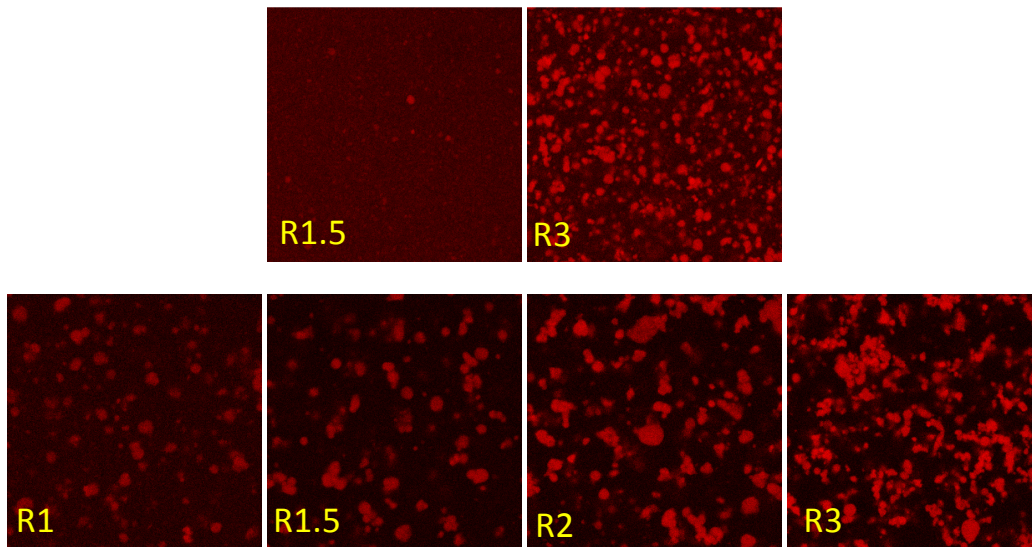


Figure 13 CLSM images ($160 \times 160 \mu\text{m}^2$ right) of particles prepared in dextran in PEO emulsions (PEO/Dex (ϕ): 97.5/2.5) with 0.5% of fractal aggregates after aggregation for 1h30min (top) and 4h (bottom) followed by dilution, centrifugation and redispersion in pure water. Calcium ratios (R) are indicated in the figure.

Conclusion

Cold gelation of microgels and fractal aggregates can be induced in PEO/dextran mixtures by modifying the pH between 6.0 and 3.5 or by adding salt at pH 7.0. Cold gelation modified the structure and evolution of the mixtures and, depending on the rate of gelation, creaming and sedimentation can be avoided or will be accelerated. It causes the interfacial layer of protein particles to become denser and leads to flocculation of droplets that increases their creaming or sedimentation rate until a network is formed in the continuous phase that withstands the buoyancy force. When the gelation was very fast, for example for fractals near the iso-ionic point, the droplets were not fully covered but the gel formed by the excess particles in the continuous phase was strong enough to avoid coalescence and creaming. These gels could support their own weight when turned up side down. The elastic modulus of the gels formed by protein aggregates slowly increased with time and was at a given time higher for the emulsions and dextran solutions than for pure water. Fractals were more efficient to avoid creaming at pH 6.0 and 4.0 while microgels were more efficient at pH 7.0 and 0.3M NaCl. Dense protein micro particles can be formed by cold gelation of dextran in PEO emulsions when the protein aggregates are partitioned to the dispersed dextran. By careful tuning the aggregation conditions stable spherical dense protein microparticles can be formed with a high yield.

Chapter 5 Results and discussion (3)

Modulating the stability of W/W emulsions by native whey proteins

Polystyrene-based latex particles have been already been trapped at the droplet interface of PEO/ dextran W/W emulsions. However the particles did not stabilize the emulsions and no difference was observed in the speed of phase separation (Balakrishnan et al. 2012). Adsorbing molecules at the surface of particles has already been shown to modulate their efficacy as was discussed in Chapter 1. Here we investigated the capacity of adsorption of native whey proteins onto the latex particles to modulate their efficacy to stabilize the emulsions. Modulating the stability of W/W emulsions could find applications in drug delivery, controlled release, microreactors and so on.

It has also been previously discussed that native whey proteins are not capable to form an interface in PEO/Dextran mixtures so no competition between the latex particles and the free native proteins will take place.

Mixtures of PEO and dextran from the same tie-line (containing 8.2 wt% PEO and negligible dextran concentration in the PEO phase and 15.8 wt% dextran with negligible PEO concentration in the dextran phase) were prepared as described in Chapter 2. Polystyrene-based spheres (Fluoresbrite® Polychromatic Red Microspheres, batch 690690) with a diameter of 0.5 μ m were added to emulsions of dispersed dextran droplets in a continuous PEO phase (D/P). The composition of emulsion A was $C_{PEO} = 7.3\%$, $C_{Dex} = 2.25\%$ with volume fractions (Φ) of 87% and 13%, respectively and of emulsion B it was $C_{PEO} = 6.3\%$, $C_{Dex} = 4\%$ (Φ : 75% and 25%, respectively). Latex particles were added to the emulsions at concentration of $6.25 \cdot 10^{-3}$ g/ml ($0.91 \cdot 10^{11}$ particles/ml) for A and $8.75 \cdot 10^{-3}$ g/ml ($1.27 \cdot 10^{11}$ particles/ml) for emulsion B. The excess particles partitioned to the PEO phase. Native WPI was added at $C_{WPI} = 0.01$ and 0.1 g/L for emulsion A and 0.1 g/L for emulsion B. The fraction (R) of latex surface that can potentially be covered by a monolayer of WPI can be calculation as described in Chapter 2. $R=0.05$ and $R=2$ for emulsion A and $R=4$ for emulsion B.

The samples that did not contain WPI presented complete phase separation after 48h. A clear layer of the dextran phase (labeled with FITC dextran) was visible at the bottom of the tubes, see Figure 1. However, when WPI was added to the mixtures complete phase separation did not occur after a week. A coverage as low as $R = 0.05$ was enough to stabilize emulsion A, but

the dextran drops of this system were so big that they could be seen by naked eye. Smaller drops were formed in mixtures containing more WPI (See: Figure 2).

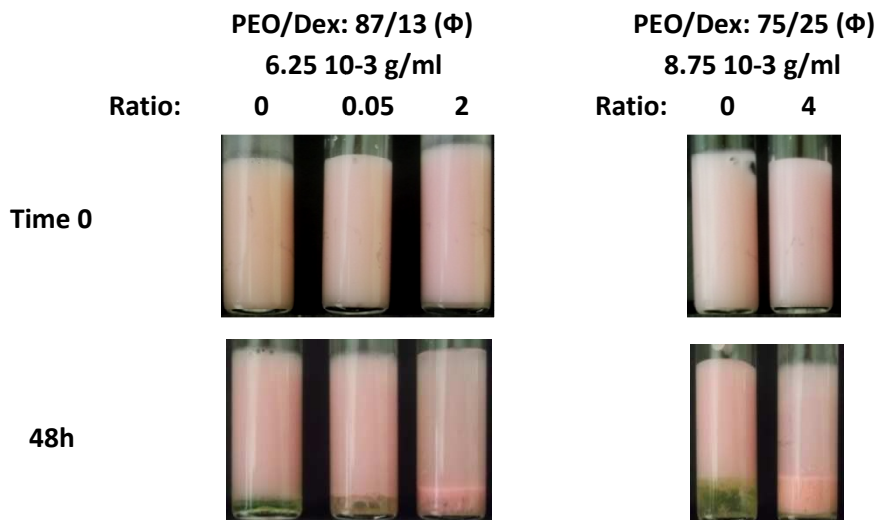


Figure 1 Photographs of dextran in PEO emulsions (PEO/Dex Φ : 87/13 left; PEO/Dex Φ : 75/25 right) after mixing and after 48h of containing latex and different amounts of WPI

Reducing the volume fraction of the dextran phase resulted in the formation of smaller drops in the presence of excess WPI. Moreover, trials with increasing amount of latex particles keeping R constant showed that a lower amount of particles (from $8.75 \cdot 10^{-3}$ g/ml to $2.5 \cdot 10^{-3}$ g/ml) was required to stabilize the mixtures when the volume fraction of dextran was reduced from 25% to 5%.

Effect of the protein concentration in dextran-in-PEO emulsions

The effect of the protein concentration on the efficacy of the latex particles to stabilize the emulsions was studied for dextran in PEO emulsions (PEO/Dex Φ : 95/5) with $2.5 \cdot 10^{-3}$ g/ml particles (0.36×10^{11} particles/ml). Figure 2 shows the evolution in time of the emulsions in the presence of WPI at different concentrations corresponding to R: 0, 0.05, 2, 4, 8, 16, 24, 32, 48, 64 and 128. With time a small bottom layer of sedimented dextran droplets was observed, which was difficult to observe at lower R due to the high turbidity of the continuous PEO top layer caused by the presence of excess latex. The turbidity of the PEO phase at steady state decreased with increasing R, see Figure 2. This indicates that less excess particles were located in this phase.

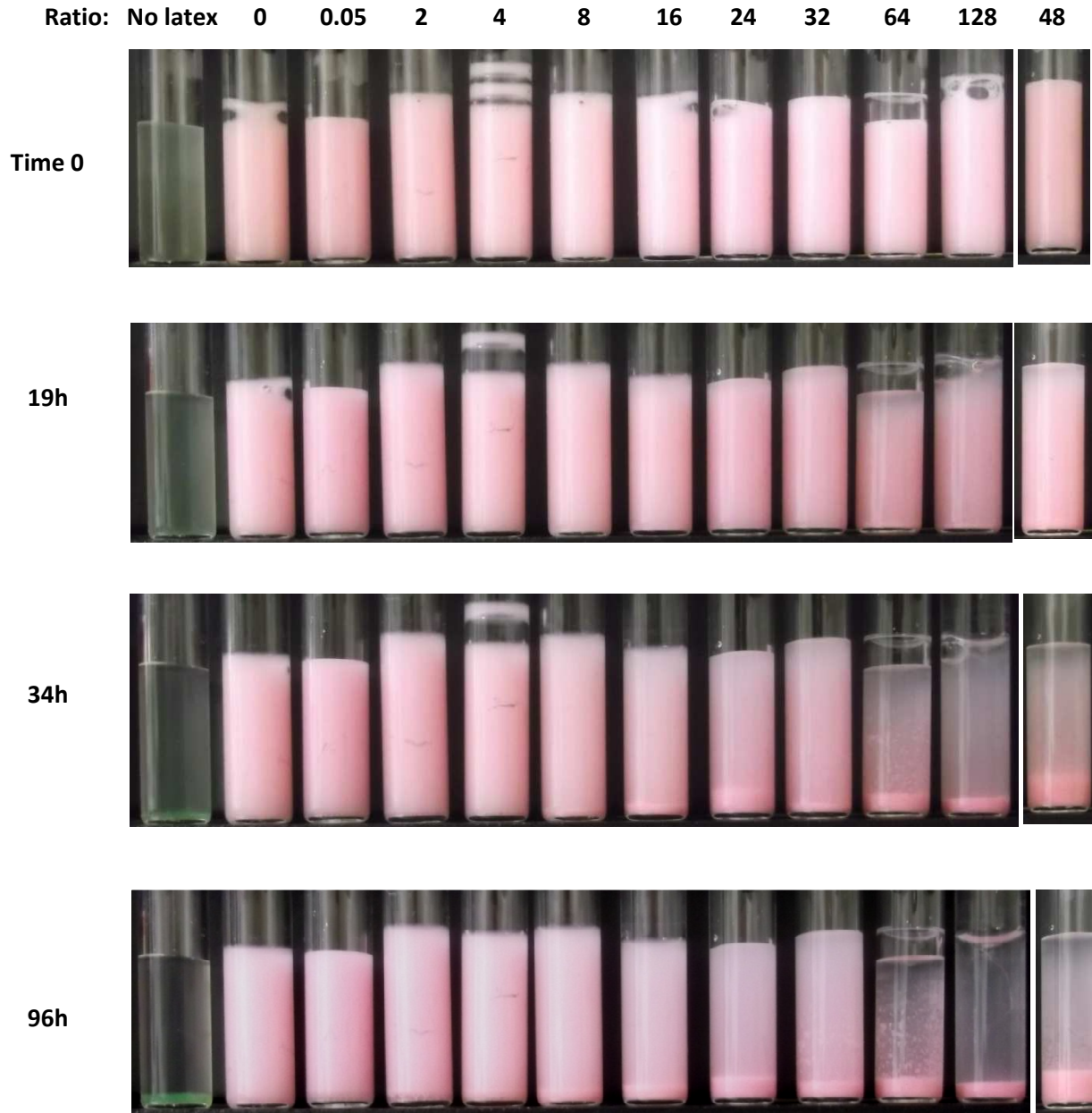


Figure 2 Photographs at different times after mixing dextran in PEO emulsions (PEO/Dex Φ : 95/5) containing $2.5 \cdot 10^{-3}$ g/ml of latex particles and different ratio of WPI (from left to right: 0 and no latex, 0, 0.05, 2, 4, 8, 16, 24, 32, 64, 128, 48)

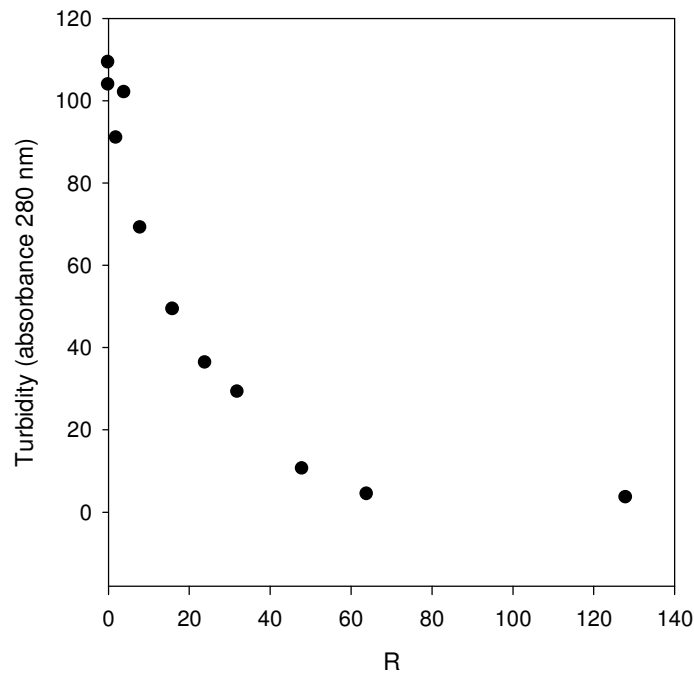


Figure 3 Turbidity (absorbance at 280 nm) after a week of the top PEO phase of the dextran in PEO emulsions (PEO/Dex Φ : 95/5) containing 2.5 10⁻³ g/ml of latex particles and different ratio of WPI

Confocal laser scanning microscopy (CLSM) images of the emulsions were taken 5-10 minutes after mixing (Figure 4). It can be observed that at lower R there was very low coverage, but for $R > 24$ the amount of latex particles at the interface increased with increasing R. For $R \geq 64$ hardly any particle was observed in the PEO continuous phase. We also imaged the sedimented layer at the bottom of microscope slides that were kept in vertical position after reaching steady-state (Figure 5). They showed that between $R = 0.05$ and $R = 64$ a layer of close packed dextran droplets stabilized by latex particles had formed, whereas at $R = 0$ and $R \geq 128$ a continuous dextran layer had formed. The latex particles were preferentially located at the PEO phase for $R = 0$ whilst aggregates of latex particles were observed at the dextran phase for $R \geq 128$. Large dextran drops are formed for $R = 0.05$, but the size progressively decreased with R up to $R = 32$. The average drop ratios were 103 ± 25 , 74.1 ± 10 , 67.7 ± 13.5 , 51 ± 8.6 , 47 ± 6.7 , 18.8 ± 2.3 and $17.5 \pm 1.5 \mu\text{m}$ for R 0.05, 2, 4, 8, 16, 24 and 32, respectively. For $R > 24$, drops could not relax completely to the spherical shape after coalescence indicating that the protein covered latex particles had formed an elastic layer at the surface.

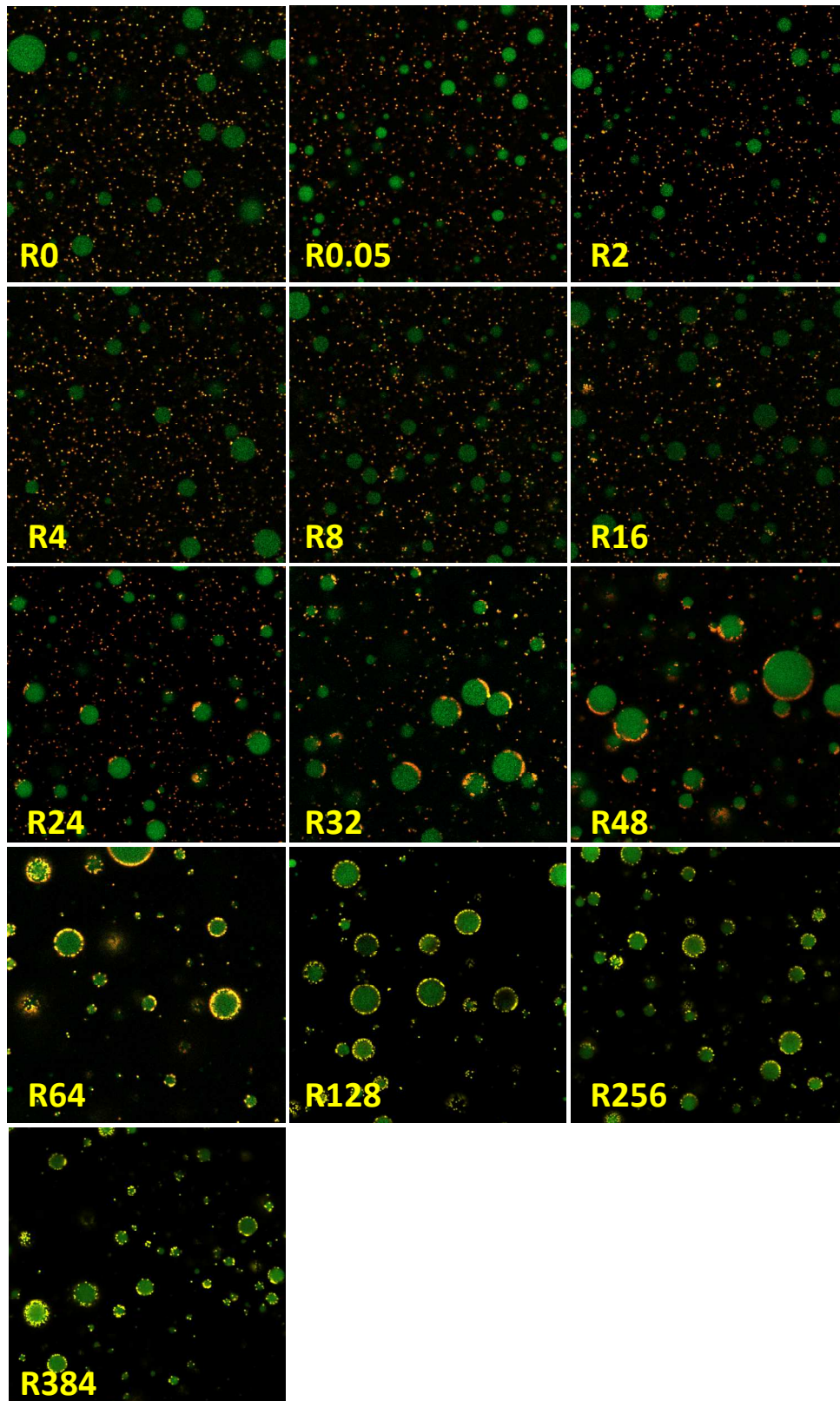


Figure 4 CLSM images ($100 \times 100 \mu\text{m}^2$) taken right after mixing the dextran in PEO emulsions (PEO/Dex ϕ : 95/5) containing $2.5 \cdot 10^{-3}$ g/ml of latex particles and amounts of WPI as indicated in the figure

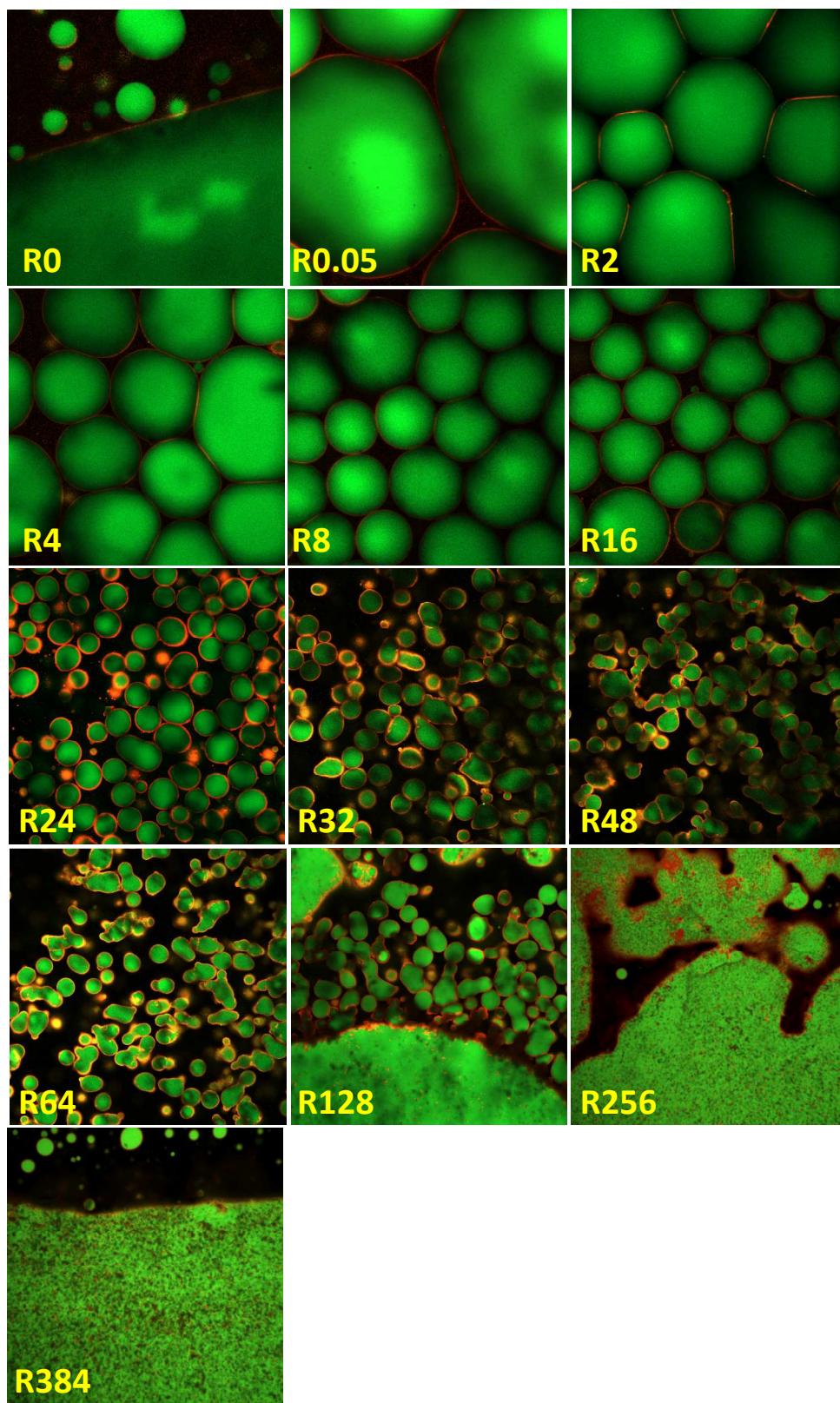


Figure 5 CLSM images ($512 \times 512 \mu\text{m}$) taken at steady state of the bottom of dextran in PEO emulsions (PEO/Dex Φ : 95/5) containing $2.5 \cdot 10^{-3}$ g/ml of latex particles and different ratio of WPI as indicated in the figure

In the absence of proteins the latex particles did not adsorb at the PEO/dextran interface, because they preferred to be in the PEO phase. This was different for latex particles used by Balakrishnan et al. (2012) that did adsorb to the interface though this did not lead to stabilization. Most likely the difference is caused by a different surface chemistry. The exact chemistry and characteristics of the surface of the latex particles were not provided by the supplier. In fact, comparing different batches of commercial particles from the same supplier gave different results in terms of their stability as a function of the WPI concentration. The latex particles used here showed a large preference for the PEO phase and adsorbed to the PEO/dextran interface only if they were covered with proteins. The reason is that proteins prefer the dextran phase over the PEO phase and therefore adsorbed proteins increased the affinity of the latex particles for the dextran phase. The very small amount of proteins that adsorbed did not allow accurate measurements of the amount of adsorbed to the latex particles at the different ratios. Nevertheless, it is clear that the latex particles were increasingly covered with proteins with increasing protein concentration increasing their propensity to adsorb at the PEO/dextran interface. As a consequence the affinity for the dextran phase increased with increasing protein concentration and for $R > 64$ all excess latex particles were situated in the dextran phase. This may explain the observed instability of the emulsions observed for $R > 64$. We stress that native whey proteins do not adsorb at the interface and are unable to stabilize the emulsion.

Modification of the affinity of particle for the two phases in W/W emulsions by modifying the particles surface was already described by de Freitas et al. (2016) for mixtures of amylopectin and xyloglucan in the presence of protein microgels. Xyloglucan adsorbed onto β -Ig microgels when the pH was lowered inverting their partition from the amylopectin to the xyloglucan phase. As a consequence, microgels adsorbed at the interface and stabilized the W/W emulsion at lower pH.

Effect of latex concentration in dextran-in-PEO emulsions

The effect of the latex concentration on their efficacy to stabilize the emulsions was studied for dextran in PEO emulsions (PEO/Dex Φ : 95/5) at $R=16$. Figure 6 shows the time evolution of the emulsions at different amounts of latex particles. In all cases a bottom layer of sedimented dextran droplets is formed. Increasing the amount of latex particles increased the turbidity of the continuous PEO phase, indicating a progressively greater amount of excess particles was localized in that phase.

Figure 7 shows the CLSM images of the emulsions a few minutes after mixing and at steady state. Initially no differences in the dextran droplet coverage can be seen, but an increase of

particle concentration in the PEO phase can be clearly observed. Images at steady state show that the size of the droplets decreased with increasing latex concentration. This effect was also visible at two other WPI concentrations: R=4 and R=64 (Figure 8). Combining higher R and higher latex concentration favored coverage of the dextran droplets, which explains the reduction of the droplets size. It is not surprising that increasing the amount of particles leads to smaller droplets as coalesce occurs until the interface is fully covered. A decrease of the droplet size with increasing particle concentration has already been reported for dextran/PEO emulsions with other types of particles (Nguyen et al. (2013) Peddireddy et al. (2016)). Small droplets were not close packed after sedimentation, but formed a sediment loosely clustered droplets. The implication is that the latex particles densely covered with protein not only stuck together at the interface, but also to particles at adjacent droplets.

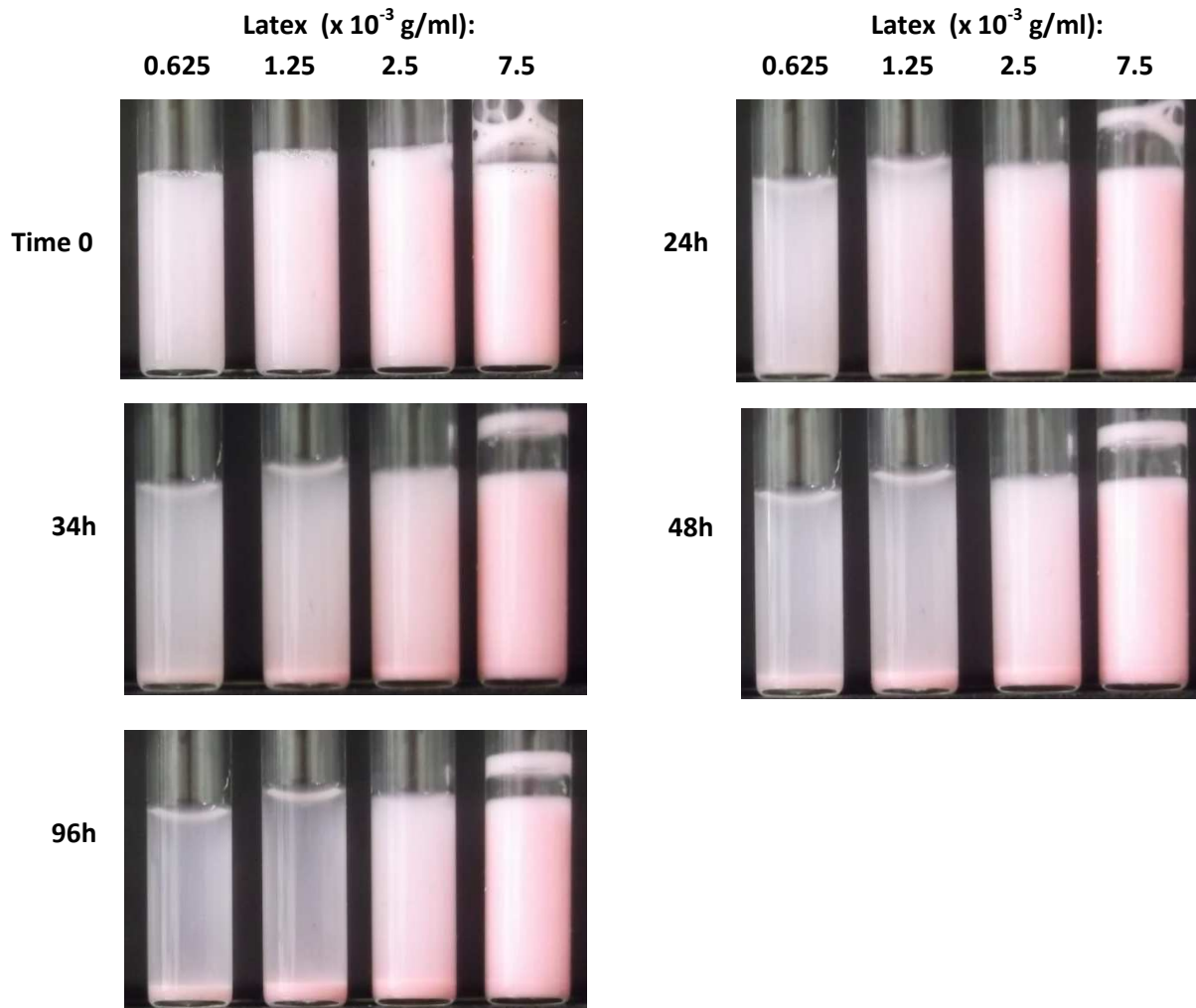


Figure 6 Photographs at different times after mixing dextran in PEO emulsions (PEO/Dex Φ : 95/5) with R=16 and different latex concentration (from left to right 0.625, 1.25, 2.5 and 7.5 x 10⁻³ g/ml)

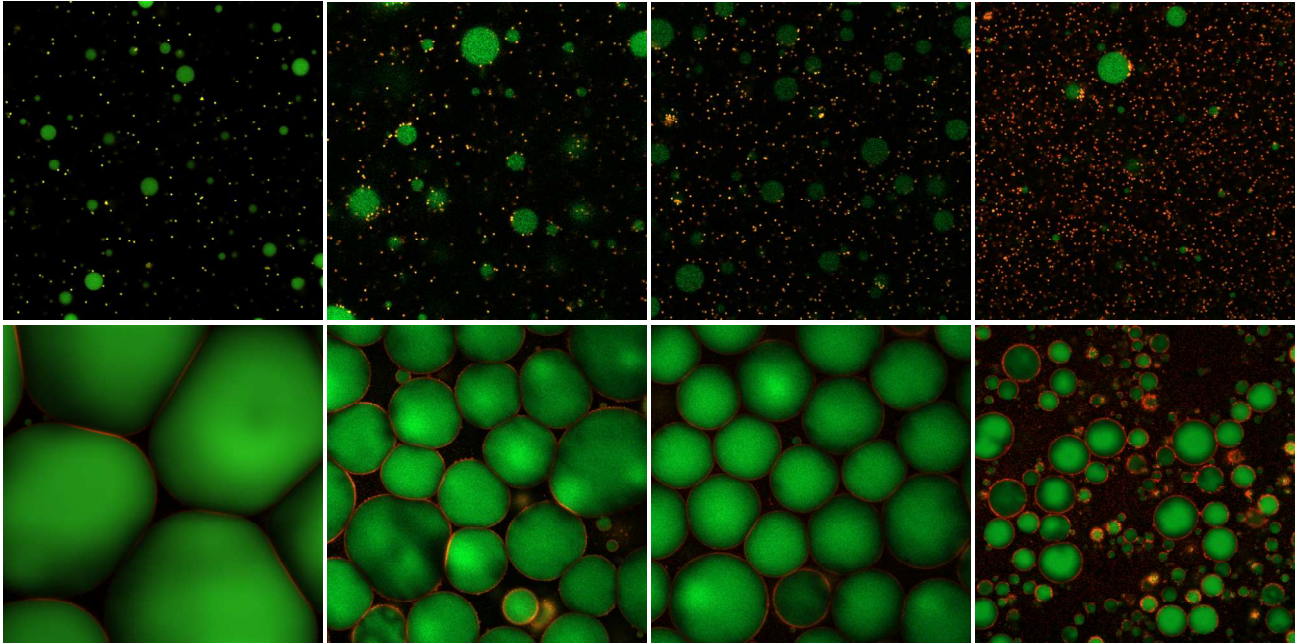


Figure 7 CLSM images of dextran in PEO emulsions (PEO/Dex Φ : 95/5) with $R=16$ and different latex concentration (from left to right: 0.625 , 1.25 , 2.5 and 7.5×10^{-3} g/ml). Top images ($100 \times 100 \mu\text{m}^2$) were taken after mixing; Bottom images ($512 \times 512 \mu\text{m}^2$) at steady state from bottom of the microscope slide

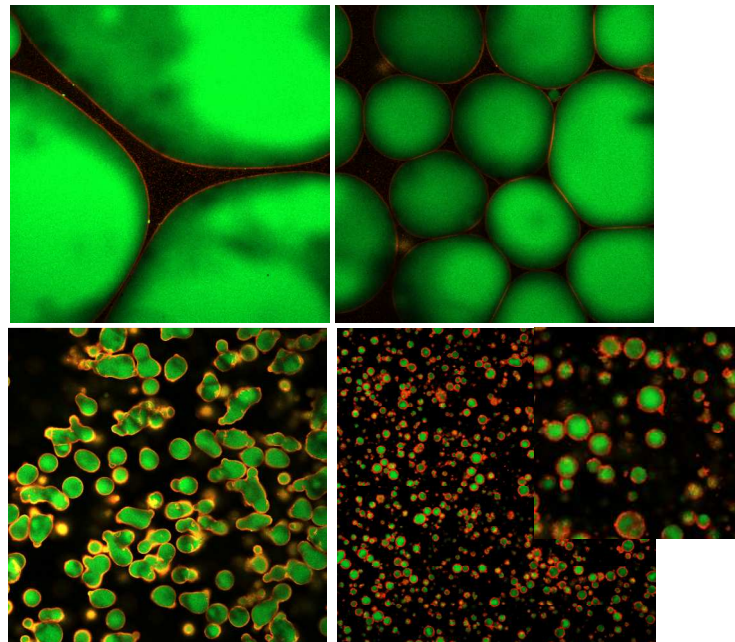


Figure 8 CLSM images of dextran in PEO emulsions (PEO/Dex Φ : 95/5) with $R=4$ (top) and $R=64$ (bottom) and different latex concentration (left $C=2.5$, right $C=7.5 \times 10^{-3}$ g/ml). The pictures ($512 \times 512 \mu\text{m}^2$) were taken at steady state from bottom of the microscope slide. The scale of the insert is $128 \times 128 \mu\text{m}^2$.

PEO-in-dextran emulsions

The results on dextran in PEO emulsions discussed so far were compared with emulsions of PEO-in-dextran (PEO/Dex Φ : 10/90) containing 2.5×10^{-3} g/ml of latex particles and different ratios of WPI (R: 0, 16, 128, 256, 384). At steady state the continuous dextran layer was transparent implying that most latex was situated in the top phase (Figure 9). The rate at which PEO droplets creamed increased with increasing R. It was especially fast for R=256 where after 7h a dense top layer was formed. The reason for this behavior is flocculation of the PEO drops which increased their effective size and therefore their creaming velocity. Due to the opacity of the top layer no difference could be appreciated macroscopically.

CLSM images were taken immediately after mixing (Figure 10). No significant difference of droplets size and coverage was observed for the different ratios right after mixing. Excess particles were situated in the PEO drops at $R \leq 128$ whereas for $R=256$ and $R=384$ they were in the dextran phase. Images of the top phase at steady state showed that a stable emulsions of PEO in dextran was formed for $R \geq 128$, whereas phase separation into two homogeneous phases occurred at $R=0$ and $R=16$. The change in the preference of the particles for the two phases may explain the change in their capacity to stabilize the emulsions. At $R=256$ and $R=384$ excess latex particles become bound in clusters to the particles at the interface and therefore creamed. This explains why the dextran phase remained transparent even when the excess particles preferred this phase. Clustering of the latex particles at high R also explains why the droplets could not fully relax to the spherical shape after coalescence.



7h



9h



19h



34h



72h

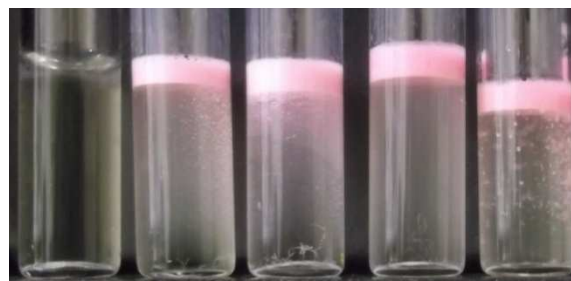


Figure 9 Photographs at different times after preparing PEO in dextran emulsions (PEO/Dex ϕ : 10/90) containing no latex or 2.5×10^{-3} g/ml and different ratio of WPI indicate in the figure

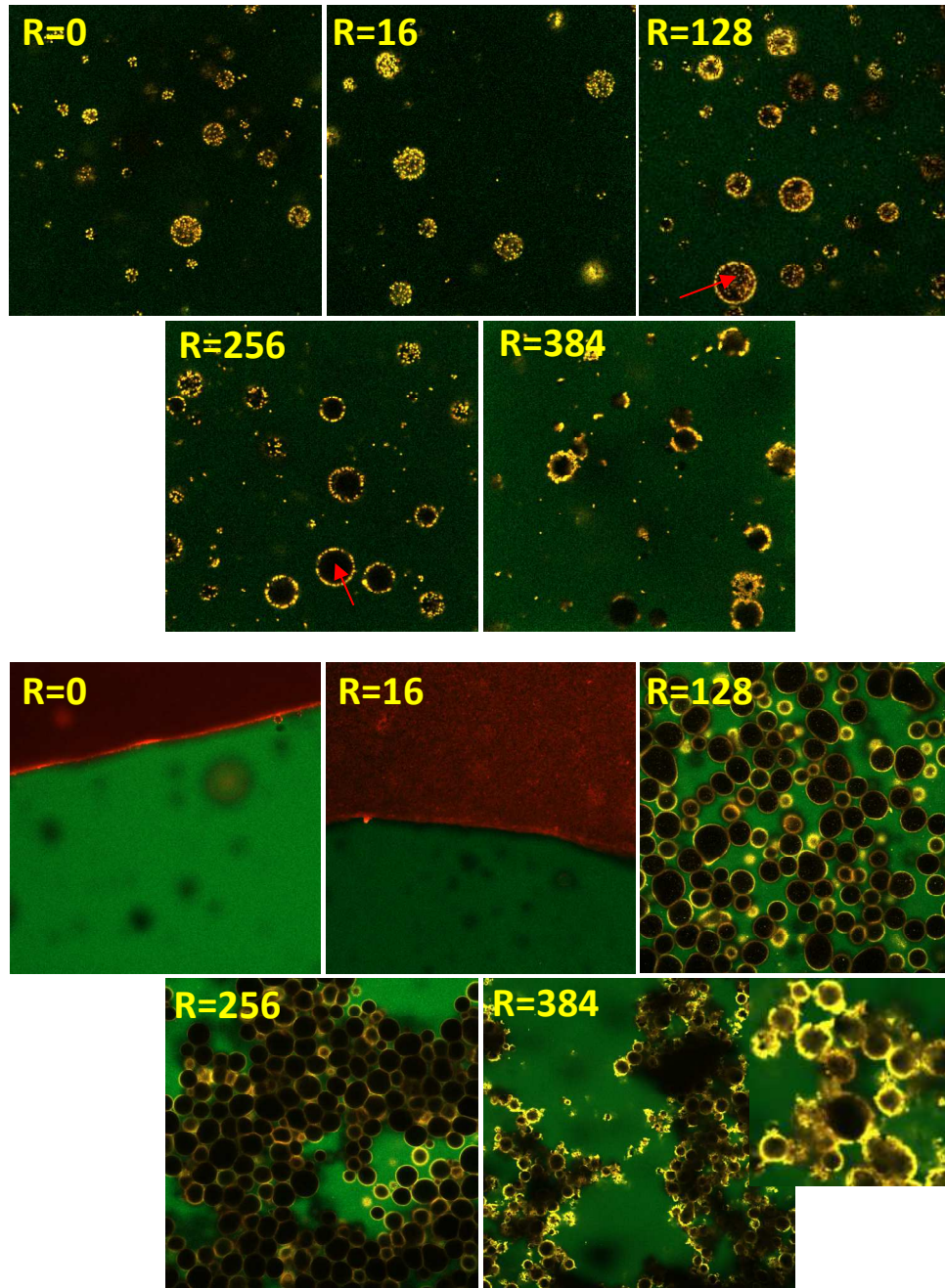


Figure 10 CLSM images of PEO/Dex emulsions (PEO/Dex Φ : 95/5) with 2.5×10^{-3} g/ml latex and different R as indicated in the figure. The first five pictures ($100 \times 100 \mu\text{m}^2$) were taken after mixing; the last five ($512 \times 512 \mu\text{m}^2$ and $125 \times 125 \mu\text{m}^2$ the insert) at steady state from top of the microscope slide (the two arrows indicate the center of a drop with and without latex particles inside)

The effect of the latex concentration was tested at R=128. Similarly to the PEO in dextran emulsions, increasing the concentration of latex led to the formation of smaller droplets (Figure 11). It should be noticed that the large deformed droplets in at $C_{\text{lat}}=7.5 \times 10^{-3}$ g/ml at steady

state image are most likely an effect of interaction with microscope slide as the dextran phase of these droplets wets the slide.

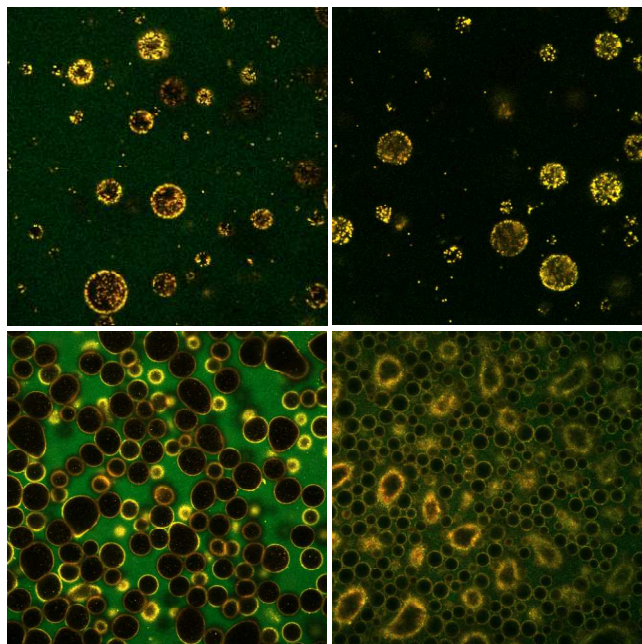


Figure 11 CLSM images of PEO in dextran emulsions (PEO/Dex Φ : 10/90) with $R=128$ and different latex concentration (left $C=2.5$, right $C=7.5 \times 10^{-3}$ g/ml). The top pictures ($512 \times 512 \mu\text{m}^2$) were taken after mixture and the bottom pictures ($100 \times 100 \mu\text{m}^2$) at steady state from top of the microscope slide

We have seen that particles with relatively high protein coverage had a tendency to stick together at the interface and form an elastic surface layer. We have investigated the interaction between latex particles with different protein coverage by observing their behavior in pure PEO and dextran solutions. 2.5×10^{-3} g/ml latex particles and WPI at $R = 32, 64, 128$ and 384 were suspended in a PEO solution at 8% or a dextran solution at 16%. Figure 12 and Figure 13 show CLSM images of the PEO and the dextran solutions, respectively, after 48 hours. Aggregation was observed only for $R=128$ and $R=384$ and was particularly strong in PEO solutions. The implication is that attractive interaction between the particles strong enough to overcome the mixing entropy necessitated high coverage. Of course, weaker attraction between the particles with lower coverage at the interface can still be important and inhibit coalescence.

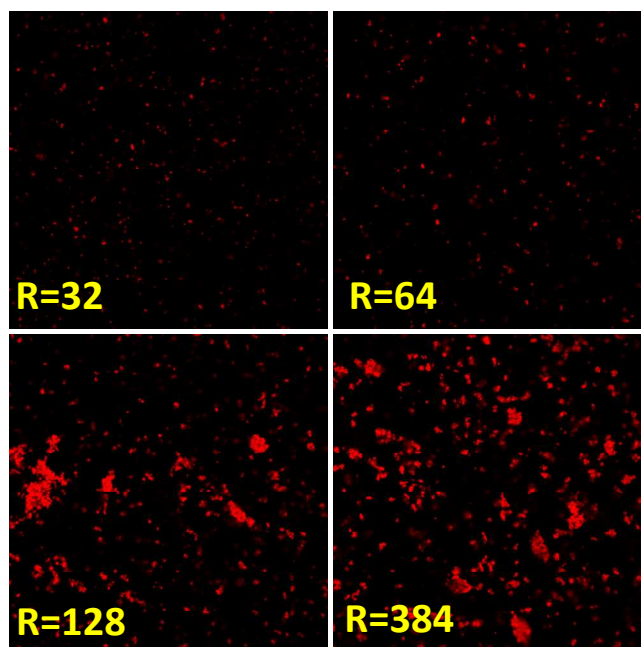


Figure 12 CLSM images of 8% PEO solutions with 2.5×10^{-3} g/ml latex particles and different WPI ratio, as indicated in the figure. Images ($100 \times 100 \mu\text{m}^2$) were taken after 48h.

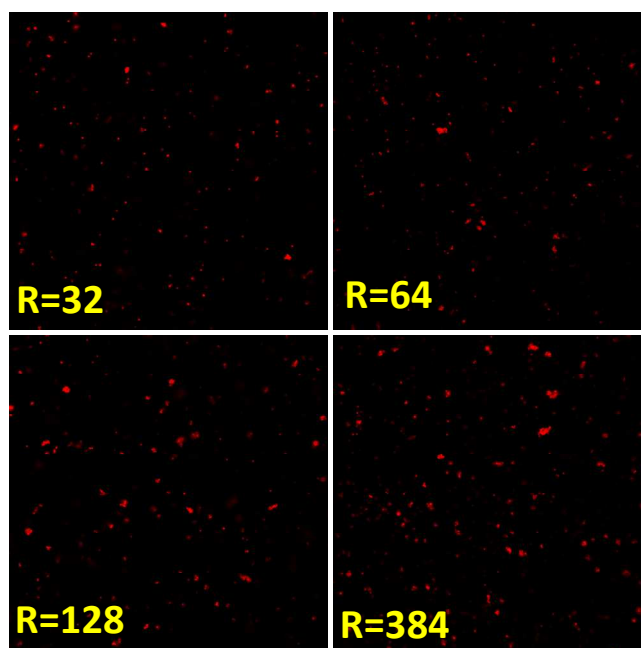


Figure 13 CLSM images of 16% dextran solutions with 2.5×10^{-3} g/ml latex particles and different WPI ratio, as indicated in the figure. Images ($100 \times 100 \mu\text{m}^2$) were taken after 48h

Conclusion

Addition of native WPI proteins modifies the capacity of latex particles to stabilize W/W emulsions by changing the surface properties of the latter. By varying the amount of proteins adsorbed to the particle it was possible to stabilize both P/D and D/P emulsions. Dextran in PEO emulsions were stable for $R \leq 64$, whereas PEO in dextran emulsions were stable for $R \geq 128$. We speculated in Chapter 3 that more stable emulsions are formed when particles have a preference for the continuous phase. The results obtained in this study corroborate this idea since P/D emulsions could be stabilized when the protein coverage was still relatively low and the particles preferred the continuous dextran phase whereas D/P could be stabilized at high coverage when the particles preferred the continuous PEO phase. Another effect of coverage by proteins was that the particles had a tendency to stick together. Attractive interaction between the particles at the interface helped stabilize the emulsions, but if the attraction is too strong it causes clustering of the droplets.

Chapter 6 Conclusions and perspectives

The research reported in this thesis was aimed at improving our understanding of stabilization of W/W emulsions by particles, which may help in the development of industrial applications of W/W emulsions in different areas such as food, pharmaceutical, cosmetic and personal care. Using a model system, consisting in mixtures of PEO and dextran, we have been able to make stable W/W emulsions by adding particles based on whey proteins. We have determined some of the factors and mechanisms that affect the stability of these types of mixtures. We have shown how the aggregation process of the protein particles can be exploited to produce particles with different morphologies, which in turn can be used to tune the stability of the W/W emulsion. In addition, we used whey proteins to modify the surface of latex particles in order to improve their efficacy to stabilizing W/W emulsions.

β -lg particles in the form of fibrils, microgels and fractal aggregates showed different efficacy to stabilize W/W emulsions. It depends not only on the concentration of protein particles, but also on their morphology, their affinity for each phase and their interaction with each other at the interface. We found that the partition of β -lg changed with the pH from favoring the dextran phase at $\text{pH} \geq 4.0$ to the PEO phase at lower pH. We conclude that W/W emulsions are more stable when particles have a preference for the continuous phase. At neutral pH, fibrils stabilized PEO in dextran (P/D) emulsions against coalescence better than microgels, whereas fractals were the less efficient. At pH 3.0 the same emulsions could not be stabilized by fibrils, but it were very stable when fractals were added. At neutral pH, dextran in PEO (D/P) emulsions phase separated very quickly for the three types of particles, but stable D/P emulsions could be formed at pH 3.0 with significantly smaller drops formed with fractals than with microgels and fibrils.

Aggregation of fractals and microgels in PEO/Dextran emulsions can be induced either by screening the electrostatic repulsion by addition of NaCl or by reducing the net charge density of the proteins by lowering the pH towards the isoionic point. In this way the emulsions with as little of 5 g/L protein could made to form weak gels by setting the pH between 6.0 and 3.5 or adding 0.3 M NaCl at pH 7.0.

Gelation modified the structure and evolution of the emulsions. Creaming or sedimentation will occur if a network was formed by excess protein particles in the continuous phase incorporating the dispersed droplets that could withstand the buoyancy force. Some of the gels formed could support their own weight when turned upside down. Creaming or sedimentation of the dispersed droplets was accelerated if gelation was slow or avoided if gelation was fast. Fractals were more efficient to avoid creaming when gelation was induced by setting the pH at 6.0 or 4.0 whereas microgels were more efficient when gelation was induced by adding 0.3M NaCl at pH 7.0.

Dense protein micro particles can be formed by inducing gelation of protein aggregates when they are partitioned to the dispersed phase. In this way protein microparticles were formed by adding CaCl_2 to D/P emulsions at pH 7. Spherical dense particles with a high yield could be formed that were stable to dilution by tuning the salt concentration and time before diluting.

Addition of native WPI proteins modifies the capacity of latex particles to stabilize W/W emulsions by adsorbing to the surface of the particles. With increasing amount of added proteins the preference of the particles shifted from the PEO phase to the dextran phase, which in turn determines the efficacy of the particle to stabilize P/D or D/P emulsions. Attractive interactions between the protein covered latex particles at the interface increased the stability. However, too high concentrations of proteins induced clustering of the droplets.

There are still many issues about the stabilization Pickering W/W emulsions that require further research. For instance, there are still open questions regarding the effect of particle morphology such as the intriguing optimal concentration found for fractals to stabilize P/D emulsions at pH 7.0 and the fact that they were the most efficient particles at pH 3.0 whereas fibrils were most effective at pH 7.0. It would also be of interest to study the effects of mixing aggregates with different morphologies on the PEO/Dextran mixtures.

Furthermore usage of particles with different chemistry (bio-compatible or synthetic) for which the shape can be modified may contribute to deeper understanding of the effect of the morphology on the stability of W/W emulsions.

A more detailed rheological study of the emulsions at different conditions would be of great interest.

Formation of dense protein microparticles exploiting W/W emulsions requires more research in order to control their size and density. Notably it would be interesting to study the effects of heating and applying shear.

From a fundamental point of view it would be very interesting to use bigger latex particles in order to relate the contact angle with the stability at different proteins ratios. It would be equally interesting to modify the conditions or the surface of the particle in-situ, i.e. under direct observation. A challenging question would be if it is possible to inverse the emulsion in this way by environmental modifications.

Here we have studied a model W/W emulsion, but for applications it will, of course, be necessary to study W/W emulsions formed by other types of polymers notably food grade polymers for potential applications in the food industry.

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Thèse de Doctorat

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Nouvelles émulsions eau/eau stabilisées par effet Pickering

New water/water emulsions stabilized by Pickering effect

Résumé

Les émulsions eau /eau (W / W) ont récemment suscité un grand intérêt en raison de leur fort potentiel d'application dans différentes industries telles que l'agroalimentaire, les produits pharmaceutiques, les cosmétiques et les soins personnels. Le caractère particulier des émulsions W / W est leur stabilisation par ajout de particules. L'objectif de ce travail de thèse est de comprendre cet aspect en étudiant une émulsion modèle W / W à base de dextran et du poly (oxyde d'éthylène) stabilisée par des particules à base de protéines du lactosérum. Dans un premier temps, nous avons étudié l'effet de la morphologie des particules protéiques et leur partitionnement sur la stabilité des émulsions W / W. En particulier, la stabilité s'est révélée dépendre de la structure des particules quand ses derniers étaient sous forme de microgels, d'agrégats fractals ou de fibrilles. Il a été montré que la stabilité s'améliorait lorsque les particules se localisent préférentiellement dans la phase continue. Deuxièmement, nous avons étudié la gélification, des microgels et des agrégats fractals, induite en réduisant le pH entre 6,5 et 3,5 ou en ajoutant 0,3 M NaCl à pH 7,0 aussi bien quand l'excès des particules se situe dans la phase continue ou dispersée. Dans le premier cas, un réseau se forme dans la phase continue de dextran, permettant d'inhiber le crémage des gouttelettes de PEO, les agrégats fractals étant plus efficaces que les microgels. Dans le second cas, des particules protéiques denses pourraient être formées par gélification des gouttelettes de dextran dispersées. Troisièmement, nous avons exploré l'adsorption des protéines natives sur les particules de latex et leur capacité à stabiliser les émulsions W/W.

Mots clés

Émulsions; Pickering; protéines de lactosérum; PEO; dextran; gélification à froid; agrégats fractals; microgels

Abstract

Water/water (W/W) emulsions have attracted great interest recently due to their high potential for applications in different industries such as food and beverages, pharmaceutical, cosmetics and personal care. An important issue is the stabilization of W/W emulsions by adding particles. The aim of the research for this thesis was to shed light on this issue by studying a model W/W emulsion formed by mixing dextran and poly(ethylene oxide) with particles based on whey proteins. Firstly, we studied the effect of the morphology of protein particles and their partitioning on the stability of W/W emulsions. The stability was different when microgels, fractal aggregates or fibrils were added. We showed that stability improved when the particles partitioned to the continuous phase. Secondly, we investigated gelation of the fractal aggregates and microgels induced by reducing the pH between 6.5 and 3.5 or by adding 0.3M NaCl at pH 7.0 with excess particles either in the continuous or the dispersed phase. In the first case, a network was formed in the continuous dextran phase, making it possible to arrest creaming of PEO droplets, fractal aggregates being more effective than microgels. In the second case, dense protein particles could be formed by gelation of the dispersed dextran droplets. Thirdly, we explored the effect of adsorbing native proteins onto latex particles on their capacity to stabilize W/W emulsions. Emulsions; Pickering; Whey proteins; PEO; dextran; cold gelation; fractal aggregates; microgels

Key Words

Emulsions; Pickering; Whey proteins; PEO; dextran; cold gelation; fractal aggregates; microgels