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Lingsam Tea, Taco Nicolaï, Frédéric F. Renou. Stabilization of Water-In-Water Emulsions by Linear Homo-Polyelectrolytes. *Langmuir*, 2019, 35 (27), pp.9029-9036. 10.1021/acs.langmuir.9b01604 . hal-03026701

HAL Id: hal-03026701

<https://hal.science/hal-03026701>

Submitted on 3 Dec 2020

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Stabilization of Water-in-Water Emulsions by Linear Homo-Polyelectrolytes.

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ABSTRACT: The effect of adding a small quantity of linear polymers on the stability of water-in-water (W/W) emulsions was studied for emulsions of dextran rich droplets in a continuous poly(ethylene oxide) phase (D/P) and vice versa (P/D). It was found that out of 16 different polymers that were tested 3 had a significant effect: chitosan (Chit), diethylaminoethyl dextran (DEAED) and propylene glycol alginate (PGA). In the presence of Chit or PGA, P/D emulsions were much less stable than D/P emulsions, but DEAED stabilized both types of emulsion. Interactions of these polymers with PEO or dextran were investigated with light scattering and the microstructure of the emulsions was studied with confocal laser scanning microscopy. The effect of the pH, polymer concentration, interfacial tension and ionic strength on the stability was investigated and was found to be different for the three polymer types. The results suggest that stabilization of W/W emulsions by linear polymers requires that they contain both charged and hydrophobic units.

Introduction

Water-in-water (W/W) emulsions are formed by mixing aqueous solutions of polymers A and B that are immiscible. Such mixtures phase separate and form an aqueous two phase system with a phase rich in polymer A and a phase rich in polymer B ¹. After shaking, an emulsion is formed of droplets of one phase suspended in the continuous second phase. The interface is broad compared to that between two molecular liquids such as oil and water as it is expressed only over distances larger than that between the polymer chain segments, i.e. larger than a few nm ². The interfacial tension (γ_{AB}) between the two polymer phases is orders of magnitude smaller than that between oil and water. For these reasons it is not possible to stabilize W/W emulsions with molecular surfactants as is done for oil/water emulsions.

However, it has been shown that W/W emulsions can be stabilized by adding particles that are sufficiently large ³⁻⁵. The particles were found to spontaneously accumulate at the interface forming a layer around the droplets that can inhibit coalescence. Particles with different shapes, morphology and chemical structure have been used successfully for different types of W/W emulsions ⁶⁻¹². Effective stabilization of oil in water (O/W) emulsions by particles has been known for a long time and has been called Pickering stabilization after one of the pioneers in this area ¹³. The driving force for the absorption of the particles at the interface is the reduction of the free energy, which for spherical particles with radius R can be written as follows ¹⁴:

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

where θ is the contact angle of the particles with the interface. The contact angle is determined by the difference between the interfacial tension of the particles with phase A (γ_{PA}) and with phase B (γ_{PB}): $\cos(\theta) = (\gamma_{PA} - \gamma_{PB}) / \gamma_{AB}$, which means that particles only spontaneously adsorb at the interface if $(\gamma_{PA} - \gamma_{PB}) < \gamma_{AB}$. If the particles are sufficiently large then ΔG can still be much larger than kT even though γ_{AB} is much smaller for W/W emulsions than for O/W emulsions. An important observation for W/W emulsions is that the spontaneous formation of a layer of particles is not always sufficient to inhibit coalescence ¹⁵. If contact is made between the dispersed phase of two neighboring droplets, the shear stresses during coalescence can be sufficient to drive the particles from the interface. It appears that interaction between the particles at the interface is required. Another particularity of W/W emulsions is

that stabilization of emulsions of phase A dispersed in phase B does not imply that emulsions of phase B dispersed in phase A can be stabilized by the same particles¹⁶.

An alternative to stabilization of W/W emulsions with particles is to use block copolymers. If the blocks each have a preference for a different phase and are sufficiently large to be able to span the interface they are expected to form a protective layer similarly to that of small surfactant in O/W emulsions¹⁷. It has been shown that this can indeed be done for O/O emulsions formed by mixtures of incompatible polymers in an organic solvent¹⁸⁻²⁰. As far as we are aware, stabilization using bihydrophilic block copolymers has only once been explored for W/W emulsions²¹. Ossenbach-Sauter and Riess²¹ studied an aqueous mixture of PEO and poly(vinyl pyridinium) in the presence of a diblock copolymer of these polymers and found that destabilization of the emulsion was slower in the presence of the copolymer. More recently, Buzza et al.²² studied W/W emulsions formed by mixing PEO and dextran in the presence of block copolymers containing one hydrophobic block and one or two hydrophilic blocks. For the triblock copolymers it was suggested that emulsions were stabilized by the formation of a layer of block copolymers with one hydrophilic block in each phase and a central layer of associated hydrophobic blocks. However, diblock copolymers containing a single hydrophilic block were also found to increase the stability of these emulsions. For the latter it is clear that polymer micelles were formed that acted as particles. It is likely that the triblock copolymers also associated in the form of polymeric micelles and stabilized the emulsions as particles. The stabilization by particles formed by self assembly of smaller molecules into liposome was also reported for the same W/W emulsions²³.

An issue that has not been addressed so far is whether linear homo-polymers can stabilize W/W emulsions. The capacity of particles to stabilize emulsions does not require them to have two sides each preferring a different phase. Therefore one might wonder whether it is necessary for polymers to contain different blocks that prefer different phases. On one hand, it is expected that polymers accumulate at the interface if $(\gamma_{PA}-\gamma_{PB}) < \gamma_{AB}$ and their radius is as large as or larger than that of particles that have been shown to successfully stabilize W/W emulsions. Therefore there appears to be no a priori reason that linear polymers cannot form a protective layer that inhibits coalescence and therefore can be used to stabilize W/W emulsions. On the other hand, polymers can easily deform and they can interpenetrate with the polymers in the emulsion. Furthermore, as was mentioned above, accumulation at the interface is not sufficient to stabilize W/W emulsions.

Here we show for the first time that certain types of linear homo-polymers can indeed stabilize W/W emulsions formed by mixing dextran and poly(ethylene oxide) (PEO) solutions. Dextran/PEO mixtures were used, because they have been studied extensively in the past and may be considered as model W/W emulsions. We have tried a range of different polymers, mainly polysaccharides. Three of them were found to have a strong stabilizing effect of the emulsions: chitosan, diethyl aminoethyl dextran and propylene glycol alginate. We will show that the stabilizing effect is not caused by an increase of the viscosity, but it appears to require both charged and hydrophobic units on the polymers.

Experimental Section

Materials

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 (Millipore). The PEO powder contained a small amount of silica particles that were removed by centrifugation of the PEO solutions.

Chitosan (Chit) is a cationic polysaccharide with a $pK_a \approx 6.5$ ^{24,25}. The Chit sample used for this study was purchased from Sigma-Aldrich (batch STBG5137V) and had a degree of acetylation of 25% as determined by NMR²⁶. Diethyl aminoethyl dextran (DEAED) is a cationic polysaccharide obtained by functionalizing dextran with diethyl aminoethyl groups that are positively charged in a wide pH range ($pK_a \approx 9.5$ ²⁷) and also has a hydrophobic character through the ethyl groups. The sample used for this study was purchased from Sigma-Aldrich and contained 80 % functionalized sugar units. Propylene glycol alginate (PGA) is an anionic polysaccharide obtained by functionalizing alginate ($pK_a \approx 3.3$ ²⁸) with propylene oxide. The sample used for this study was purchased from Kimica and contained 70 % functionalized sugar units. The structures of these polysaccharides are shown in fig. 1. The water content of the samples was determined by thermo gravimetric analysis was found to be 5.5%, 3.7% and 11% for Chit, DEAED and PGA, respectively.

Stock solutions of chitosan, DEAED and PGA were prepared in Millipore water by stirring overnight. The pH of the chitosan stock solution was set at 2.5 in order to facilitate its solubilization. The stock solutions were filtered through 0.2 μm pore size filters (Anatope). The emulsions were prepared by mixing stock solutions with the required amounts and vortexing. The pH and NaCl

concentration was subsequently set to the desired values by addition of aliquots of 0.1 M HCl or 0.1 M NaOH or 1 M NaCl. No effect of the order of mixing or the speed of vortexing was observed, neither were the results different if the pH or the ionic strength was set for each polymer stock solution before mixing or for the mixture. The average molar mass (M_w) and hydrodynamic radii were determined by light scattering techniques as described in detail for Chit in ref. ²⁶. Results obtained at different scattering angles and polymer concentrations were used to extrapolate to zero angle and zero concentration. In addition NaCl was added in order to screen electrostatic interactions. We obtained $M_w = 3.4 \times 10^5$ g/L and $R_h = 58$ nm for Chit, $M_w = 8 \times 10^5$ g/mol and $R_h = 39$ nm for DEAED and $M_w = 8 \times 10^5$ g/mol and $R_h = 62$ nm for PGA. M_w of Chit was independent of the pH up to pH 5.0 and increased slightly at pH 5.5. At pH 6.0 large aggregates were formed and for $\text{pH} \geq 6.5$ macroscopic flocculation could be observed.

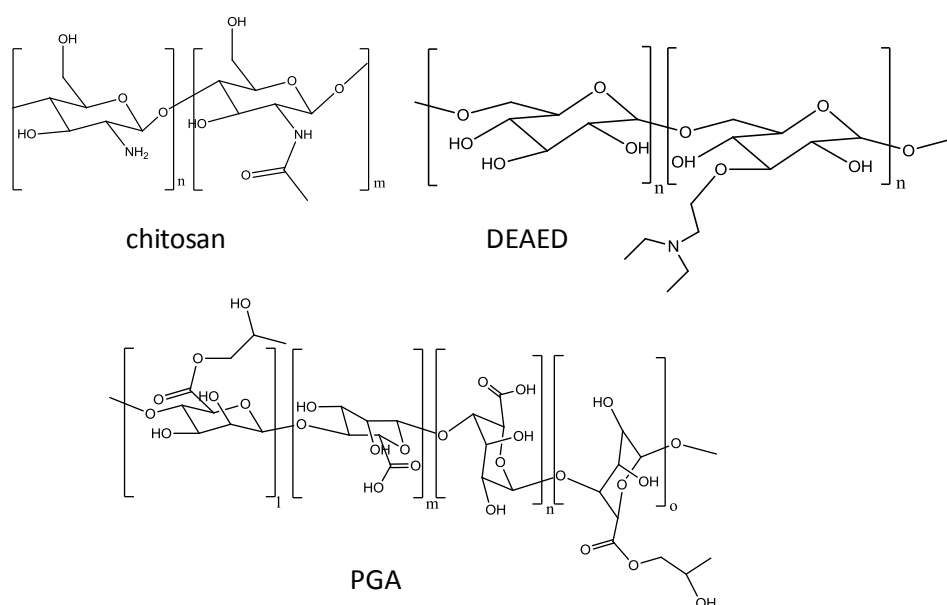


Fig. 1 Chemical structure of chitosan, DEAED and PGA.

Unsuccessful trials were made with: alginate, κ -carrageenan (Car), carboxymethyl cellulose, methyl cellulose, hydroxymethyl propyl cellulose, polystyrene sulfonate, fish gelatin, amylopectin, polyvinyl alcohol (from sigma chemical Co, USA) gum arabic (Alland & Robert, France), pectin, scleroglucan (Cargill, France), xyloglucan (from UEM, Brazil) and hydroxyethyl cellulose (Fluka, Switzerland)

Emulsions of dextran droplets in a continuous PEO phase (D/P) and vice versa (P/D) were produced by mixing 4 wt% dextran with 6.3 wt% PEO and 12 wt% g/L dextran with 1.9 wt% g/L PEO, respectively. Both emulsions were on the same tie-line with an interfacial tension $\gamma = 75 \mu\text{N/m}$ ¹⁵. The volume fraction of the dispersed phase was in both cases 25%. To these emulsions polymer was added at different concentrations of the powder up to $C = 1 \text{ g/L}$ after which the solutions were mixed by vortexing.

Methods

Light scattering

Light scattering measurements were done using a commercial apparatus (ALV-CGS3, ALV-Langen). The light source was a He–Ne laser with wavelength $\lambda = 632 \text{ nm}$. The temperature was controlled by a thermostatic bath to within $\pm 0.2 \text{ }^\circ\text{C}$. Measurements were done as a function of the scattering wave vector: $q = (4\pi n/\lambda) \cdot \sin(\theta/2)$, with n the refractive index of the solution, and θ the scattering angle.

Confocal scanning laser microscopy

Images were obtained with a Zeiss LSM800 (Carl Zeiss Microscopy GmbH, Germany) with two different water immersion objectives: HC×PL APO 63× (NA = 1.2) and HC×PL APO 25× (NA = 0.7). The solutions were inserted between a concave slide and a cover slip and hermetically sealed. The dextran phase was visualized by adding a small amount of dextran labeled with FITC. In order to observe the partitioning of Chit and DEAED, a small amount of Chit and DEAED labeled with FITC or rhodamine B was added. Labeling of these polymers was done following the method reported by Heilig et al.²⁹.

Results

The effect of adding linear flexible homo-polymers on the stability of D/P and P/D was tested for a range of polymers at 1 g/L: alginate, methyl cellulose, hydroxymethyl propyl cellulose, hydroxyethyl cellulose, xyloglucan, gelatin, gum arabic, pectin, scleroglucan, k-carrageenan (Car), poly(vinyl alcohol), polystyrene sulfonate, fish gelatin, amylopectin, polyvinyl alcohol, PGA, Chit and DEAED. For most polymers only a limited slow-down of the destabilization of the emulsions was observed

visually that could be explained by the increase of the viscosity. Only for the last three polymers did we observe a strong stabilizing effect. This is illustrated in Fig. 2 where we compare the evolution of D/P emulsions in the absence of polymer and in the presence of Car, Chit, DEAED and PGA. Emulsions in the presence of Car completely destabilized within 6 h compared to 3 h for emulsions without polymer. Emulsions containing Chit or DEAED showed no signs of destabilization after 7 days, whereas emulsions containing PGA showed sedimentation of dextran droplets without formation of a homogeneous dextran layer. Here we consider emulsions stable as long as they do not show a visible continuous layer of the dispersed phase, even if the droplets cream or sediment.



Fig. 2 D/P emulsions at pH 5 with and without polymer added at $C = 1 \text{ g/L}$ at different times after preparation as indicated in the figure. For clarity the phase boundary is marked with a white line for a few samples.

The microstructure of the emulsions was visualized by CLSM after adding a small fraction of fluorescently labeled dextran. They showed droplets of the dextran phase in a continuous PEO phase for the D/P emulsions, see fig. 3. From the images it appears that the droplets are smaller and more monodisperse in the presence of DEAED. This was confirmed by analyses of the droplet size distribution, see figure S1 of the supplementary information. The number average diameters were $8 \pm 5 \mu\text{m}$, $3 \pm 2 \mu\text{m}$ and $6 \pm 4 \mu\text{m}$ for Chit, DEAED and PGA, respectively. The volume average diameters

were $14 \pm 6 \mu\text{m}$, $6 \pm 2 \mu\text{m}$ and $10 \pm 5 \mu\text{m}$ for Chit, DEAED and PGA, respectively. The droplet size grew rapidly for emulsions without added polymer or with 1 g/L Car, whereas no significant evolution of the droplet size was observed within one week in the presence of 1g/L Chit or DEAED. However, the droplet size in the presence of 1 g/L PGA slowly increased to $70 \pm 30 \mu\text{m}$ after standing one week.

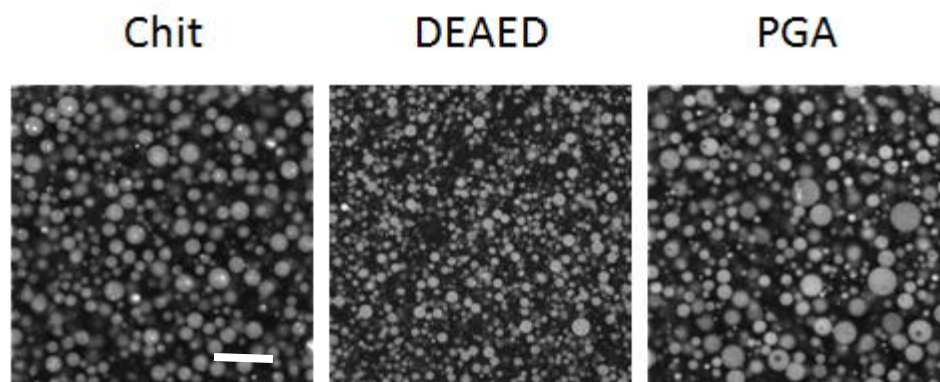


Fig. 3 CLSM images of D/P emulsions at pH 5 in the presence of 1 g/L Chit, DEAED or PGA. The scale bar represents 50 μm .

The shear rate dependent viscosity of these emulsions is shown in fig. 4. The viscosity at low shear rates is 2-3 times higher in the presence of polymer, but this increase is by far not sufficient to explain the stability in the presence of Chit, DEAED or PGA. In fact, the viscosity of unstable suspensions containing Car was higher than that of stable suspensions containing DEAED. We draw attention to the decrease of the viscosity above a critical shear rate, which is characteristic for emulsions and has been discussed elsewhere for unstable W/W emulsions³⁰. Further decrease of the viscosity at high shear rates is caused by shear thinning and is also observed for solutions of PEO with or without added polymer. We will discuss the shear rate dependence of the viscosity of these emulsions in more detail elsewhere.

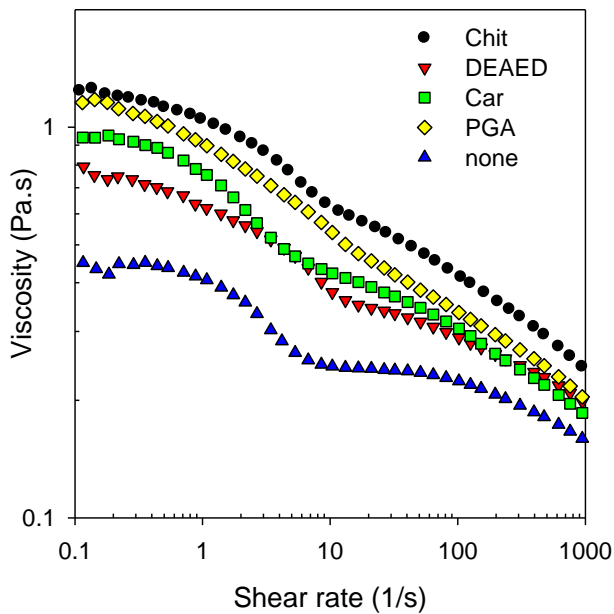


Fig. 4 Viscosity as a function of the shear rate for D/P emulsions with and without different types of polymer added at $C = 1 \text{ g/L}$ as indicated in the figure.

Effect of the pH

Fig. 5 shows photographs of D/P emulsions in the presence of 1 g/L Chit, DEAED or PGA at different pH taken 7 days after mixing. Emulsions with Chit were stable for at least one week between pH 3 and pH 6. As was mentioned above, Chit is not soluble at higher pH. Emulsions with DEAED formed an almost transparent dextran bottom phase after one week at pH 3 and at $\text{pH} \geq 7$, but remained stable at intermediate pH. Except at pH 10, the top phase was still turbid after standing for a week implying that it still contained small dextran droplets that had not yet sedimented. Emulsions with PGA showed destabilization only at pH 9 and 10, but the dextran droplets had sedimented to different extents at different pH. Emulsions without polymers or with Car destabilized within 6 h at all pH values tested between 3 and 10. The viscosity of the emulsions was found to be the same at all pH within the experimental error and therefore cannot explain the observed differences. DEAED is fully charged below pH 8 and PGA is fully charged above pH 5, but the stability of the emulsions containing these polymers varied. This means that the stability is not directly related to the charge density of the added polymers. However, differences were observed in the droplet size and the partitioning of the polymers between the two phases that will be discussed below.

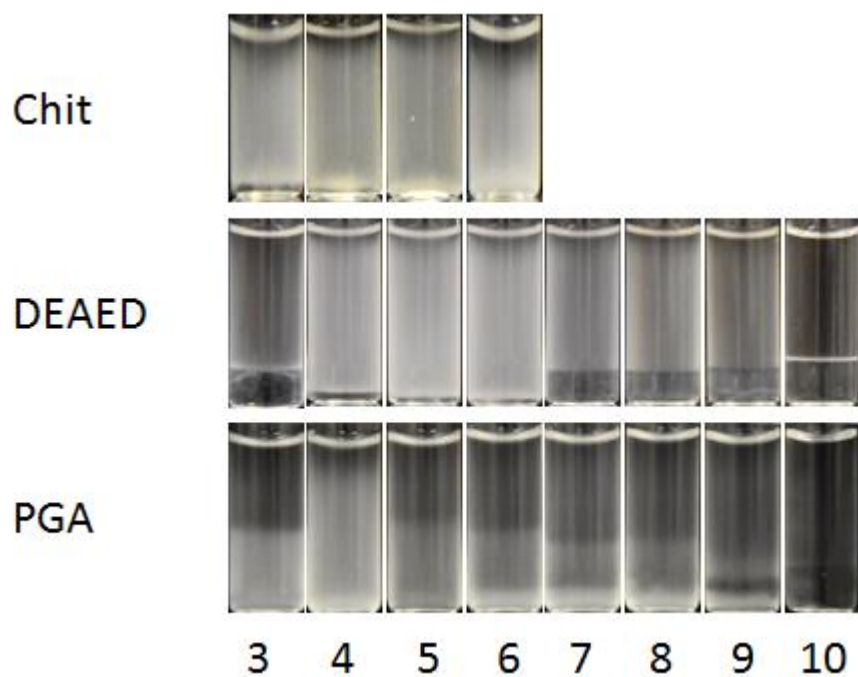


Fig.5 D/P emulsions at different pH in the presence of 1 g/L Chit, DEAED or PGA one week after preparation.

Effect of the added polymer concentration

The effect of the added polymer concentration is shown in figure 6 for emulsions in the presence of Chit or DEAED at pH 5 and for PGA at pH 4, where the emulsions are most stable. Destabilization was observed below 0.6, 0.4 and 0.2 g/L for Chit, DEAED and PGA, respectively. In the presence of DEAED, dextran droplets sedimented after a few days at $C = 0.4$ g/L and in the presence of PGA the rate of sedimentation increased progressively with decreasing concentration. The effect of the concentration on the evolution of the samples was studied at different pH for D/P emulsions with Chit, see fig. S2 of the supplementary information. The stability of emulsions with $C < 1$ g/L decreased both at $\text{pH} < 5$ and at $\text{pH} 6$. There appears to be an optimum pH range to form stable emulsions with Chit as was already observed at $C = 1$ g/L for DEAED and PGA.

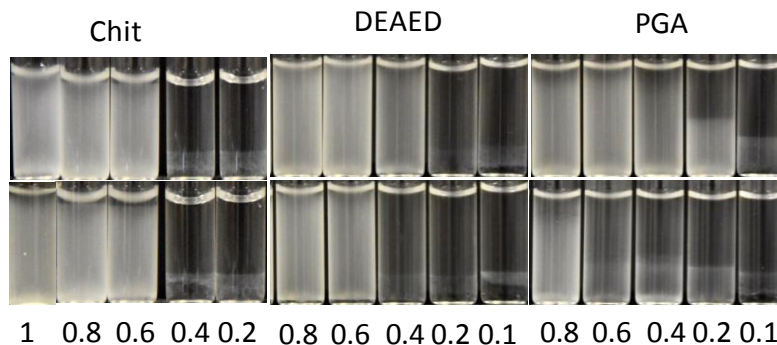


Figure 6. D/P emulsions at 3 days (top) and 7 days (bottom) after preparation containing different polymer concentrations (g/L) as indicated in the figure. The pH of the emulsions was 5 for Chit and DEAED and 4 for PGA.

Effect of adding NaCl

Figure 7 shows D/P emulsions at different NaCl concentrations containing 1 g/L Chit, DEAED or PGA. Addition of salt decreased the stability of emulsions with DEAED that formed a clear dextran phase very rapidly in the presence of 0.1 M NaCl. The emulsions with Chit or PGA remained stable, but addition of salt increased the droplet size and the sedimentation rate. The emulsions with PGA were found to be most resistant to addition of salt.

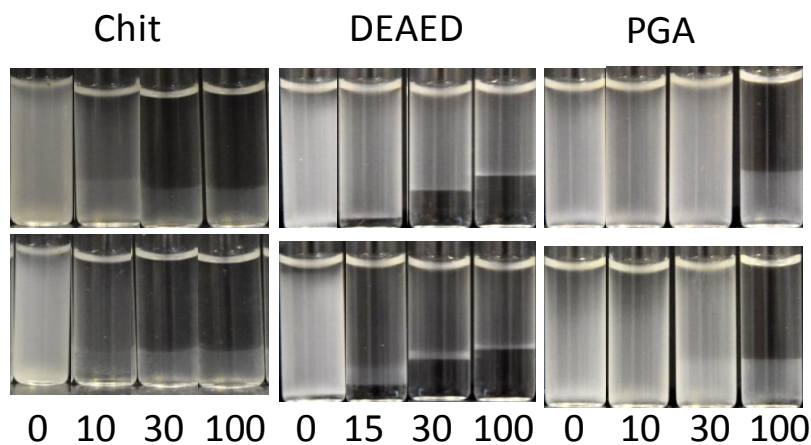


Figure 7. D/P emulsions figure 3 days (top) and 7 days (bottom) after preparation containing $C = 1$ g/L polymer and different NaCl concentrations (mM) as indicated in the figure. The pH of the emulsions was 5 for Chit and DEAED and 4 for PGA.

Effect of dilution

Diluting the emulsions leads to a decrease of the interfacial tension¹⁵. Figure 8 shows the effect of dilution on the stability of the emulsions keeping the Chit, DEAED or PGA concentration fixed at 1 g/L. The emulsions with Chit, DEAED and PGA remained stable for at least one week down to dilutions by a factor 2.5 ($\gamma \approx 2 \mu\text{N/m}$), 1.5 ($\gamma \approx 15 \mu\text{N/m}$) and 1.2 ($\gamma \approx 37 \mu\text{N/m}$), respectively.

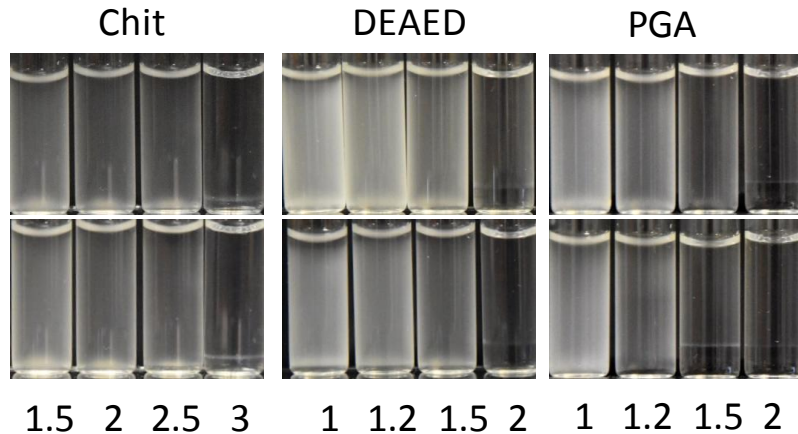


Figure 8. D/P emulsions 3 (top) and 7 (bottom) days after preparation at different dilutions indicated in the figure while keeping the Chit, DEAED and PGA concentration constant at $C = 1 \text{ g/L}$. The pH of the emulsions was 5 for Chit and DEAED and 4 for PGA.

Comparison of D/P and P/D emulsions

As was mentioned in the introduction, stabilization of W/W emulsions by a given type of particles was found to depend on which phase was continuous. For example, protein particles were found to be efficient stabilizers for P/D emulsions, but not for D/P emulsions³¹. We observed that P/D emulsions were much less stable than D/P emulsions in the presence of 1 g/L PGA or Chit, see fig.9. A continuous layer of the PEO phase was formed after 3 days for PGA at all pH and for Chit at pH 3 and 4. P/D emulsions with Chit were no longer stable after a week at all pH, but in the presence of 1 g/L DEAED they were stable for at least one week between pH 4 and 7 although the droplets had started to cream.

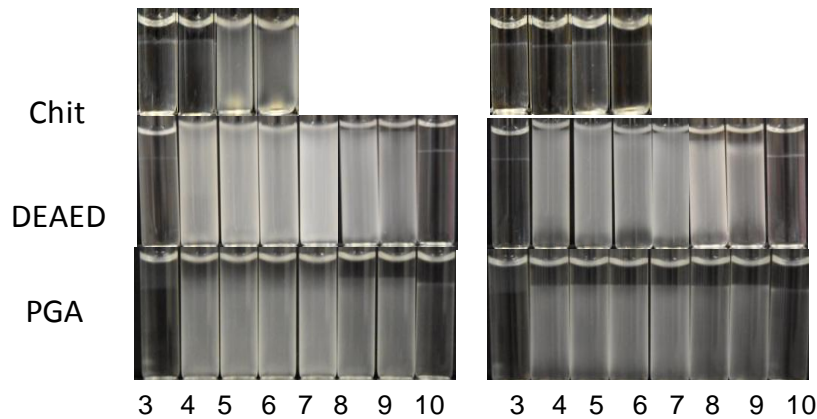


Figure 9 P/D emulsions 3 days (left) or 7 days (right) after preparation in the presence of 1 g/L Chit, DEAED or PGA at different pH indicated in the figure.

Partition

The partition of the polymers between the two phases was determined at different pH by adding a small amount of fluorescently labeled polymer. CLSM images show differences in fluorescence intensity corresponding to differences in added polymer concentrations, see Fig. 10. We verified that the fluorescence intensity was proportional to the polymer concentration, which allowed us to determine the polymer concentration in each phase. A layer of labeled DEAED around the dextran droplets was faintly visible, but the concentration of Chit or PGA in the layer was too small to give a visible contrast. In order to establish if there was a bias to the partitioning due to the labeling, the polymers were labeled with FITC or with RITC that have by themselves a preference for the dextran or the PEO phase, respectively. We did find a slightly larger preference for the PEO phase for polymers labeled with RITC. Therefore we decided to take the average of the values obtained with the two labels. No systematic difference was found in the partition between D/P and P/D emulsions. However, a systematic effect of the pH was observed. Fig. 11 shows the ratio (R) of the polymer concentration in dextran to that in PEO. Excess of Chit was partitioned preferentially in the dextran phase and the difference increased with increasing pH. Excess DEAED was partitioned more equally showing a slight increase at low and high pH. Excess PGA was situated more in the dextran phase except at pH 3 and pH 10, where it preferred the PEO phase. The effect of the pH on the partition shows that the interaction between the added polymers and the two phases depends on the pH, but there is no clear relationship between the partition and the stability of the emulsions.

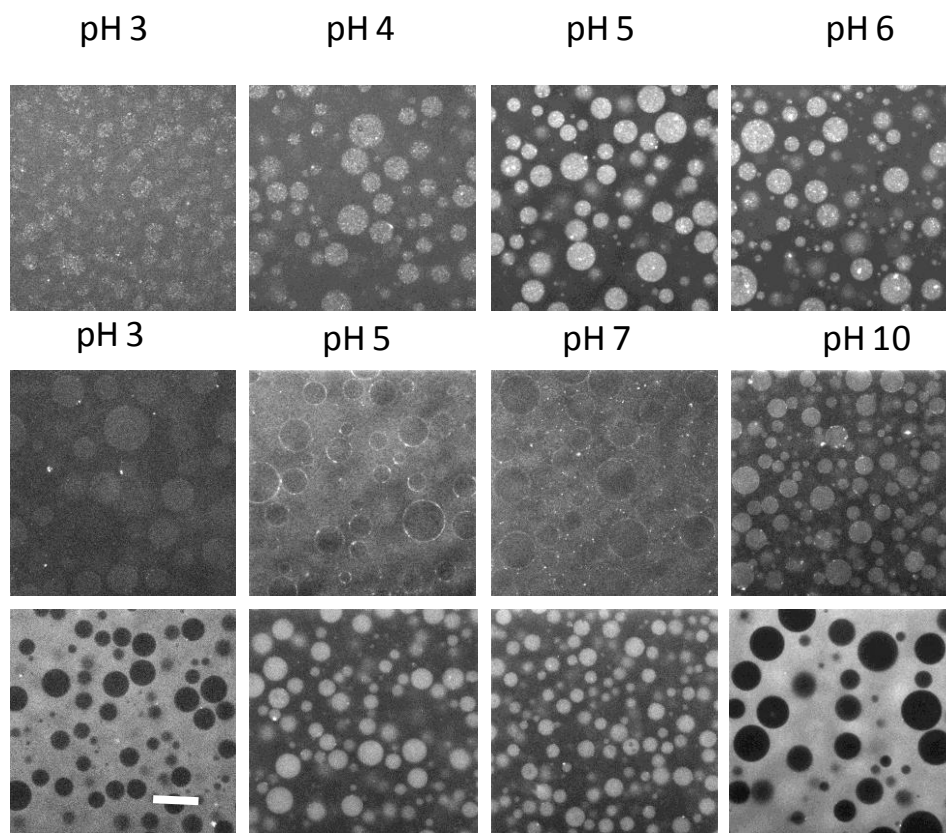


Figure 10. CLSM images of D/P emulsions in the presence of 1 g/L Chit (top), DEAED (middle) or PGA (bottom) at different pH as indicated in the figure. A small amount of FITC labeled polymers had been added. The scale bar represents 20 μm .

Relatively minor differences were observed for the initial droplet size distribution at different pH. In the presence of Chit the number average diameter decreased weakly with increasing pH from $12 \pm 5 \mu\text{m}$ at pH 3 to $8 \pm 5 \mu\text{m}$ at pH 5 and 6. In the presence of DEAED larger droplets were formed at pH 3, 7 and 10 (number average $7 \pm 4 \mu\text{m}$) than at pH 5 ($3 \pm 2 \mu\text{m}$). In the presence of PGA the initial droplet size distribution was similar at pH 5, and 7 ($6 \pm 4 \mu\text{m}$), but at pH 3 and 10 it showed a bidisperse distribution of smaller ($7 \pm 3 \mu\text{m}$) and larger droplets ($15 \pm 5 \mu\text{m}$).

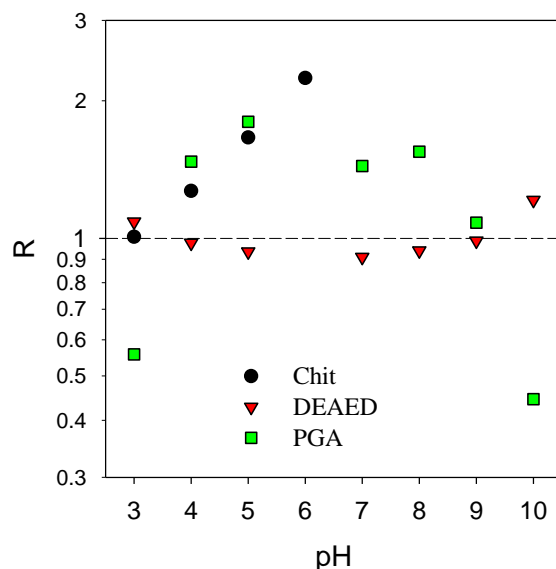


Fig. 11 Average ratio of the polymer concentration in the dextran and the PEO phase as a function of the pH for D/P and P/D emulsions containing 1 g/L of chit, DEAED or PGA.

We found that addition of salt also had an influence of the partition of the polymers, but this was not studied in more detail since addition of salt reduced the stability of the emulsions. Interestingly, the layer of DEAED was observed more clearly in the presence of 0.1 M NaCl than in the absence of salt, see fig. S3 of the supplementary information. The fact that a layer is clearly formed even in the presence of salt shows that formation of a layer does not guarantee stability for W/W emulsions as was mentioned in the introduction.

Discussion

We have demonstrated here that certain types homo-polymers can be utilized to stabilize W/W emulsions. One possibility is that the added polymers interact specifically with dextran or PEO leading to the formation of particles that in turn stabilize the emulsions. Visually individual PEO and dextran solutions remained transparent when Chit, DEAED or PGA were added even up to $C = 10$ g/L indicating that colloidal particles were not formed in significant concentrations and that no phase separation had occurred. The interaction of the added polymers with dextran or PEO was investigated in dilute solutions using light scattering. If the interaction is attractive the scattering intensity of the mixture is larger than the sum of the individual solutions at the same concentration as in the mixture,

whereas it is smaller if the interaction is repulsive. As an example, results are shown for the case of chitosan at pH 5 in fig. 12. The intensity of mixtures with Chit or DEAED was in all cases the same or less than the sum showing that the interaction was either negligible or weakly repulsive. For mixtures with PGA the intensity of the mixtures was found to be up to 40% larger indicating weak attractive interaction. The principal conclusion is, however, that interactions between the added polymers and dextran or PEO was weak and that no complexation had occurred, which implies that the stability was caused by the presence of individual polymers. The present situation is thus different from that reported by Vis et al.³² who observed stabilization of W/W emulsions formed by mixtures of dextran and gelatin in the presence of FITC dextran. In that case FITC-dextran formed complexes with oppositely charged gelatin that accumulate at the interface.

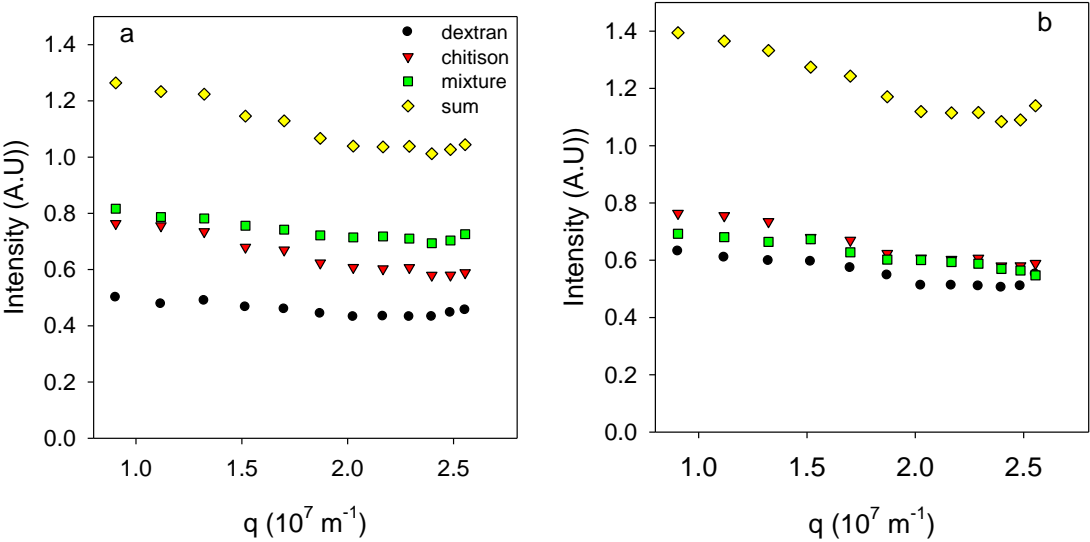


Fig.12 Scattering intensity as a function of the scattering wave vector for aqueous solutions of chitosan (1 g/L), dextran (0.05 g/L), PEO (0.1 g/L) at pH 5 and mixtures of Chit with PEO (a) or with dextran (b) containing the same amounts of polymer. The sum of the scattering intensity of the pure solutions is also shown for comparison with the mixtures.

Electrostatic interaction appears to be essential for stabilization of W/W emulsions by polymers as screening these interactions by addition of salt either caused more extensive coalescence leading to the formation of larger droplets or to fast destabilization. A strong effect of adding salt on the stability of W/W emulsions has earlier been reported for polyelectrolyte microgels¹⁰. One possible mechanism for stability is electrostatic repulsion between droplets that contain different amounts of polyelectrolyte

compared to the continuous phase. However, in that case emulsions containing other types of polyelectrolytes such as Car should also stabilize the emulsions as they partition much more strongly than DEAED. However, no correlation was found here between the partitioning of the polyelectrolytes and the stability. In addition, native proteins or Rhodamin B that partition to the dextran or PEO phase, respectively, do not stabilize the emulsions. Furthermore, this mechanism does not explain why often the stability of D/P is different from that of P/D. Therefore we suggest that Chit, DEAED or PGA form a layer at the surface of the droplets that inhibits coalescence through electrostatic and steric repulsion. The layer at the interface is clearly visible in CLSM images in the presence of DEAED, but the excess concentration of labeled Chit or PGA in the layer was too low to be seen as a layer in the images. The driving force for the formation of the layers is most likely the same as for particles, i.e. reduction of the interfacial tension. However, the osmotic pressure exerted by the two polymer phases on opposite sides of the layer renders it unlikely that the conformation of the polymers at the interface remains unperturbed. Most likely the polyelectrolyte chains at the interface are compressed and interpenetrated each other. Since Chit, DEAED and PGA are all readily miscible with dextran and PEO the surface layer will probably be also interpenetrated to some extent by the polymers used to form the emulsion.

The pH range where the emulsions were stable depended on the type of added polymer. In the presence of 1 g/L Chit the emulsions were stable in the whole range where Chit is soluble (pH 3 - 6). But from measurements at lower Chit concentrations it is clear that the emulsions are less stable both at pH 3 and at pH 6. Emulsions in the presence of DEAED were less stable both at pH 3 and above pH 6. Finally, for PGA we found that the stability decreased above pH 8. The effect of the pH on the stability is not directly related to the charge density of the polymers as DEAED and PGA are fully charged at acidic and basic pH, respectively, where differences in stability were observed. Neither can it be related directly to the pH dependence of the partition of the polymers between the two phases as we did not observe a correlation between the stability of the emulsions and the partitioning of the polymers between the two phases.

The emulsions with Chit, DEAED and PGA all destabilized when they were diluted, which is to be expected as the interfacial tension that drives the polymers to the interface decreased. However, emulsions with Chit remained stable when diluted 2.5 times, whereas emulsions with DEAED or PGA destabilized after diluting by a factor 2. On the other hand, less PGA than Chit was needed to produce stable emulsions. D/P emulsions with DEAED were more sensitive to the presence of salt than those with PGA or Chit, but only DEAED could stabilize P/D emulsions.

Perhaps the most important question is why only a few of the polymers that were tested stabilized the emulsions. Charge appears to be required, but it is not sufficient as several polyelectrolytes that were tried did not stabilize the emulsions. An additional feature of the 3 stabilizing polyelectrolytes was that they contained hydrophobic groups. Since neutral polymers containing hydrophobic groups such as methyl cellulose did not stabilize the emulsions, it appears that both charge and hydrophobicity are required. Furthermore, interaction of the added polymers with each other at the interface is likely to be different for the different polymers and to depend on the conditions. Clearly more research is needed in order to relate the configuration of the polymers and the interaction to their capacity to stabilize W/W emulsions.

Conclusion

W/W emulsions formed by mixing dextran and PEO can be stabilized by adding a small amount of linear polymers that adsorb at the interface and form a layer around the dispersed droplets. It appears that only polymers that are charged and contain hydrophobic groups can stabilize these emulsions by a combination of electrostatic and steric hindrance. Two cationic (Chit and DEAED) and one anionic polysaccharide (PGA) have so far been identified as having a strong stabilizing effect on D/P emulsions. However their behaviour as a function of the pH, ionic strength, polymer concentration and emulsion concentration was found to be different. Only DEAED could stabilize P/D emulsions. The different behaviour of these 3 polysaccharides is probably related to differences in the interaction between the polymers at the interface.

ASSOCIATED CONTENT

Supporting Information. Number and volume weighted size distributions of dextran droplets in D/P emulsions. Photographs of D/P emulsions at different pH and Chit concentrations taken at different times after preparation. CLSM images of D/P and P/D emulsions at 0.1 M NaCl in the presence of 1 g/L polymer. CLSM images of a D/P emulsion containing DEAED in the presence of 0.1 M NaCl.

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