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Effect of charge on the stabilization of water in water emulsions by thermosensitive bis-hydrophilic microgels

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Abstract

Hypothesis

Molecular surfactants are not able to stabilize water-in-water (W/W) emulsions, unlike nano or micro-particles, which can achieve this in some cases. However, the effect of electrostatic interactions between particles on the emulsion stability has rarely been investigated. We hypothesize that introducing charges modifies the stabilization capacity of particles and renders it both pH- and ionic strength-dependent.

Experiments

Charge was introduced into bis-hydrophilic and thermoresponsive dextran/poly*N*-isopropylacrylamide microgels by replacing a small fraction of poly*N*-isopropylacrylamide with acrylic acid groups. The size of the microgels was obtained by dynamic light scattering. The stability and microstructure of dextran/poly(ethyleneoxide)-based W/W emulsions, was studied as a function of pH, NaCl concentration and temperature using confocal microscopy and by analytical centrifugation.

Findings

The swelling degree of charged microgels depends on the pH, ionic strength and the temperature. In the absence of salt, charged microgels do not adsorb at the interface and has little stabilizing effect even after neutralization. However, the interfacial coverage and the stability increases with rising concentration of NaCl. Salt induced stabilization of these emulsions was also observed at 50°C. Increasing the temperature strongly influences the emulsion stability at low pH.

1. Introduction

Water-in-water (W/W) emulsions, also referred to as "aqueous-two phase systems" (ATPS) are generated by mixing two incompatible types of polymers in aqueous solution, which form two distinct phases [1]. These systems have attracted interest as a simple route to form aqueous microcompartments [2]. Many molecular species tend to partition preferentially to one of phases, which means that they can spontaneously localize and concentrate within the dispersed phase [3]. As such, they are also considered as interesting tools to study rudimentary forms of artificial cells [4]. However, stabilization W/W emulsions is challenging. Compared with oil-in-water (O/W) emulsions, the interface separating aqueous phases is thicker and has a much lower interfacial tension[5]. For these reasons small surfactants are inappropriate to stabilize W/W emulsions, but particles of sufficiently large size may in some cases spontaneously adsorb at the interface leading to an effective stability of W/W emulsions [6-8]. The driving force for the adsorption is the reduction of the total interfacial tension when a particle is located at the interface. This means that the particles need to have some affinity with both phases. In fact, the optimum situation for adsorption is when the particles prefer each phase equally and therefore partition equally between the phases. It has been found, however, that adsorption at the interface is not by itself sufficient to obtain stability and interaction between the particles at the interface appears to play a crucial role. Another interesting feature of W/W emulsions is that the stability generally depends on which phase is the dispersed phase even if the emulsions are situated on the same tie-line and therefore the interfacial tension is the same [9-11].

Examples of amphiphilic block copolymers micelles [12, 13], liposomes [14] and even recently lipidic bilayers [15] have been reported. Different types of particles have been studied for their ability to stabilize W/W emulsions, such as polymers [16, 17], protein aggregates [9, 10, 18], fat particles [19], mineral particles [20, 21] and synthetic microgels [22]. Among them, bis-hydrophilic microgels formed by crosslinking dextran (Dex) grafted with poly(N-isopropylacrylamide) (pNIPAM) show interesting effects on the stability of W/W emulsions formed by the mixture of poly(ethylene oxide) (POE) and Dex [11]. The two components of these microgels present a different affinity for each phase of this system. In addition, the thermosensitivity of pNIPAM causes these microgels to shrink when heated above 32°C and was found to have an effect on the stability. Notably, at room temperature, emulsions of the DEX rich phase dispersed in the PEO rich phase (D/P) were found to be much more stable than

the inverse emulsions (P/D), whereas at 45°C P/D emulsions were more stable than D/P emulsions even though the emulsions were situated on the same tie-line.

The aim of this investigation was to study the effect of electrostatic interactions on the stabilization of the same model W/W emulsion by these microgels. Beyond the fundamental character aimed at understanding the stabilization mechanisms of water/water emulsions, this study shows that modulating the pH, ionic strength or temperature can be used as triggers to modulate the stability and the behavior of the emulsions for potential applications Acrylic acid (AA) groups were incorporated into the otherwise neutral microgels during the synthesis. AA was chosen, because the copolymerisation reaction can be easily done with this monomer, it is well-described in the literature [23-27] and its protonation can be modulated by varying the pH. In this manner, the charge density of the microgels could be modified by varying the pH while the strength of electrostatic interactions could be varied either by changing the charge density or by adding salt. We will show the effect of electrostatic interactions on the size of the microgels and their capacity to stabilize W/W emulsions as a function of the temperature. Dynamic light scattering measurements were used to determine the size of the microgels and the stability of the emulsion was evaluated by measuring the transmission profile during centrifugation. The evolution of the microstructure of the emulsions with time was probed by confocal laser scanning microscopy (CLSM), which also allowed us to measure the partition of the microgels between the two phases and at the interface. We will compare the effect of the charged microgels with that of neutral microgels with the same bis-hydrophilic composition [11].

2. Materials and methods

2.1. Materials

All reagents were purchased from Sigma-Aldrich including Dextran (M_w=4.5-6.5x10^5 g/mol) and POE (Mw=2x10^5 g/mol). Dextran from Leuconostoc spp. (Mw = 6000 g/mol) was further substituted with methacrylic anhydride according to Tea et al. [12]. Before ensuing synthesis, N-isopropylacrylamide (NIPAM, purity 97%) was recrystallized and dried overnight under vacuum. Acrylic acid (purity 99%) was used as received.

2.2. Synthesis of microgels

The bis-hydrophilic microgels were synthesized by aqueous free-radical precipitation polymerization of DEX functionalized with methacrylic anhydride (Dex-MA) and *N*-isopropylacrylamine (NIPAM) as described in detail by Merland et al. [11]. The degree of substitution (DS) of Dex-MA by the methacrylate groups was 12 mol%. The mass fraction of Dex-MA in the microgel synthesis was 27 wt%. In order to introduce the charge, a fraction of the NIPAM units were replaced by acrylic acid (AA) during the synthesis, see Fig. 1. Fluorescently labelled microgels were obtained by adding acryloyl fluorescein (0.2 wt.%) to the monomer mixture. Microgels with 14 mol% NIPAM units replaced by AA units were studied in most detail, but microgels with 5 mol% and 28 mol% AA units were also investigated. For the sake of simplicity, the microgels without AA will be called "neutral" and the ones with AA will be called "charged" microgels whatever the pH, although most AA are neutral at pH 3. Transmission Electronic Microscopy images show that microgels are approximately spherical (See Fig. S1 in the SI). However due the necessary treatment, the microgels were aggregated which DLS shows not the case in aqueous suspensions, see below.

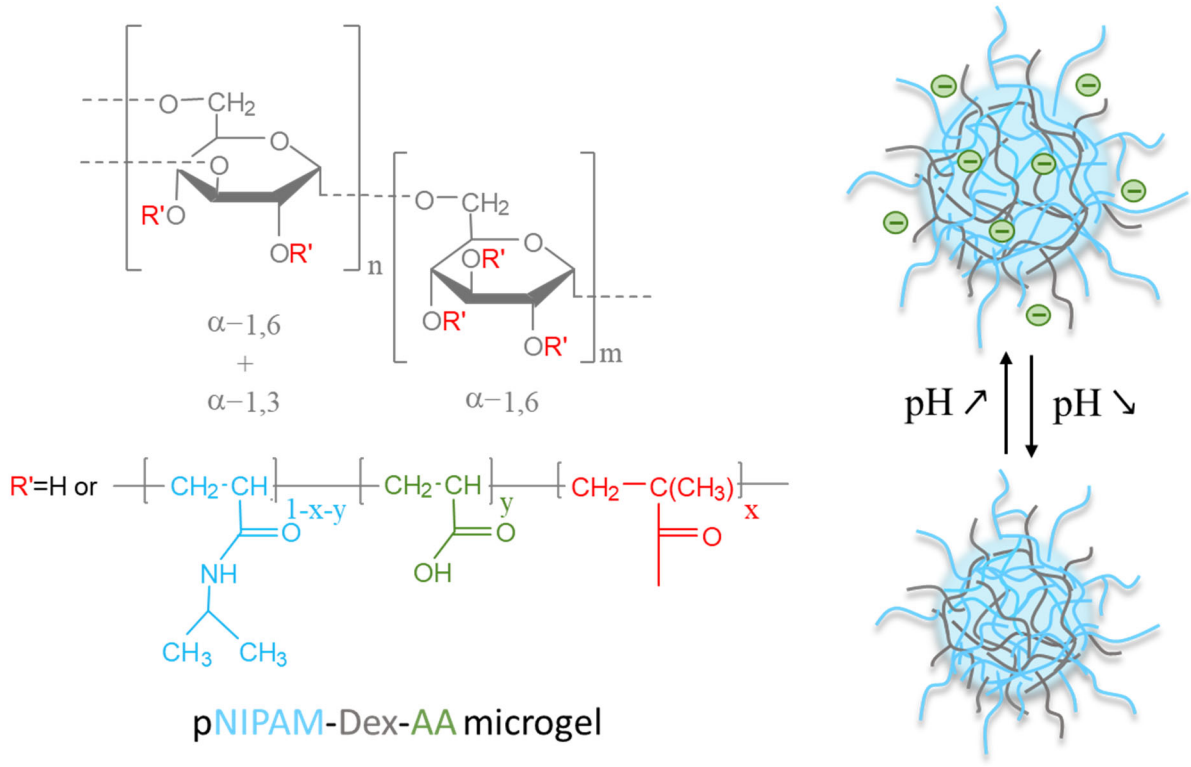

Figure 1. Scheme of the synthesis and pH-dependent swelling of charged microgels

2.3. Emulsion preparation

Aqueous stock solutions of DEX and PEO were prepared by dissolving the powders in ultrapure water (Milli-Q®, Millipore). A small amount of silica particles are present in the PEO powder that was removed by centrifuging the PEO solutions at $5x10^4$ g for 4 h. The phase diagram of the PEO and Dex used here has already been published by Gonzalez-Jordan et al. [28] and is reproduced as Fig. S2 in the supporting information (SI). Emulsions with the Dex phase dispersed in the continuous PEO phase (D/P) and the reverse (P/D) were prepared with a volume fraction of the dispersed phase equal to 25% at compositions situated on the same tie-line as indicated in Fig. S2. The two phases were almost pure in Dex (8.2 wt%) and POE (15.8 wt%), respectively. The concentration of microgels was fixed at 0.05 wt%.

2.4. Methods

2.4.1 Dynamic light scattering

The hydrodynamic diameter of the microgels was determined by dynamic light scattering using an ALV-5004 correlator in combination with an ALV-CGS3 goniometer (ALV-Langen). The light source is a He-Ne laser with a wavelength of 632.8 nm. Measurements were made as a function of the scattering wave vector ($q = (4\pi n/\lambda).\sin(\theta/2)$, where n is the refractive index of the solution and θ is the scattering angle between 20° and 150°. The temperature was controlled by a thermostated bath.

The measured intensity autocorrelation functions could be fitted to a single relaxation mode with an average relaxation time (τ) that was used to calculate the translational diffusion coefficient: $D = 1/(\tau.q^2)$ of the microgels [29]. The average hydrodynamic diameter (d_h) was determined from D extrapolated to q=0 (D_0) using the Stokes-Einstein relation: $d_h = kT/(3\pi\eta D_0)$. Measurements were done at several concentrations of microgels to determine the effect of interactions, which was important for the highly charged microgels in water without added salt. It was found that in this case the microgels needed to be diluted down to 0.02 wt% in order to be able to neglect interactions. The necessity to do the measurements at very low concentration explains why the error bar on the measured d_h is larger for the highly charged microgels in water, see Fig.4. Alle suspensions were filtered through a 0.45 μm pore size filters.

2.4.2 Confocal laser scanning microscopy

A confocal microscope Zeiss LSM 800 (Carl Zeiss Microscopy GmbH, Germany) was used with water immersion objectives (X25 and X63) to image the microgels. The microgels were covalently labeled with the fluorochrome FITC (Fluorescein IsoThioCyanate). A 490 nm wavelength laser was used to excite FITC and the emitted light was detected between 490 and 700 nm.

For observation, the samples were inserted between slides and coverslips and sealed using a double-sided sticker. The sealed slides were stored vertically in a holder and then observed horizontally at different positions to probe effects of creaming or sedimentation.

A device called RheOptiCAD® was used to control the temperature of the samples during observation with the confocal microscopy [30] (CAD Instruments, Les Essarts-le-Roi, France). In order to avoid strong temperature gradients an air objective (X50) was used for observation at high temperatures.

2.3.3 Emulsion stability analysis

The emulsion stability was analyzed by measuring the transmission profile of the samples along the length of the tubes as a function of time. In order to speed up creaming/sedimentation and destabilization, measurements were done during centrifugation. A commercial device (LUMisizer®, LUM GmbH, Berlin, Germany) was used, the operation of which is explained in detail elsewhere [31, 32]. Its application to study the stability of W/W emulsions was discussed in detail by Tea et al. [33]. The tubes are positioned horizontally in the rotor of a centrifuge. A light beam passes through the tubes during centrifugation and the transmitted intensity is measured along the length of the tube. The device allows temperature control between 4 and 60°C.

Fig. S3a and S3b in the SI show examples of transmission profiles during centrifugation at 470g for D/P and P/D emulsions that are not stabilized by microgels. Notice that the centrifugal force is here from the left to the right, which corresponds to from the top (100%) to the bottom (0%) of the sample under gravity. The transmission increased both at the top and the bottom due to the formation of a homogeneous transparent phase of POE and Dex, respectively. This can be explained by the sedimentation and coalescence of the Dex phase droplets in the D/P emulsion and the creaming of the PEO phase droplets in the P/D emulsion. The sedimentation/creaming front is not sharp because the droplet size is dispersed. The

droplets coalesce and form a continuous layer, which height increases with time until the equilibrium value of 25% of the total height is reached after 30 min. A similar development of the transmission profile was observed for a D/P emulsion in the presence of the microgels, see Fig. S3c, but the evolution with time was much slower and equilibrium had not yet been reached after 30 min, suggesting that the microgels retard droplet coalescence.

The rate at which the droplets coalesce into a continuous phase can be quantified by measuring as a function of time the transmission of the section of the sample where the dispersed phase forms a continuous phase at equilibrium (0-25% for D/P and 75-100 % for P/D). An example is shown in Fig. S4 in the SI for a P/D emulsion without microgels. The transmission gradually increases until it reaches a plateau around 85%, which corresponds to the value for the homogeneous phase of POE. A characteristic time (t_c) is defined as the time at which 50% of the transmission variation is reached. Repeat measurements were done for a number of samples and the uncertainty of the results can be estimated from the spread of the data for the same emulsions shown in Figs 7 and 9.

3. Results

3.1 Characterization of the microgels

The hydrodynamic diameter (d_h) of the charged microgels in which 14% of the NIPAM units was replaced by acrylic acid units was determined by dynamic light scattering as a function of the temperature at different pH, with and without 0.1 M NaCl, see Fig. 2. At 20°C, d_h = 260 nm at pH 7 and pH 5, but decreased to 170 nm at pH 4 and 120 nm at pH 3. The decrease can be explained by the deswelling due to a decrease of the charge density of the microgels, given that the pKa of acrylic acid is 4.3.

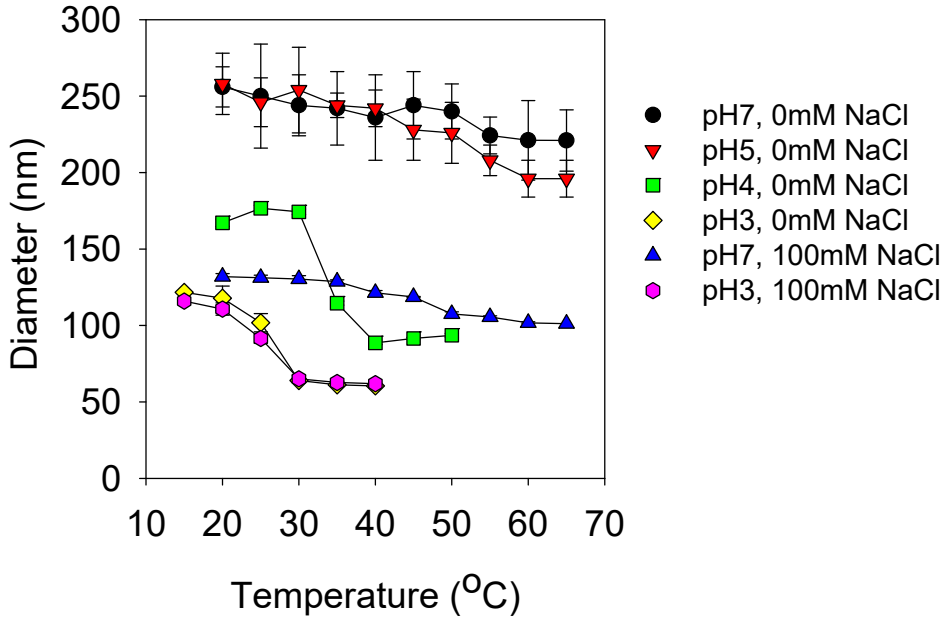

Figure 2. Temperature dependence of the diameter of charged microgels (F_{AA} = 14%) at different pH with and without added NaCl as indicated in the figure. The error bar is based on the spread of the data as a function of the scattering wave vector.

At pH 7 and 5, the size of the microgels decreased only weakly, down to 230 nm and 200 nm, respectively, when increasing the temperature to 65°C. However, shrinkage of the microgels was observed at pH 4 starting at 30°C and at pH 3 starting at 20°C. At both pH, d_h decreased by about 2-fold. It appears that the high charge density at pH 5 and pH 7 inhibited shrinkage, and countered the hydrophobic attractions between the NIPAM segments at higher temperatures. The lower charge density at pH 3 compared to at pH 4 may explain why the shrinkage started at a lower temperature at pH 3 than at pH 4.

Addition of NaCl led to shrinkage of the strongly charged microgels at pH 7 and further weak shrinkage during heating starting at 35°C, see Fig. 2. A more detailed study of the effect of adding salt at 20°C and 50°C showed that shrinkage was important already at 5 mM NaCl, see Fig. S5 of the SI. The effect of NaCl can be explained by screening of electrostatic repulsion. At pH 3, the microgels have very little residual charge, which explains why NaCl had almost no effect on the size of the microgels.

Electrostatic repulsion can be reduced either by lowering the charge density through lowering the pH or by screening through salt addition. Both led to shrinkage of highly charged microgels at 20°C. However, the effect of heating was found to be very different. Lowering the charge density had a much stronger effect on the temperature dependence than screening. The difference is that in the case of screening the AA units are still charged, but the electrostatic repulsion between the charged is screened, whereas at low pH the AA units are no longer charged. It appears that the presence of charges inhibits temperature induced deswelling even if screened, perhaps because in this case the AA units cannot approach closely. The temperature at which shrinkage began for microgels with low charge density was even lower than for equivalent neutral microgels. This observation shows that neutralized acrylic acid groups promoted shrinkage.

The effect of heating on d_h was also studied for microgels for which the fraction of AA units was F_{AA} = 5% or 29%. At 20°C the d_h of these microgels was, respectively, 280 nm and 250 nm at pH 7 and 140 nm and 90 nm at pH 3. Fig. 3 shows that at pH 7 deswelling is more gradual and occurs at higher temperatures when the microgels contain more charges both in pure water and with 100 mM NaCl. The most strongly charged microgels showed no significant deswelling up to 70°C. At pH 3 where the AA units are neutral, the extent of deswelling did not depend significantly on F_{AA}, but the temperature where deswelling started decreased with increasing F_{AA} from about 35°C at F_{AA} = 0% to about 25°C at F_{AA} = 29%. It should be noted that the microgel suspensions remained colloidally stable whatever the salinity and temperature, thanks to the steric stabilization provide by the dextran chains, as discussed by Merland et al. [11].

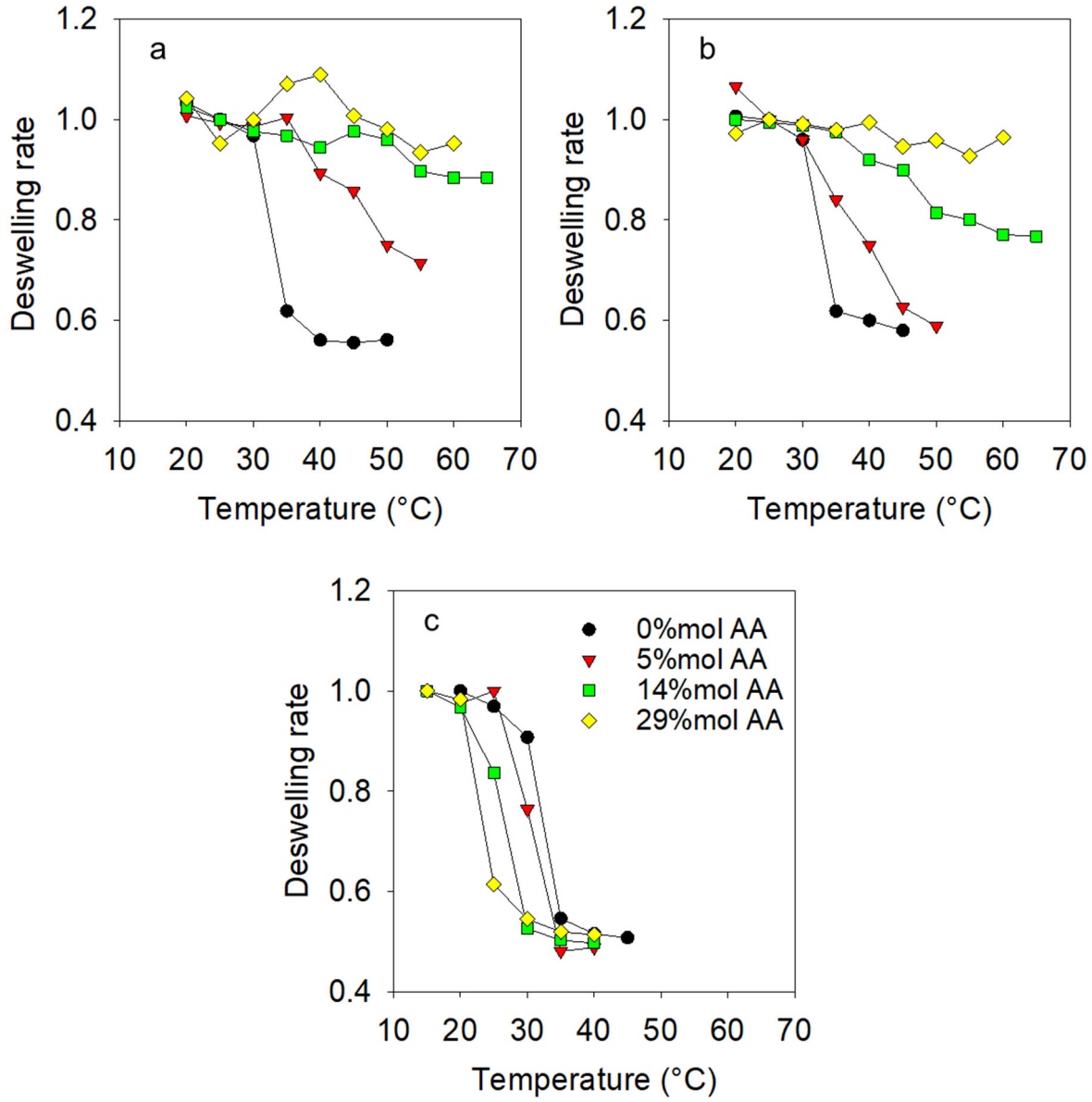

Figure 3. Deswelling of microgels with different fractions of AA units as a function of the temperature at pH 7 in pure water (a) or in 100 mM NaCl (b) or at pH 3 in pure water (c).

3.2 Emulsion microstructure and stability

In first instance, we will focus on the properties of emulsions containing microgels with F_{AA} = 14%. We will present results on the effect of varying the pH without added salt, the effect of the NaCl concentration at pH 7 and the combined effects of reducing the pH to 3 and adding 100 mM NaCl. Measurements were done at 20°C and 50°C to see the effect of increased hydrophobic interactions between the NIPAM units. Finally, the effect of varying the fraction of AA units will be discussed.

3.2.1 Effect of the pH

The microstructure of D/P and P/D emulsions with charged microgels did not depend significantly on the pH, see Fig. 4. No distinct layer of charged microgels at the interface was observed at any pH. This is in stark contrast with emulsions containing corresponding neutral microgels for which the layer is clearly visible, see Fig. 4. In all systems, the microgels preferred the PEO phase over the DEX phase. The partition of the microgels between the two phases was quantified using fluorescence intensity profiles across droplets, see Fig. S6 of the SI. The ratio of the microgel concentration in DEX over that in PEO was approximately 0.65 for charged microgels in D/P emulsions and 0.39 in P/D emulsions independent of the pH, see Fig. S7 of the SI. The same partition was approximately found for the neutral microgels. The fact that the partition depends on the relative volume fractions of the phases suggests that the composition of the microgels is disperse, so that not all microgels partition the same between the phases.

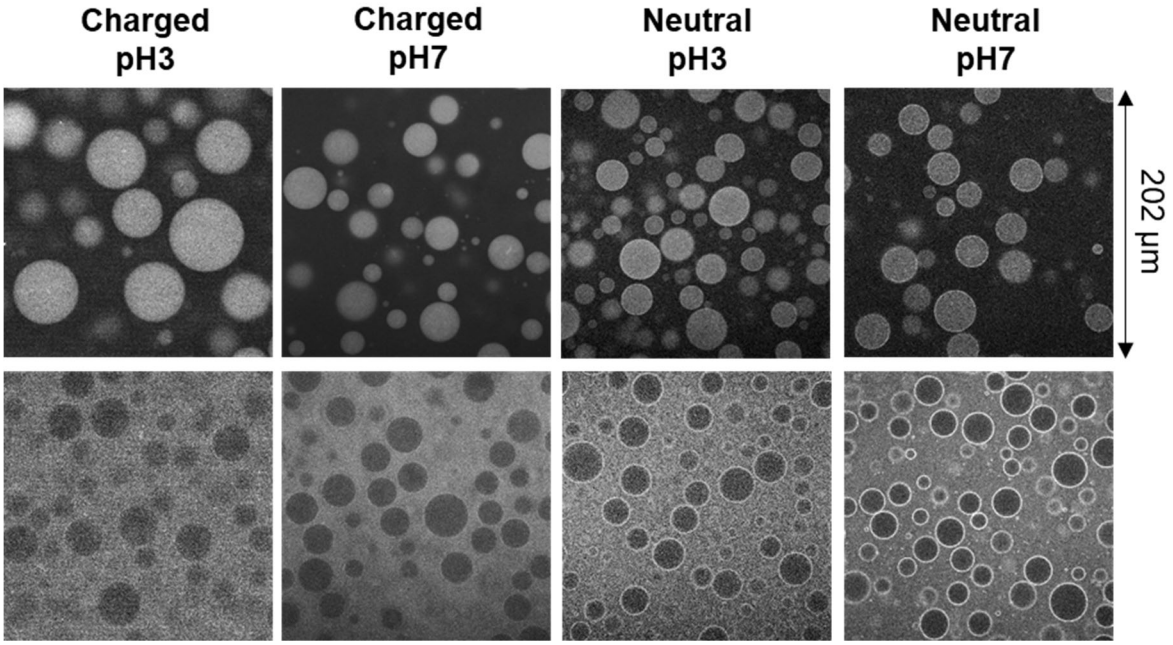

Figure 4. CLSM images of P/D (top) and D/P (bottom) emulsions at 20°C with neutral microgels and charged microgels (F_{AA} = 14%) at different pH as indicated in the figure.

The stability of the emulsions was quantified by measuring the transmission profiles at different times during centrifugation at 470 G and determining a characteristic time (t_c), as was explained in section 2.3.3. Centrifugation accelerates destabilization and allows us to distinguish between systems that are stable for more than a week under gravity, which is the

case when $t_c > 10^3$ s, see Fig. S8 and Fig. S9 in the SI. The results at 20°C show that the stability of D/P or P/D emulsions during centrifugation was not clearly improved by the presence of charged microgels with $t_c \approx 200$ s independent of the pH, whereas $t_c \approx 150$ s in the absence of microgels, see Fig. 5a. Adding neutral microgels was also not very effective in stabilizing P/D emulsions, but it was more effective in D/P emulsions for which $t_c \approx 500$ s. There was no significant effect of the pH in the presence of neutral microgels. It iss worth to notice that the trends are similar at 1G and 470G. However, t_c are ate least two orders of magnitude higher at 1G than under acceleration for neutral particles, see Fig. S8 in the SI. In addition, D/P emulsions present a higher stability at 1G, especially with neutral particles.

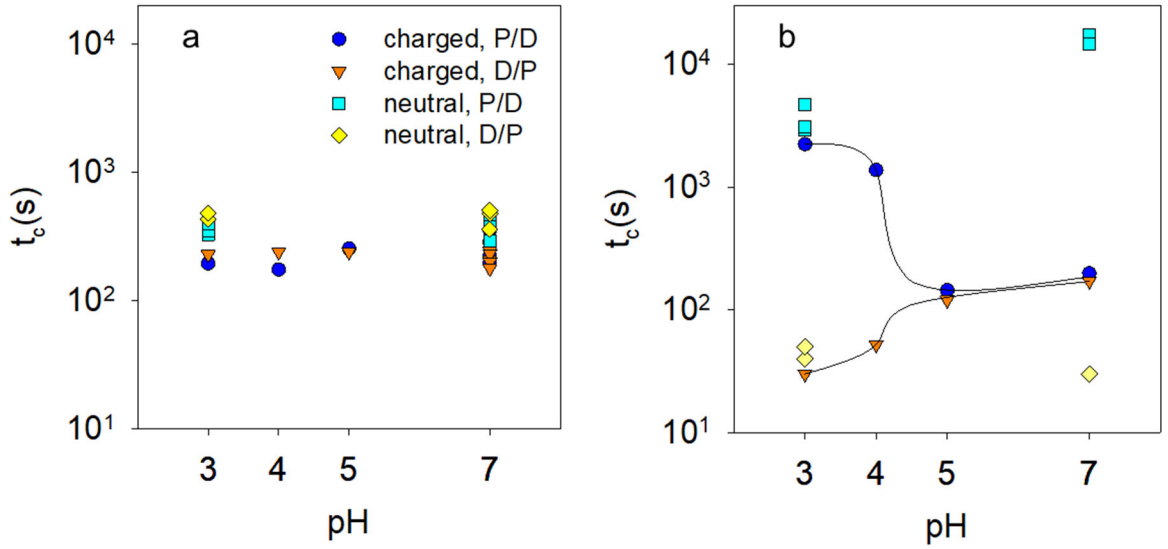

Figure 5. Characteristic stabilization times of P/D and D/P emulsions at 20°C (a) or 50°C (b) with neutral microgels at pH 7 or charged microgels (F_{AA} = 14%) at different pH as indicated in the figure.

The emulsions that were formed at 20°C did not change their morphology after heating to 50°C, see Fig. S10 of the SI. However, some aggregates of microgels were formed at 50°C in the D/P emulsions at pH 3 and pH 4, which had a tendency to adsorb at the interface, but we don't think that these aggregates had an influence on the stability of the emulsions. The partitioning of the microgels between the phases remained the same after heating to 50°C within the experimental error, see Fig S11 of the SI.

At pH 5 and 7, heating to 50°C did not have a significant effect on the stability of the D/P and P/D emulsions with charged microgels, see Fig.5b. However, at pH 3 and pH 4, the P/D emulsion was much more stable after heating, whereas the D/P emulsion was less stable.

At pH 3, the effect of heating on the stability of the P/D and D/P emulsions with charged microgels was similar to that with the equivalent neutral microgels, but at pH 7 the D/P emulsion was much more stable in the presence of neutral microgels. The strong difference of stability between D/P and P/D emulsions at pH 7 with neutral microgels was not observed with charged microgels, which may be related to the fact that the latter did not shrink much during heating, see Fig. 2.

3.2.2 Effect of salt

Fig. 6 shows CLSM images of emulsions containing fully charged microgels at pH 7 after adding different amounts of NaCl. It is clear that screening the electrostatic interactions by adding NaCl induced adsorption of charged microgels at the interface in both P/D and D/P emulsions, but more strongly in the latter. In addition, the average size of the DEX droplets in D/P emulsion decreased strongly when NaCl was added from 23 μm on average without salt to 10 μm if more than 20 mM NaCl was added, see Fig. S11 of the SI, whereas for the P/D emulsion, it remained constant at approximately 22 μm. Screening electrostatic interactions through salt addition did not have the same effect as reducing the charge by lowering the pH. The microstructure of emulsions with neutral microgels was not influenced by the addition of salt.

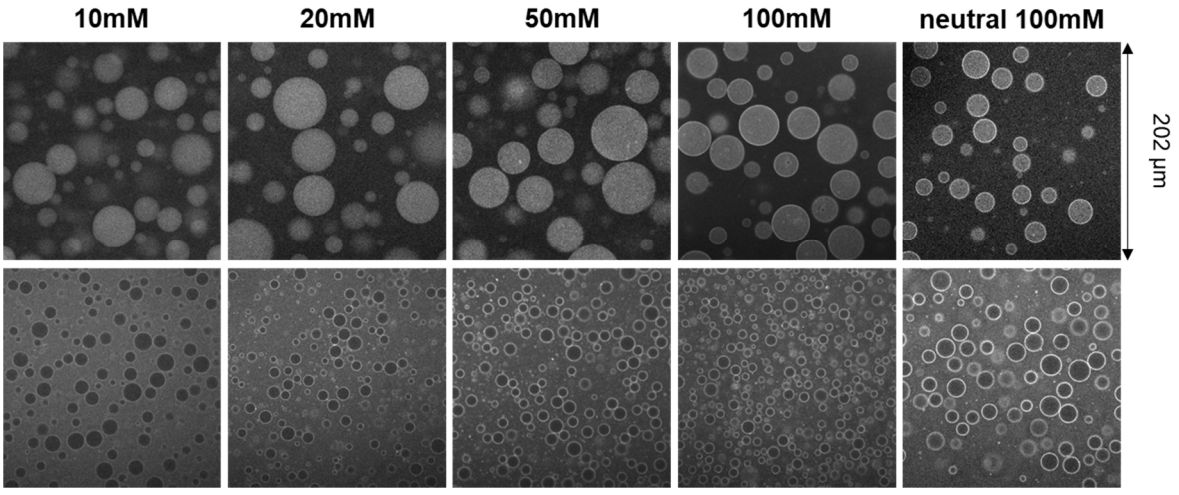

Figure 6. CLSM images of P/D (top) and D/P (bottom) emulsions at 20°C with charged microgels (F_{AA} = 14%) at different NaCl concentrations at pH 7, as indicated in the figure. Results with neutral microgels at 100 mM NaCl are also shown for comparison.

The concentration ratio of the charged microgels between the DEX and PEO phases increased weakly with increasing NaCl concentration up to 0.89 at 100 mM for the D/P

emulsions and remained at 0.39 for the P/D emulsions, see Fig. S12 of the SI. For the D/P emulsion, the excess fluorescence intensity due to charged microgels at the interface increased progressively with increasing NaCl concentration to about 2.5 times the intensity in POE phase at 100 mM NaCl, see Fig. S13 of the SI. For the P/D emulsion, the excess at the interface was only significant at higher concentrations of NaCl (about 1.5 times that in POE phase at 100 mM NaCl). It's worth noting that the excess fluorescence at the interface was larger for neutral microgels (about 3.5 times in D/P and 2.2 times in P/D).

The D/P emulsion was found to be much more stable when NaCl was added, whereas the effect was weak for the P/D emulsion. D/P emulsions with charged microgels at 100 mM NaCl were stable for at least one week showing no significant change in the microstructure and no signs of sedimentation under gravity. The characteristic stabilization times are plotted as a function of the NaCl concentration in Fig. 7a showing that the stability improved markedly with increasing NaCl concentration for the D/P emulsion, but the effect was negligible for the P/D emulsion. The effect of adding salt on the stability of emulsions with neutral microgels was small for both P/D and D/P emulsions. At 1G, the trends are the same as at 470G, except that t_c is much larger especially for charged particles where above 10 mM, no change was observed even after a week, see Fig S9 of the SI.

Heating solutions prepared at 20°C to 50°C did not have a significant effect on the morphology of the emulsions, see Fig. S14 of the SI, nor on the partitioning, see Fig. S12 of the SI. The stabilizing effect of adding salt for the D/P emulsion at 20°C was also observed at 50°C, albeit to a lesser extent, see Fig. 7b. In fact, the charged microgels behaved similarly at 20°C and 50°C, which could be explained by the weak effect of heating on the size of the microgels, see Fig. 2.

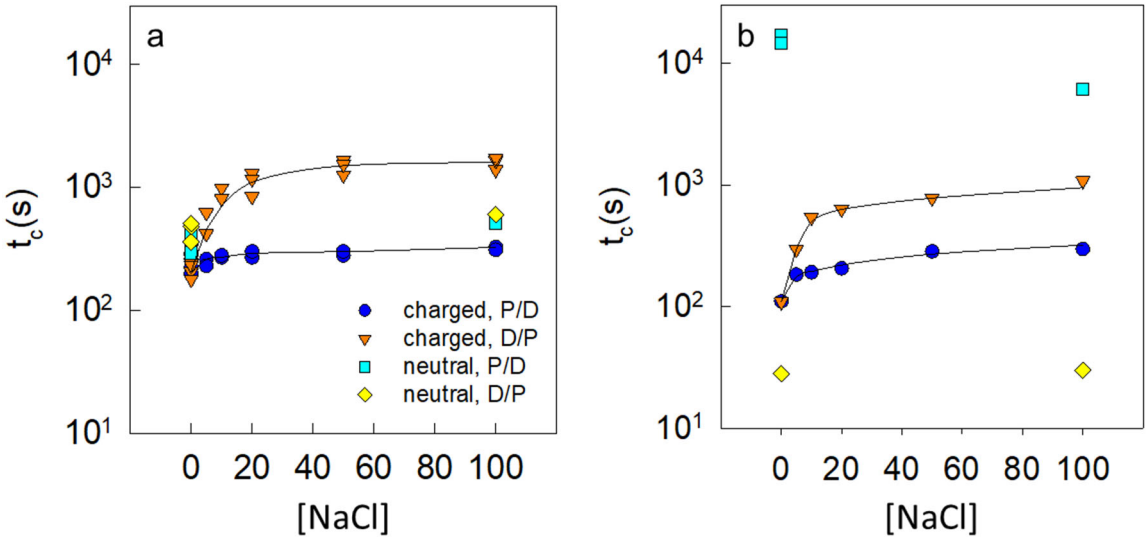

Figure 7. Characteristic stabilization times of emulsions with neutral and charged microgels (F_{AA} = 14%) in different NaCl concentration at 20°C (a) and 50°C (b).

3.2.3 Combined effect of pH and salt

To study the combined effects of low charge density and screening, emulsions were prepared at pH 3 and 100 mM NaCl. Fig. 8 shows that adding salt favored adsorption of the microgels at the interface also at pH 3 for both D/P and P/D emulsions even though their charge density at pH 3 was very small. However, adding salt at pH 3 had little effect on the droplet size or the stability either at 20°C or at 50°C. Thus, the spectacular effect of the addition of salt on the D/P emulsion observed with the fully charged microgels at pH 7 was not reproduced at pH 3, which means that a high density of charges is necessary to induce the effect. We expect that the effect of salt on the stability of the D/P emulsion will be intermediate at intermediate pH between very strong at pH 7 and very weak at pH 3.

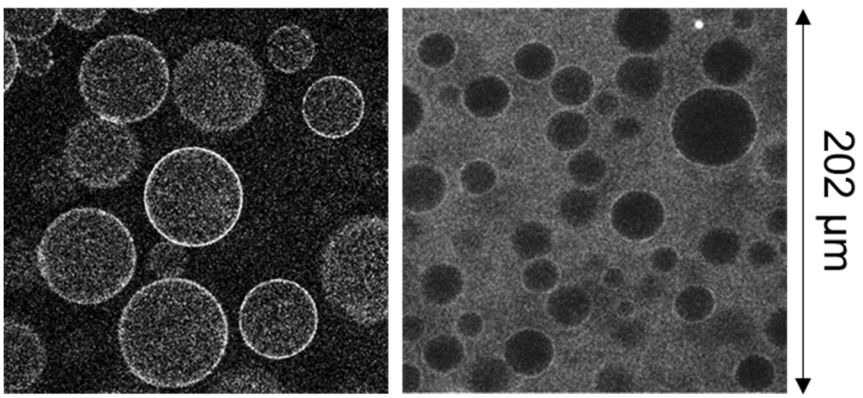

Figure 8. CLSM images of P/D (left) and D/P (right) emulsions at 20°C with charged microgels (F_{AA} = 14%) pH 3 and 100 mM NaCl.

3.2.4. Effect of the fraction of acrylic acids units

So far we have discussed the properties of emulsions containing microgels with F_{AA} = 14%. The effect of varying the charge density was investigated by comparing emulsions containing microgels with different fractions of AA units at pH 7. In the absence of salt even including only 5% AA units inhibited the microgels to accumulate at the interface, see Fig. S15 of the SI. In the presence of 100 mM NaCl, the microgels accumulated at the interface at all charge densities and the size of the dispersed droplets decreased with increasing charge density.

At 20°C, the effect of the fraction AA in the microgels on the emulsion stability in the absence of salt was insignificant at pH 7, see Fig. 9a. In section 3.2.2 we showed that the strong increase of the stability of the P/D emulsion with neutral microgels after heating to 50°C disappeared when 14% AA units were introduced into the microgels. Fig. 9a shows that the effect of heating on the stability is still important at F_{AA} = 5%, albeit less than with neutral microgels. The addition of 100 mM NaCl did not improve the stability of the P/D emulsion at 20°C, but the stability of D/P increased sharply with increasing fraction of charged units, see Fig. 9b. Heating to 50°C decreased the stability of the P/D emulsion at low charge density and the D/P emulsion at high charge density. At pH 3, where the AA units are neutral the effect of F_{AA} on the stability was relatively small showing a weak decrease of the stability with increasing fraction of AA units, see Fig. S16 of the SI.

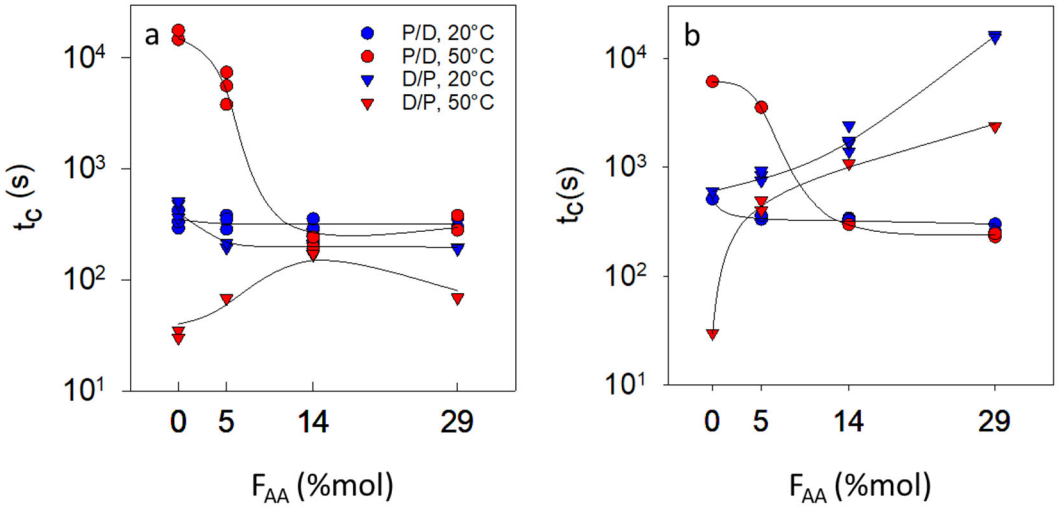

Figure 9. Dependence on F_{AA} of the characteristic stabilization time for P/D and D/P emulsions at pH 7 without (a) or with NaCl 100mM (b) at 20°C or 50°C, as indicated in the figure.

4. Discussion

Introducing AA groups into neutral bis-hydrophilic microgels led to a strong reduction of their adsorption at the W/W interface of POE-Dex the emulsions in pure water, probably due to electrostatic repulsion between the microgels. This would explain why adding salt favored adsorption of the microgels at pH 7. However, one would expect that neutralization of the charged groups by lowering pH to 3 would also enhance adsorption at the interface, which was not clearly observed. The observation that charged microgels do not adsorb at the interface cannot be explained just by electrostatic interactions, as reducing the charge density by decreasing the pH did not have a significant effect even though it did have an effect on the size of the microgels. After adding salt, the microgels adsorbed at the interface, which had a strong stabilizing effect on D/P emulsions at pH 7. The stabilizing effect on D/P emulsions was stronger with microgels that contained more AA units, but adsorption of microgels had no stabilizing effect on the P/D emulsion. As we mentioned in the Introduction, adsorption of the microgels at the interface does not in itself guarantee a stabilization of the emulsions. Clearly, there are additional contributions coming onto play, which cause the different effect on the stability of P/D and D/P emulsions even though the microgels are adsorbed at the interface in both cases.

It is clear that the stability of the emulsions by the microgels cannot be explained either by the partitioning or by the density of the microgels at the interface. This is most obvious when we compare the stability at 20°C and at 50°C. At pH 3 and pH 4, heating led to an increased stability of P/D emulsions and a decreased stability for D/P emulsions even though the interfacial tension of the P/D and D/P emulsions was the same and there was no effect on the microstructure nor on the partitioning of the microgels between the phases and the interface. We therefore conclude that the effect of the microgels on the stability is most likely related to overall interaction between the microgels at the interface, not just the electrostatic interaction, which we believe needs to be net attractive to obtain stability.

It is of interest to compare the present results with those obtained by Nguyen et al. [22] with microgels formed by copolymerisation of ethyl acrylate, methacrylic acid (MAA) and 1,4-butanedioldiacrylate. These microgels also showed strong shrinkage when the charged MAA units were neutralized, decreasing from 220 nm at pH ≥ 7.2 to 60 nm at pH ≤ 6.5. The microgels partitioned strongly to the PEO phase below pH 7, but the relative concentration in the DEX phase increased when the microgels became charged, whereas no effect of the pH on the partition was observed in the present study. The emulsions containing these microgels were very stable at pH ≤ 7.5, but not at higher pH. The observed destabilization at pH > 7.5 could not be directly attributed to electrostatic interaction, because the microgels were already strongly charged at pH 7.5. Addition of salt did not lead to strong shrinkage for these microgels, but adding as little as 10 mM NaCl caused destabilisation of the very stable D/P emulsions formed at pH 7.2 and 7.5. It also shifted the preference of the microgels from the PEO phase to the DEX phase. This destabilizing effect of adding salt is opposite to the strong stabilizing effect reported here for D/P emulsions. The principal conclusion of this comparison is therefore that there does not appear to be a general effect on the stability of W/W emulsions by microgels that can be attributed to electrostatic interactions. The stability appears to be controlled by interactions between the microgels that depend on the pH and the ionic strength, but are specific to the chemical composition of the microgels.

Compared to equivalent neutral microgels, introducing AA units rendered the effect of the bis-hydrophilic microgels on the stability pH and salt sensitive in addition to the temperature sensitivity already observed for the neutral microgels. The effect of these parameters on the stability of D/P and P/D emulsions is best seen by inspecting figs. 5, 7 and 9. In particular, the equivalent neutral microgels stabilized the P/D emulsion only at high temperatures, whereas the charged microgels stabilized this emulsion both at low and high temperatures if more than 20 mM NaCl was added. The strong temperature effect on the stabilization of P/D emulsion shown by the neutral microgels was recovered with the charged microgels at pH 3 and pH 4 in the absence of salt, but with charged microgels the emulsion destabilized after increasing the pH. It is clear that introducing a small amount of acidic units can be useful to control and manipulate the stability of W/W emulsions.

5. Conclusions

Charged microgels swell in water due to electrostatic interactions. Decreasing charge density by decreasing the pH or screening the interaction by the addition of NaCl causes deswelling of the microgels. Merland et al. [11] have already shown that neutral bis-hydrophilic microgels shrink when the solution is heated above 32°C [11], because pNIPAM units dehydrate. Here we find that introducing charges into these microgels inhibits deswelling during heating due to strong electrostatic repulsion both in pure water and in the presence of salt. The effect of deswelling during heating is recovered when the charge density is reduced by lowering the pH. At low pH, deswelling starts at lower temperatures when more NIPAM units are replaced by neutral AA units.

Different types of particles have been used in the past to stabilize W/W emulsions [19-22, 28], but none showed a dependence both on temperature and pH. Elsewhere, Merland et al. [11] showed that by using bis-hydrophilic microgels containing both NIPAM and dextran the stability of a W/W emulsion depends on the temperature. Here we show that by introducing AA units into the bis-hydrophilic microgels the stability depends both the temperature and the pH. Charged bis-hydrophilic microgels are less efficient in stabilizing emulsions than the corresponding neutral microgels that were studied in and adsorb less at the interface. Screening the electrostatic repulsion between the microgels at pH 7 by adding salt increases the density of the microgels at the interface and improves the stability, in particular for the P/D emulsion. Adding salt also causes a decrease of the dispersed drop size in P/D emulsions. The stability is not improved at pH 3, where electrostatic repulsion is very small, even though the addition of salt promotes the adsorption of the microgels at the interface also at pH 3.

Elsewhere, it was shown that heating strongly improved the stability D/P emulsions with neutral microgels and decreased the stability of P/D emulsions [11]. No such effect was observed for strongly charged microgels, but it was recovered when the microgels were neutralized by decreasing the pH. The stabilizing effect of adding salt for D/P emulsions was also observed after heating.

The effects of varying the pH or adding NaCl on the stability of the emulsions cannot be related directly to the partition of the microgels between the phases and the interface as the latter depended little on the pH and the temperature. In an earlier study of the same W/W emulsion with another type of charged microgels, Nguyen et al. [22] showed different and

contrary effects of varying the charge density and adding salt on the stability and the partition. The implication is that the chemistry of the microgels is more important than electrostatic interactions.

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