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Emulsification and Stabilization Mechanisms of O/W Emulsions in the Presence of Chitosan

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We study emulsification of paraffin oil in aqueous solutions of chitosan without adding any other surfactant. By monitoring the surface tension of the water–paraffin interface, we show that chitosan itself has only a weak surface activity. Nevertheless, chitosan dissolved in the aqueous phase allows the dispersion of oil by increasing the matrix viscosity and provides stabilization of the oil–water interface by forming a dense polyelectrolytic brush on the water side of this interface. We characterize emulsions with varying oil content, and concentrations of chitosan, and follow their long-term stability. Finally, we show that by precipitating the chitosan the rigid elastic network is formed in the aqueous phase, making a very stable suspension.

1. Introduction

Emulsions are intensely studied because of their potential use in many industrial applications in food technology, personal products, paints, agrochemicals, pharmaceuticals, etc. Emulsification consists of dispersing one immiscible fluid into another (typically, although not uniquely, oil and water) via creation and stabilization of an interface. Therefore, emulsions are never at thermodynamic equilibrium and their stability is rather a kinetic concept. Their production requires the addition of mechanical energy typically provided using a shear flow, as well as the use of surface-active agents (surfactants, or amphiphilic molecules) to help reducing the energy of the interface area.

By adsorbing at the liquid–liquid interface, the low molecular weight (LMW) surfactants reduce the interfacial tension σ between oil and water. In the same way, many proteins are surface active (amphiphilic) and widely used in food industry to produce emulsions. Polysaccharides also may be used in preparation of emulsion, but not all of them have high surface activity.^{1,2} In fact, they often promote the emulsion production by increasing the viscosity of the water phase η_e in which they are dissolved. Thus, the dispersion of oil into droplets of size a occurs when the viscous stress in the suspending liquid $\eta_e \dot{\gamma}$ overcomes the characteristic Laplace pressure σ/a , neglecting the role of the viscosity of the oil phase.³ In the same way, increasing the viscosity of the water phase (by the addition of polysaccharide, surface active or not) slows down the diffusion of the dispersed droplets and therefore contributes to the emulsion longevity. It is worth noting that using biopolymers, which help emulsification and/or stabilization of emulsion, allows to reduce the concentration of LMW surfactant used (with economic and environmental benefits) and also leads to dispersed systems with different properties (mechanical, for example) that we would not obtain by using only LMW surfactant.

In this study, we are interested in chitosan, a ubiquitous biopolymer (second most abundant biopolymer after cellulose). It is a linear polysaccharide obtained by deacetylation of chitin, Figure 1. The original chitin is not soluble in water due to the strong hydrogen bonding between its aminoacetyl groups

(NHCOCH_3).⁴ By replacing the majority of these groups by the amine moieties (NH_2) chitosan molecules can be dissolved in water, but only in acidic conditions. In such solutions, chitosan is a positively charged polyelectrolyte due to the protonation of the amine groups (the NH_2 group in chitosan has a pK_a of around 6.4). Chitosan has biological characteristics (e.g., biodegradability, nontoxicity, musco-adhesion) and physicochemical properties (polyelectrolytic, rheological thickening), which make it attractive for many potential applications in areas such as foods, pharmaceutical industry, and bioengineering.^{5,6} Particularly, the texture of chitosan solution may be varied from simple liquid to weak gel to solidlike through the formation of reversible physical cross-linking (via ionic, hydrogen-bond, or hydrophobic interactions) or direct covalent cross-linking. For instance, chitosan in its hydrogel form is widely studied for application as controlled drug delivery systems^{7–10} or to mimic extracellular membranes.⁶

There are several papers available describing the stabilization of O/W emulsion where chitosan is adsorbed on oil surface through interaction with an added anionic surfactant or protein.^{11–13} The main aim of this study was to evaluate the dispersion of mineral oil in aqueous phase where chitosan was added without the help of any surfactant or protein, and how this polymer promotes the stability of the obtained emulsions. Two main parameters which usually affect emulsification and stabilization of emulsions were examined: the O/W interface tension and the viscosity of the external phase (chitosan solution). The variation of these two parameters was performed by changing the polymer concentration. The entanglement concentration of chitosan in solution was determined by rheological measurements. By comparing the effect of chitosan on concave and convex oil/water interfaces, we conclude that steric hindrance of a dense

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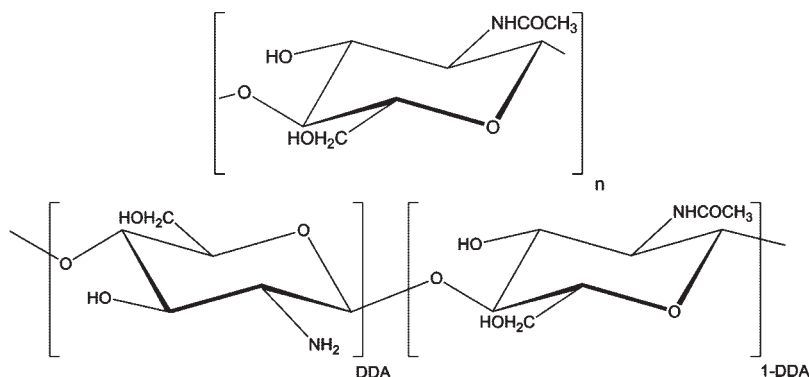


Figure 1. Chitin and chitosan molecules. The degree of deacetylation (DDA) characterizes the proportion of hydrophilic (NH_2) compared to hydrophobic (NHCOCH_3) groups on the polymer chain. DDA was 80% in our case.

polyelectrolytic brush in the water phase is responsible for the emulsion stability while the inverted emulsion is not stable. Finally, we investigated the solid (elastomeric) emulsion by precipitating (and physically cross-linking) droplets of emulsion solution and studied the ability of this system to contain oil for different concentration of paraffin.

2. Experimental Section

2.1. Materials. Chitosan was purchased from Acros with a broad molecular weight distribution of 100 000 to 300 000 g/mol. A deacetylation degree DDA $80 \pm 3\%$ (average value of three measurements) was measured according to Brugnerotto's method, using FTIR spectroscopy.¹⁴ Chitosan was dissolved in 0.1 M acetic acid aqueous solution (prepared from deionized water) to a concentration of 2 wt %. In this condition, chitosan is largely dissolved by protonation of its amine groups. The degree of protonation was determined to be approximately 0.95, using the method of Rinaudo et al.¹⁵ The solution was then filtered on a Swinex 25 support equipped with Whatman ashless filter paper. This stock solution was then dissolved to desired lower concentrations with acetic acid solvent. The pH was adjusted after dissolution to 5 with NaOH.

As a "model oil", liquid paraffin (mixture of n-alkanes) was provided by Gallenkamp, Griffin and George Ltd. and further purified by distillation under vacuum at 190 °C. Its density and viscosity were determined to be 869 kg/m³ and 0.14 Pa·s at 20 °C, respectively.

Emulsions were prepared by homogenizing paraffin and chitosan solution during 4 min at room temperature using a Silverson L4R laboratory mixer with a shear rate of 2800 s⁻¹. The volume fraction of paraffin varied across the whole range, between 0.05 and 0.9. The concentration of chitosan varied between 0.05 and 2 wt %.

The protocol used to prepare cross-linked beads of precipitated chitosan was previously described by Martinez et al.¹⁶ We adapted it to prepare also beads of O/W emulsions: pure solutions of chitosan, or various O/W emulsions freshly prepared in the water–chitosan solutions, were dropped into 10 (wt/vol)% sodium hydroxide solution through a flat-tip needle, by means of a peristaltic pump. As a result, the positively charged amine moieties of chitosan, which caused swelling in the acidic water phase, were neutralized in NaOH environment, making the polymer insoluble. However, since the hydrogen-bonding aminoacetyl groups are in the minority, the resulting chitosan becomes an elastic rubber (contrary to the rigid chitin). The droplet-wise delivery results in more or less spherical shape of precipitated chitosan beads (homogeneous, or containing oil droplets). The beads were collected and washed with distilled water until the solution they were suspended in recovered neutrality.

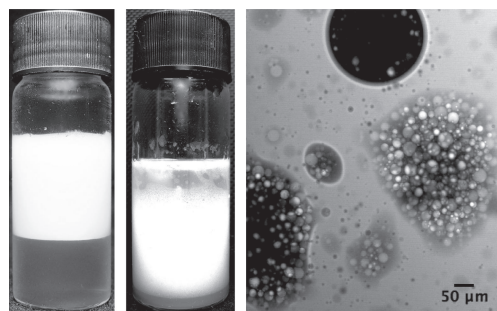


Figure 2. Creaming of an emulsion of paraffin in a 2 wt % chitosan solution, volume fraction = 0.34, 2 months of age (left image). Unstable O/W/O double emulsion of paraffin in a 2 wt % chitosan solution, volume fraction = 0.70, 1 day of age (the O/W emulsion sediments in the external oil phase) (middle image). Microscopic image of O/W/O double emulsion of paraffin in a 2 wt % chitosan solution, 0.70 volume fraction, aged for 1 day (right image).

2.2. Measurements. To characterize the interfacial properties of chitosan, we determined the time evolution of interfacial tension between air or paraffin, and aqueous chitosan solutions at different concentrations of polymer. These measurements were performed with a pendant-drop FTA32 instrument, equipped with a CCD camera to dynamically acquire images of a drop suspended from a needle. The shape of the drop is determined by the competition of specific gravity and the interfacial pressure. The surface tension σ between the constituent liquid of the drop and the surrounding liquid (or air) can thus be computed by analyzing this shape. In our case, the pending drop was an aqueous solution in air or paraffin. The drops were formed with a pump rate of 0.1 μL/s. The measurements were performed at 25 °C with an error of 1 mN/m.

The freshly prepared emulsions were observed under a confocal microscope (Zeiss LSM 510) using Nile Red A, a poorly soluble and weakly fluorescent probe in water (purchased from SIGMA). We checked that the addition of Nile Red A did not change the storage stability for all the studied emulsion. The observation under confocal microscope revealed the type of the emulsion: oil in water (O/W) or water in oil (W/O) or double emulsion. The microscope images were analyzed with the software ImageJ to determine the size distribution of the droplets.

The stability of the emulsions was judged in two aspects: against droplet coalescence and against creaming. Both were estimated visually at 20 °C in a graduated tube of 10 mL. The creaming rate, and its extent, were measured by the thickness of a layer of water underneath of the creamed emulsion, cf. Figure 2, while the coalescence was observed by the formation of a layer of oil on top of the emulsion.

Rheological measurements were performed using a DSR rheometer (Rheometrics Ltd.) equipped with a parallel-plate geometry (diameter 25 mm, gap 0.05 mm), cone-plate geometry having a diameter of 25 mm (cone angle 0.1 rad, gap 0.035 mm), or a couette geometry

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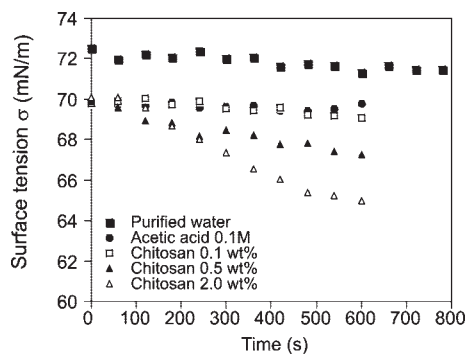


Figure 3. Surface tension of interface between air and the aqueous chitosan solution with 0.1 M acetic acid, at 25 °C, as a function of time.

(cup diameter 32 mm, bob diameter 29.5 mm, bob length 45 mm) according to the viscosity range. The temperature was kept at 24 °C during all measurements. Both frequency sweeps (to test the degree of gelation and stress relaxation) and dynamic stress sweep tests (at the frequency $\omega = 1$ rad/s, to verify the limits of linear response) were conducted. From steady stress sweep measurements, a shear thinning behavior was observed in the shear rate dependence of the viscosity after a shear-independent plateau at low shear rates for chitosan concentration varying from 0.01 to 2 wt % (see Supporting Information for chitosan 1, 1.5, and 2 wt %). In the results, we only report the values of zero-shear or specific viscosity, to characterize the solution at different chitosan concentrations; more detailed rheological measurements will be described in further work. It is worth saying that, in the range of concentration of this study, solutions of chitosan behave like viscoelastic liquids (e.g., elastic modulus lower than loss modulus for a wide range of frequency and strain).

The compression of elastic chitosan beads was carried out in a homemade device comprising two parallel PTFE platforms attached to a micrometer and a dynamometer (Pioden Systems Ltd.) to measure the normal force. Assuming the beads are nearly spherical, this corresponds to the Hertzian contact geometry on both sides.^{17,18} From the size of the compressed bead force-compression dependence, we extract the Young modulus of the chitosan matrix. Of course, in this way we can only obtain an approximated value of this modulus, which is affected by small asphericity, the residual water content in chitosan, and the degree of effective cross-linking (related to the DDA value) and, most importantly, on the amount of time allowed for stress relaxation (which is very large, as in any random physically cross-linked elastomer).¹⁹ We use this estimate as a guideline only, to illustrate the physical strength of swollen chitosan beads.

3. Results and Discussion

3.1. Interfacial Properties of Chitosan. The time evolution of interfacial tension characterizes the surface activity, i.e., the kinetics of adsorption and reconfiguration of chitosan at interface. The effect of increasing chitosan concentration in the aqueous phase is illustrated in Figure 3 for the air–water interface, for reference, and in Figure 4 for the paraffin–water, which is relevant for the remainder of this paper. We observed that chitosan has no surface activity at both the air and paraffin interface for concentration below 0.1 wt %. These results are in accordance with Babak's work²⁰ who studied the adsorption properties of chitosan and hydrophobically modified chitosan at air/water interface at low concentration of polymer (below 0.1 wt %).

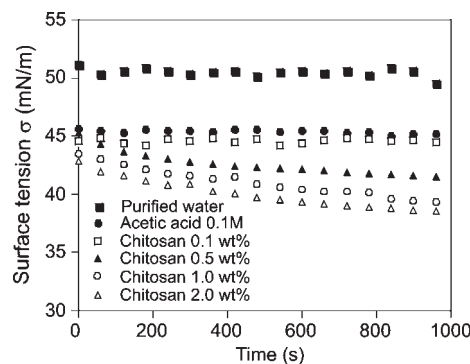


Figure 4. Surface tension of interface between paraffin oil and the aqueous chitosan solution with 0.1 M acetic acid, at 25 °C, as a function of time.

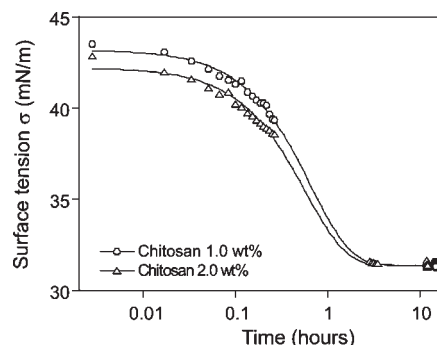


Figure 5. Long-time evolution of surface tension of interface between paraffin and high-concentration aqueous chitosan solutions (1 and 2 wt %, cf. Figure 4). Solid lines are the exponential relaxation fits (see text); note that the saturation value is the same.

For higher concentrations of chitosan, the interface tension (for both air–water and paraffin–water) decreases, but relatively slowly, and the overall decrease is small compared to traditional amphiphilic molecules. For comparison, a LMW surfactant Span 80²¹ makes the tension of paraffin/water interface $\sigma \approx 3$ mN/m, while the assembly of milk proteins²² gives $\sigma \approx 8$ –20 mN/m for concentrations lower than 0.1 wt %. For solutions with 1 and 2 wt % of chitosan, we measured the saturation surface tension of only 31 ± 1 mN/m after 16 h, Figure 5, which is in fact similar to the values reported with other polysaccharides, such as glucopyranoside and maltose.²³ Low interfacial activity between alkane and two other polysaccharides alginate or xanthan was also observed.²⁴

This relatively low amphiphilic activity may be understood by examining the molecular structure of many polysaccharides, and in particular chitosan (Figure 1). The molecule is highly hydrophilic in solution due to the presence of amine groups NH_2 . The short-range hydrophobic interactions may exist through the acetyl amine groups^{25–27} but in our case they are in small proportion (20%) compared to the amine groups. Also, the

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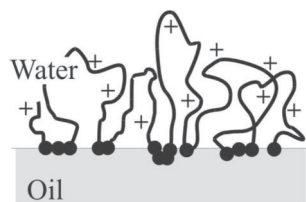


Figure 6. A sketch of chitosan assembly on the oil/water interface with a few hydrophobic anchoring points (black points), producing a dense brush of hydrophilic loops and chain ends on water side, and a relatively small effect on the oil side.

Table 1. Storage Stability of Emulsions as a Function of the Volume Fraction of Paraffin, with a Constant 2 wt % Chitosan Aqueous Solution

storage time (day)	volume fraction of paraffin									
	0.05	0.11	0.22	0.34	0.40	0.54	0.60	0.63	0.73	0.90
				O/W				O/W/O	W/O	
1	100	100	100	100	100	100	100	not studied		
10	11	26	46	86	96	100	100	not studied		
28	11	26	46	61	67	100	100	not studied		
38	11	26	46	61	67	92	96	not studied		
66	11	26	46	61	67	92	96	not studied		

hydrophobic groups are distributed randomly on the chains, which may reduce their accessibility to the oil/water interface due to the steric hindrance and electrostatic repulsion.

It is interesting to examine the time dependence of chitosan assembly on the interface and the associated change of its surface tension. Fitting the long-time evolution of surface tension of two high-concentration chitosan solutions interfaced with paraffin oil, Figure 5, gives the exponential relaxation law. Remarkably, both 1 wt % and 2 wt % chitosan solutions (both above the overlap concentration, see below) saturate at the same surface tension $\sigma_{eq} \approx 31$ mN/m, while the relaxation is described by $\sigma(t) = \sigma_{eq} + s \cdot \exp(-t/\tau)$ with the amplitude $s_1 = 11.8$ mN/m and $s_2 = 10.8$ mN/m and relaxation time $\tau_1 = 2376$ s = 0.66 h and $\tau_2 = 2074$ s = 0.58 h, for the two samples, respectively. Such a long time indicates a complex molecular rearrangement on the interface: we assume that this involves the optimization of the number of hydrophobic groups exposed to the oil interface and results in the effective brush of dense loops of the deacetylated amine-containing chain sections. We shall see in the next section that this chain configuration on the interface creates a strong steric barrier on the water side, but has little effect on the oil side of the interface, see Figure 6.

3.2. Emulsion Stabilization. In this study, we produced emulsion of paraffin in water in the presence of chitosan. We found that there was no need of any classical amphiphilic molecules (surfactant or protein) to promote the homogenization. In Table 1 we illustrate the type and the stability of the emulsions as function of the volume fraction of paraffin for a constant concentration of chitosan of 2 wt % (we shall see later that this solution has a relatively high viscosity of ~ 1.7 Pa.s). For the purpose of this table, the storage stability of emulsion is defined as the percentage of the remaining emulsion compared to the volume of the fresh emulsion.

As usual, we observe three types of emulsions depending on the volume fraction of paraffin oil. For oil fraction below 0.60, the emulsion is of the O/W type. For volume fractions between 0.63 and 0.73, we found the double emulsion of the O/W/O type, which corresponds to the emulsion of O/W (inner emulsion) dispersed in oil (the majority phase) (see Figure 2). Finally, the emulsion with volume fraction of 0.93 is of the W/O type, with small water droplets dispersed in oil.

For the O/W type emulsions, no layer of oil appeared (characteristic of significant coalescence) at any time during aging; creaming was the only effect; see Figure 2. The kinetics of creaming was as expected, dependent on the volume fraction of paraffin: the lower was the volume fraction, the faster was the creaming, which is simply the effect of steric hindrance of droplets rising through the viscous medium. In addition, microscopic observations suggest that the droplet size distribution was independent of the paraffin volume fraction (for the fixed chitosan content in water); essentially the droplet size is determined by the local shear stress, i.e., the function of the continuous phase viscosity; see Figure 9.

For the double emulsion of the O/W/O type, between 0.63 and 0.73 paraffin volume fraction was not stable (see Figure 2); in the hour following the homogenization, the main outer oil part coalesced to form a continuous oil layer on top of an O/W emulsion. The rest of the outer oil was entrapped in millimeter-sized “pockets” in the O/W region due to the high viscosity of this inner emulsion. Therefore, measuring the quantity of the different phases precisely was impossible with our method. For the high volume fraction of paraffin oil, the W/O emulsion was not stable: the droplets are millimeter in size (due to the attempted dispersion of the high-viscosity chitosan solution in the low-viscosity majority phase) and their coalescence occurred immediately after the end of homogenization. The model of chitosan assembly on the interface agrees with these observations. In the O/W emulsion, small droplets of oil dispersed in the continuous water phase are “decorated” by the dense brush of hydrophilic chitosan segments, which present a significant barrier for coalescence—both steric and electrostatic. In contrast, the inverted W/O emulsion droplets cannot be stabilized and coalesce freely.

As we found by measuring the surface tension, chitosan itself does not have sufficient surface activity at the paraffin/water interface, and the stabilization of oil droplets against coalescence is mostly due to the repulsion of hydrophilic chitosan segments anchored on the water side of this interface. This also means that the homogenization (dispersion of droplets) was principally due to the viscous stress in the majority phase. It is worth noting the completely different results obtained by Rodriguez et al.²⁸ when studying the production of emulsion of sunflower oil in chitosan solution without surfactant. For similar viscosities of the water phase compare to our studies, they obtained emulsions only for an oil ratio of 0.2 and these emulsions were W/O/W type. The discrepancy between their results and ours may be explained by the difference of interaction between chitosan and the oils. Indeed, it is well-known that chitosan interacts strongly with fatty acids²⁹ and therefore the surface activity may outweigh the viscosity effect, inducing the type of results obtained by Rodriguez et al.

To explore the effect of viscous stress on emulsion type and stability, we varied the viscosity of the solution of chitosan by changing the concentration of chitosan.

3.3. Effect of Chitosan Concentration. The values of linear shear viscosity η_0 of the bulk chitosan solution are analyzed in Figure 7 and also listed in Table 2. One of the classical results of polymer physics states that the specific viscosity $((\eta_0 - \eta_{sol})/\eta_{sol})$ scales as a power law with the concentration, and the exponent of this scaling depend of the range of concentrations between the diluted, unentangled semidiluted, and entangled semidiluted states of the polymer solution.³⁰ For a polyelectrolyte in good

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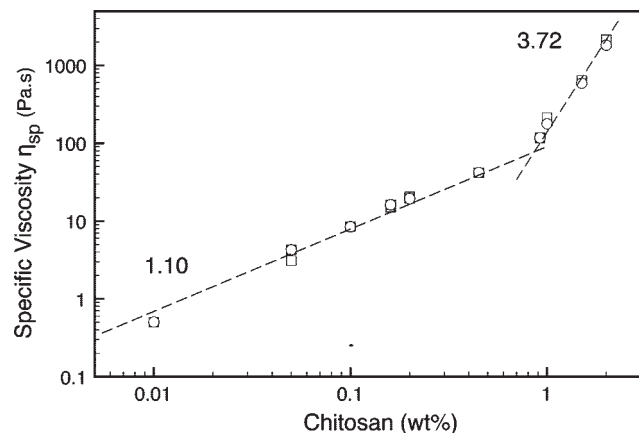


Figure 7. Scaling of the specific viscosity with concentration of chitosan in 0.1 M acetic acid and at pH = 5, from two separate measurement sessions. Viscosity of 0.1 M acetic acid is 0.93 mPa·s. The double-logarithmic scale reveals two clear power-law regimes, with the exponents ~ 1 and 3.8 and the crossover at ~ 0.8 wt %.

Table 2. Storage Stability of Emulsions as a Function of Chitosan Concentration, with a Constant 0.5 Volume Fraction of Paraffin^a

	concentration of chitosan solution (wt %)						
	0.05	0.16	0.20	0.45	1.00	1.50	2.00
η_0 (mPa·s)	8	16	19	40	166	550	1690
storage time (day)							
1				100	100	100	100
5	no emulsion			74	83	93	100
11				27 [cx]	78	81	100
38				17 [cx]	78	81	92

^a The storage stability of emulsion (against creaming) is defined as the percentage of remaining emulsion compared with the fresh one. [cx]: coalescence is observed.

solvent, exponents of $1/2$ and $3/2$ are predicted from the reptation theory for the two latter regimes.^{31,32} In Figure 7 we find the approximate scaling exponents of, respectively, 1.1 and 3.7. The high-concentration exponent is very close that the value $15/4$, predicted for a neutral entangled polymer,³⁰ while the low-concentration linear scaling with concentration suggests the pure dilute regime with only a single crossover. These findings may be understood by remembering that $\sim 20\%$ of the chitosan chain segments are the hydrophobic aminoacetyl groups, which are effectively attracted in the aqueous phase (as mentioned by Nyström et al.²⁵) which should reduce the intrachain electrostatic repulsion, and cause the weak dynamic cross-linking in the dense regime. The crossover concentration C_e of chitosan in 0.1 M acetic acid was estimated around 0.8 wt % as the point that separates the regimes of unentangled from that of entangled semidilute polymer solutions.

For chitosan concentrations between 0.05 and 0.20 wt %, we found no stable emulsion; the droplets were too big (hundreds of μm) and coalescence occurred as soon as the shearing was stopped. This is consistent with our view of chitosan assembled on oil/water interfaces, with insufficient surface coverage at low concentrations. At higher concentrations, the aqueous phase was increasingly more viscous and shear mixing has produced effective droplet dispersion. Confocal microscope images of the stable emulsions for chitosan concentration of 0.45, 1, 1.5, and

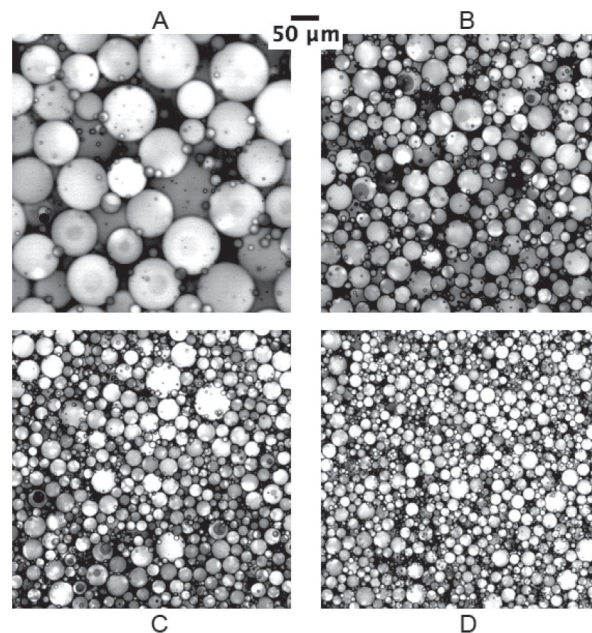


Figure 8. Microscope images of emulsions with 0.6% v/v paraffin and different concentrations of chitosan, 1 h after homogenization: (A) 0.45 wt %, (B) 1.00 wt %, (C) 1.50 wt %, and (D) 2.00 wt %.

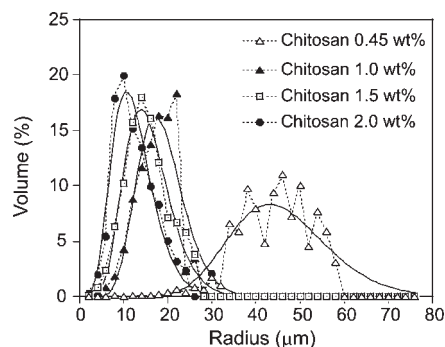


Figure 9. Size distribution of emulsions for different chitosan concentrations: 0.45, 1.00, 1.50, and wt %. Solid lines are fits by the Poisson distribution. The volume fraction of paraffin is constant at 0.6.

2 wt % are given in Figure 8. The drop size distribution for these concentrations is reported in Figure 9. We can clearly see that the increase of the concentration of chitosan promotes the fragmentation of oil into smaller droplets.

In Table 2 we list the storage stability of emulsions of paraffin in aqueous solution with concentration of chitosan varying between 0.05 and 2 wt %, with an equal 0.5 volume fraction of paraffin. The creaming rate and the height of creamed layer were found to be strongly dependent on the concentration of chitosan, which affects the droplet size and the viscosity of the continuous phase. Droplet coalescence was found for the aged samples with 0.45 wt % of chitosan, while at concentrations above the entanglement crossover (cf. Figure 7) no layer of oil was observed. Therefore, the network of chitosan chains reduces the droplet diffusion in concentrated solutions and stabilizes the contact between droplets, improving the emulsion stability.

It is worth noting the work of Benna-Zayani et al. which produced W/O/W double emulsions stable against creaming by using weak gels as the external aqueous phase.³³ By varying certain parameters such as the molecular weight, DDA, and pH,

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Figure 10. Reference beads (left) and emulsion beads (right) with the paraffin volume fraction of 0.25, with 2 wt % chitosan in aqueous phase for both. Beads are suspended in water with pH = 7.

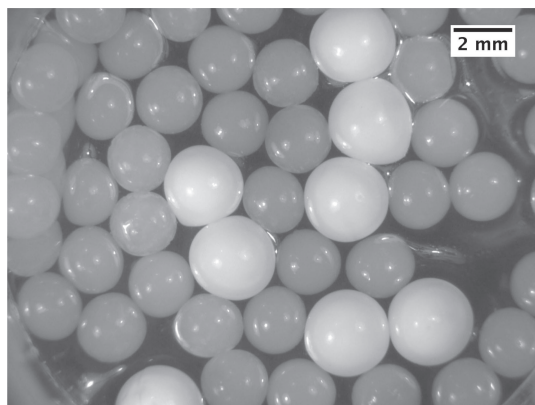


Figure 11. Mixed reference and emulsion beads in very little water. The smaller clear spherical beads are the reference beads, and the bigger white slightly asymmetric beads are the emulsion beads with the paraffin volume fraction of 0.25.

Table 3. Geometric Characteristics of Beads Asymmetry, with Changing Oil-Phase Content in the Precipitated Emulsion

paraffin v/v	0	0.25	0.40	0.50
$d \pm 0.2$ mm	2.5	3.0	2.9	2.8
$D \pm 0.2$ mm	2.5	3.1	3.2	3.4

we should be able to achieve the same effect in weak gels of chitosan.²⁷ It would be interesting to study the possibility of preparing W/O/W emulsion with chitosan (or other polysaccharides) without surfactant.

3.4. Solid O/W Emulsions. Solid monodispersed beads were obtained from the pure solution of chitosan (called here the reference beads) or paraffin/water emulsions (called emulsion beads). Images of these beads are seen in Figures 10 and 11. The concentration of chitosan in the aqueous phase for both types of beads was kept at a constant 2 wt %. The volume fraction of paraffin changed between 0.25, 0.40, and 0.50. The reference beads and the emulsion beads are clearly different in density (Figure 10), opacity, dimensions, and shape (Figure 11). In Table 3 we list the main characteristics of the precipitated beads: d , diameter at the apex, and D , elongation of the beads (i.e., its highest dimension). The reference beads were spherical while the emulsion beads had less regular shapes (with more or less pear-shaped prevalence).

It is known that the shape and dimensions of drops strongly depend of the characteristics of the solutions: viscosity, surface tension, and density.³⁴ These three parameters vary with the

volume fraction of the dispersed paraffin; the combined effect of these factors may explain the increasing asymmetry of the emulsion beads. In terms of stability, all of the beads were monitored for 7 months, showing no observed change of their density, their opacity, dimensions or shape.

We have carried out compression measurements only on the reference beads (because the emulsion beads are nonspherical), using the Hertz solution^{17,18} for the elastic contact of a sphere and a flat surface that provides the contact load force $F = (16/9)YR^{1/2}h^{3/2}$, where Y is the Young modulus of the sphere (assumed incompressible) of initial radius R , compressed by the amount h . Compressing the spheres of 2.5 mm diameter in small steps of 50 μm every 60 s, we obtain an estimate of the modulus $Y \approx 900$ Pa; however, this value strongly depends on the time allowed for stress relaxation in chitosan network. Although the measurements were done in different conditions, we can compare this value with the one obtained by Khalid et al.³⁵ For a chemically cross-linked hydrogel of chitosan (1.42×10^6 g/mol of average molecular weight) at swelling equilibrium (like in our case), they found $Y = 1200$ Pa by means of indentation measurements. The fact that the Young modulus they found is higher than what we found can be justified by the difference of molecular weight of chitosan but specially the type of cross-linking. It is not surprising that chemically cross-linked hydrogels are more rigid than the physically cross-linked ones.

4. Conclusion

We showed that it is possible to have stable emulsions of paraffin (used here as a model oil) in the aqueous solution by adding only chitosan without any additional surfactant. Adding the thickener chitosan to the water phase increases the shear stress applied during the emulsion homogenization and thus improves the dispersion of oil in water although the oil–water interface tension is quite high and the adsorption kinetics of chitosan at this interface slow. At the oil–water interface, the highly hydrophilic chitosan has a low density of adsorption but forms a dense network of polyelectrolytic brushes on the water side (see Figure 6). The O/W emulsion is thus stabilized against coalescence by steric and electrostatic hindrance, in contrast to the W/O (convex) interface, which is shown to have low barrier for coalescence. In this study, the rigidity of this network is improved by increasing the density of polymer entanglements (i.e., with the concentration of chitosan) or by precipitation of chitosan network on removing the solution acidity. There are many other ways to change the rigidity of chitosan networks. For instance, one can affect the electrostatic repulsion (by varying pH, ionic force) and the hydrophobicity of the chain (through DDA for example) or one can add covalent gelling agents. Thus, different kinds of emulsions with different mechanical properties and shapes might be produced without the help of surfactant for the great benefit of environment.

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Supporting Information Available: Apparent viscosity of chitosan solutions as a function of shear rate. Chitosan solutions exhibit thinning behavior at high shear rate and low shear rate Newtonian plateau in the range of concentrations of this paper. This type of curves allowed the determination of the zero-shear viscosity used in this paper. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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