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SHORT COMMUNICATION

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Emulsification properties of chitosan

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Abstract The chitosans use as an emulsifier in food emulsions was explored. The properties of chitosan (air/solution surface activity, electrical conductivity, HLB) were studied. The obtained emulsions were stable multiple w/o/w emulsions, whose characteristics were explained on the basis of the emulsifier structure and solution properties. The reaction with an anionic surfactant, sodium dodecylsulfate, was also studied, giving a water-insoluble complex at a given surfactant/chitosan ratio.

Key words Chitosan – HLB – food emulsions – cationic polyelectrolytes – multiple emulsions

Introduction

Chitosan, or β-(1-4) linked 2-amino-2-deoxy-D-glucose, is a linear polymer conventionally prepared by partial alkaline deacetylation of chitin. Poly(*N*-acetyl-D-glucosamine) [1] is the main structural component of the cuticles of crustacean, insects and molluscs and the cell walls of microorganisms [2]. Chitosan is a natural positively charged polysaccharide having a $pK_a \approx 6.3–7$ [3], and a potential use in several areas, including applications in cosmetics [4, 5], biotechnology [6, 7] and medicine [7–9]. In these applications, chitosan interacts in most cases with biomembrane surfaces. For instance, chitosan can be used as a hypocholesterolemic agent [10]. One advantage is that, unlike other established hypocholesterolemic agents, chitosan can be used in a soluble form. Further, its polycationic nature leads to strong interactions with lipids having the opposite charge. One possible mechanism for the hypocholesterolemic effect of chitosan is a combination of electrostatic and hydrophobic interactions between the polyelectrolyte and lipid droplets in the intestine. That such interactions occur is evident from previous reports describing the effects of chitosan on lipids in micelles [11] and liposomes [5]. Chitosan probably will cover negatively charged lipid particles in the intestine, and thus this process is likely to influence the resorption of lipids (particularly, bile salts) in the intestinal tract. This process is one possible reason for the hypocholesterolemic effect reported for chitosan [1]. Polysaccharides are promising biodegradable polymers and are under intensive investigation for industrial applications [12].

Fäldt et al. [1] studied the interaction between chitosan and soybean oil emulsions coated with phospholipid and glycocholic acid as a function of pH. A structure consisting of adsorbed cationic polysaccharides on the anionic emulsifier surface is probably formed.

Calvo et al. [8] made chitosan-coated polyester nanocapsules and submicron-emulsions. All particles displayed a high positive surface potential (+30 up to +60 mV).

Oily droplets surrounded by a polymer coat have been revealed as interesting systems for the administration of hydrophobic drugs by the ocular and oral routes [13].

In an attempt to supply positive charges to the colloidal systems used to deliver drugs, the use of positive phospholipids and other cationic surfactants in the preparation and stabilization of submicron emulsions and

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liposomes have been proposed [14]. Positive drug carriers will favor the interaction with negative biological membranes, thus improving the capacity for transporting and transfering the drugs [15]. As an alternative, Calvo et al. [8] used chitosan to stabilize the oil/water interface. Chitosan was chosen to coat these colloidal systems due to its cationic character and because it has some interesting properties such as mucoadhesivity [16] and biocompatibility [17]. Furthermore, several authors have reported that there are no signs of toxicity upon oral and nasal administration [18] and there low toxicity following intravenous administration of chitosan [19].

The goal of this work was to determine the emulsifying properties of chitosan for use in food emulsions. The results may also be used for the preparation of emulsions, with applications in pharmacology and cosmetics.

Experimental

Chitosan was obtained by treating chitin from shrimp outer shells, with NaOH 41% w/w at 136 °C for 1 h. The chitosan obtained was a powder with 3.0% water content, ash 0.17% and 89.0% deacetylation degree. The deacetylation degree was measured with a method reported elsewhere [20].

Stock solutions containing 1% w/v chitosan were prepared. The chitosan was stirred in 1% v/v aqueous acetic acid. After resting for 24 h, the undissolved chitosan was separated by filtration through a medium porosity filter.

The surface tension of chitosan solutions was measured with a Du Noy (Krüss) tensiometer on solutions with different concentrations of chitosan in 1% aqueous acetic acid solution at 25 °C.

The conductivity of chitosan solutions in an aqueous 1% acetic acid solution was measured with a CRIBABB conductimeter and an immersion cell at 25 °C.

To determine the chitosan HLB value, the diameter of a toluene drop put on the surface of the chitosan in 1% acetic acid aqueous solution was compared with those put on solutions of several surfactants with different hydrophile–lipophile balance (HLB) and the same concentration (1% w/v). The diameter of the drop at rest is related to the HLB value of the surfactant in the solution [21]. The type and volume of emulsion produced by chitosan and by several surfactants with different HLB in solutions with the same concentration (1% w/v) and preparation conditions were also compared.

The interaction with sodium dodecyl sulfate (SDS) was studied by adding solutions of SDS with different concentration to the same amount (2 ml) of chitosan 1% w/v in 1% v/v aqueous acetic acid solution. The precipitate was filtered and weighted after dehydration in a vacuum oven at 105 °C. It was also studied by optical microscopy.

Emulsions were produced by adding a variable amount of sunflower oil (20–120 g) to a constant amount of 1% w/v chitosan solution (80 g). Samples were stirred in a food processor for 3 min, and then the same volume (65 ml) was put in a 100 ml probet. To determine the optimal chitosan/oil ratio the samples were left to rest and the time elapsed until the phase separation was recorded. When phase separation occurred, three layers appeared: oil, white emulsion and a dilute emulsion of very small oil droplets in water. When no phase separation occurred, sedimentation with time produced a concentrated, coarse emulsion on the top and a dilute emulsion of very small droplets at the bottom of the samples. Then the samples were centrifuged and the volume of remaining oil was recorded against the oil/chitosan (w/w) ratio.

The emulsion type was determined by adding a drop of a water-soluble dye solution to a sample of emulsion, and a drop of an oil-soluble dye solution to another emulsion sample.

Emulsions with different chitosan/oil ratio were prepared in the same conditions and the droplet diameter distribution was determined by microscopical observation in a Leitz microscope with polarized and unpolarized light.

Double-distilled water was employed in all solutions.

Results and discussion

Chitosan properties

The surface tension of chitosan solutions were higher than that of the solvent (1% v/v acetic acid aqueous solution). This implies that chitosan is strongly excluded from the air/solution interface. This agrees with its chemical structure: a polysaccharide with cationic-NH_3^+ and alcoholic-OH groups distributed along the hydrocarbon structure. The structure has no large hydrophobic groups which may be adsorbable at the air/solution interface.

The specific conductivity may be represented by the equation [22]:

$$\kappa = \kappa^\circ + \Lambda^\circ c + c\Phi(c)\ , \tag{1}$$

where κ and κ° are the specific conductivities of the solution and the solvent, respectively, and $\Phi(c)$ accounts for the effects of interionic interactions on the conductivity. Thus, at high dilution, a plot of κ versus c should be linear with slope Λ° [23]. According to Vink [22, 24] an accurate determination of Λ° by this procedure requires that the curve representing κ be linear over a sufficiently wide range of concentration, otherwise $d\kappa^\circ/dc$ and $d[\Phi(c)]/dc$

can be neglected in comparison with Λ°, The specific conductivity as a function of the concentration of chitosan at constant concentration of acetic acid (1% v/v) follows a linear relationship up to 2 wt% chitosan:

$$\kappa\ (\mu\text{S cm}^{-1}) = 340 \pm 18 + (1425 \pm 28)c\ (\%\ \text{w/v})\ ,$$

$$r = 0.9959\ .$$

The equivalent mass of chitosan is given by

$$M = 0.89 M_{\text{Deac.}} + 0.11 M_{\text{Ac}}\ , \tag{2}$$

where 0.89 is the degree of deacetylation/100, M_{Deac} and M_{Ac} are the molar masses of the deacetylated and the acetylated D-glucosamine units, respectively. Along with the equivalent mass the equivalent conductivity of chitosan was calculated with

$$\kappa(\text{S cm}^{-1}) = (3.40 \pm 0.18) \times 10^{-4} + (2.438 \pm 0.048) \times 10^{-2} c\ (\text{Equiv. dm}^{-3})$$

in which the slope is $\Lambda^\circ_{\text{P.Ac}}$, the equivalent conductivity of the polyelectrolyte salt. The intercept (3.4×10^{-4} S cm^{-1}) is lower than the solvent conductivity (8.6×10^{-4} S cm^{-1}) and reflects the effect of the polyelectrolyte on κ°. Similar results have been described for other polyions [22–24]. The value of $\Lambda^\circ_{\text{P.Ac}} = (2.438 \pm 0.048) \times 10^{-2}$ S cm^2 Equiv^{-1} is very low compared with other polyelectrolytes, such as sodium polymethacrylate (200 S cm^2 Equiv^{-1}) and poly(1,1-dimethyl-3,5-dimethylene piperidynium) (188.9–356.5 S cm^2 Equiv^{-1}) [23]. This implies that the interaction between acetate and the polyelectrolyte is strong, and the effective charge of the kinetic unit having n positive sites is much less than n. The conductance of the polyelectrolyte solution can be expressed as [23]:

$$\Lambda^\circ_{\text{P.Ac}} = f(\lambda^\circ_{\text{P}} + \lambda^\circ_{\text{Ac}})\ , \tag{3}$$

where f is an interaction parameter [24], and λ°_{P} and $\lambda^\circ_{\text{Ac}}$ refer, respectively, to the limiting equivalent conductance of the counterion and the polyion. According to Manning's limiting law for the conductance of polyelectrolytes [25], the parameter f is

$$f = 0.866\xi^{-1}\ , \qquad \xi < 1\ , \tag{4}$$

where ξ is the linear charge density (B/l), where B is the Bjerrum length (0.7135 nm) and l is the distance between charged groups in the polyion structure in its fully extended state. ξ^{-1} may be interpreted as α, the degree of ionization [24]. In view of the low value of $\Lambda^\circ_{\text{P.Ac}}$, it follows that ξ^{-1} must be very small.

The crystalline structure of chitosan has been described as an extended two-fold helix stabilized by intramolecular hydrogen bonds [26]. A geometrical value of l calculated from the crystalline configuration of the molecule gives 0.625 nm, with values of the C–C and C–N distance bonds of 0.15 nm and C–C–C, C–C–N and C–O–C angles of 109.5°. ξ^{-1} is calculated as 0.98 and f as 0.85. These values correspond to a fully ionized molecule. It may be concluded that the kinetic entity must include a large amount of counterions, because the experimental f value is much lower than the theoretical one.

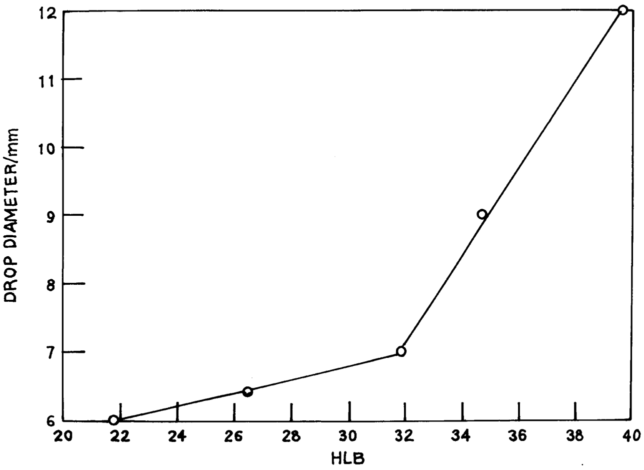


Fig. 1 Diameter of the toluene drops on solutions of 1 wt% of surfactants with different HLB values

The intramolecular hydrogen bonds are probably maintained in solution, thus keeping the helical structure found in solid chitosan. Wu et al. [27] stated that the chitosan chain is slightly extended in aqueous solution and that even in dilute solution chitosan still forms a small amount of large sized aggregates. So the bulky helical structure may reduce the mobility of chitosan fibers by enhancing the drag and by trapping acetic acid inside the helix, which reduces the net charge.

Figure 1 shows the relation between the diameter of toluene drops laid on aqueous surfactant solutions and the surfactant HLB. The drop over the chitosan solution had 10 mm diameter, giving an HLB value of about 36.7. This high HLB value for chitosan was confirmed by the type and volume of the emulsion produced with this emulsifier and surfactants with different HLB. However, in view of the chitosan structure, the expected HLB value would be higher than the determined one. The low ionization degree may be the cause of the relatively low HLB value measured here.

Interaction with anionic surfactant

The chitosan interactions with sodium dodecyl sulfate (SDS) was studied because this surfactant is a strong acid salt, which remains charged even in acid solutions. This ensures that the interactions between the polyelectrolyte and SDS are ionic and hydrophobic. When the solution of chitosan in acetic acid was poured in to the aqueous SDS solution, a white precipitate was formed. This precipitate has the appearance of thin films, with no optical birefringence. When the chitosan solution was poured in concentrated SDS solutions, the drops of chitosan solution were immediately coated by a shell of the chitosan–dodecylsulfate complex which prevents the reaction between the chitosan in the liquid content of the coated drop and dodecylsulfate ions in the surrounding solution (Fig. 2). This shell was quite resistant to squeeze with the fingers. Figure 3 shows a microphotography of one of the shells after break. When the coated drops were left in air without the surrounding liquid, the water content evaporated through the membrane, but liquid water did not permeate through it.

Figure 4 shows the amount of precipitate formed with different chitosan/SDS ratios. It was noted that there was a linear relationship between the amount of precipitate and the concentration of SDS, if chitosan was in excess. There was a maximum when chitosan and SDS react in the appropriate proportion. When SDS was in excess, the precipitate redissolved. This behavior is similar to that of the complexes produced by interaction of proteins and

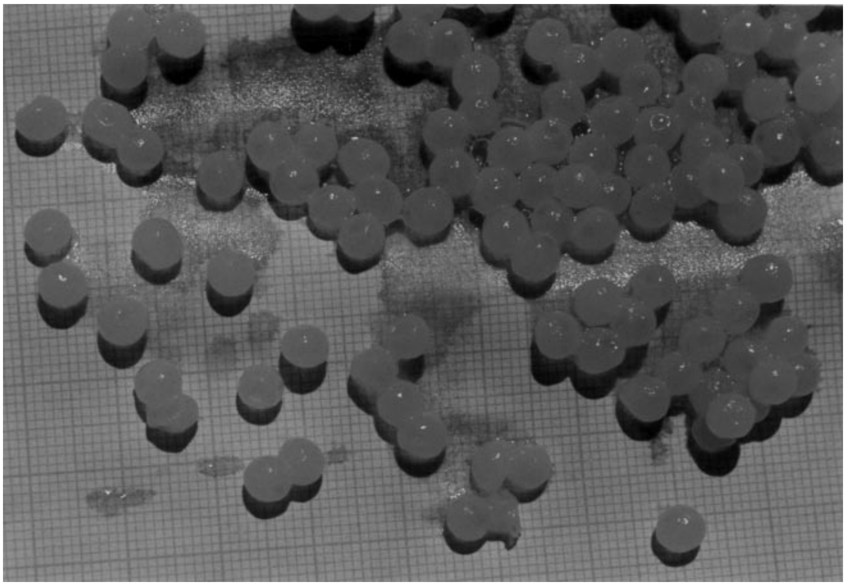


Fig. 2 Drops of chitosan solution coated with a film of chitosan–SDS complex

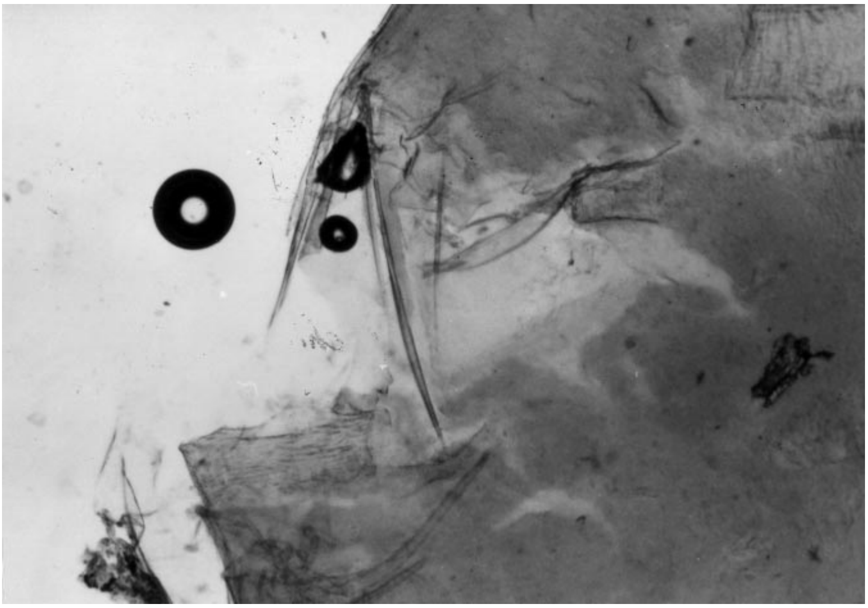


Fig. 3 Photomicrograph of a blown drop coated with chitosan–SDS complex, showing the very thin film

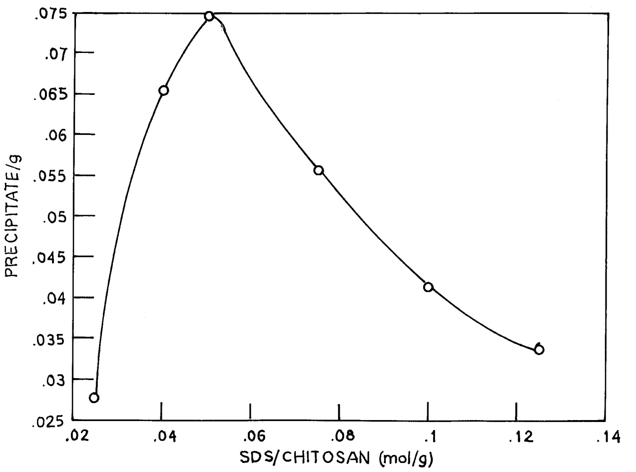


Fig. 4 Weight of the precipitated chitosan–SDS complex vs. the SDS/chitosan ratio

oppositely charged surfactants [28]. The maximum precipitation occurred when one mol of SDS reacted with 20 g of chitosan.

Emulsion

A drop of a water–soluble dye solution loaded in the emulsion dissolved readily in it, whilst a drop of the oil–soluble dye solution remained on the emulsion surface, indicating that water was the continuous phase. Observation of the emulsion with the optical microscope revealed that samples were water/oil/water (w/o/w) emulsions (see Fig. 5).

When the emulsions were left to rest, the system produced two or three layers. If there was an excess of oil, the surplus appeared as a supernatant layer. The emulsion underwent creaming with time producing a concentrated, w/o/w emulsion on the top and a dilute w/o/w emulsion of very small droplets at the bottom of the samples.

Multiple emulsions were first described by Seifritz in 1925 [29]. These types of emulsions are complex systems in which the droplets of the dispersed phase contain even smaller dispersed droplets of the continuous phase themselves. The two major types of multiple emulsions are the water–oil–water (w/o/w) and oil–water–oil (o/w/o) double emulsions. Multiple emulsions have been prepared mainly by one-step emulsification and two-step emulsification [30]. The most common and better controlled preparation of double emulsions are based on the two-step emulsification process, where a hydrophobic emulsifier is used for the primary w/o emulsification, and a hydrophilic emulsifier is used for the preparation of the o/w emulsion. Complicated calculations of the apparent and weighted HLBs at each interface have been done [31, 32]; also, sophisticated combinations of emulsifiers have been considered [33]. However, it was concluded that the classical double emulsions prepared with two sets of monomeric emulsifiers cannot provide long-term stability to the double emulsion. The w/o/w emulsions with relatively large droplets and short-term stability that are commonly obtained cannot be used in practice [34], because they are intrinsically thermodynamic unstable [35].

To increase the stability of these multiple emulsions macromolecules have been used in combination with surfactants [36].

Fig. 5 Dilute w/o/w emulsion of sunflower oil stabilized with chitosan

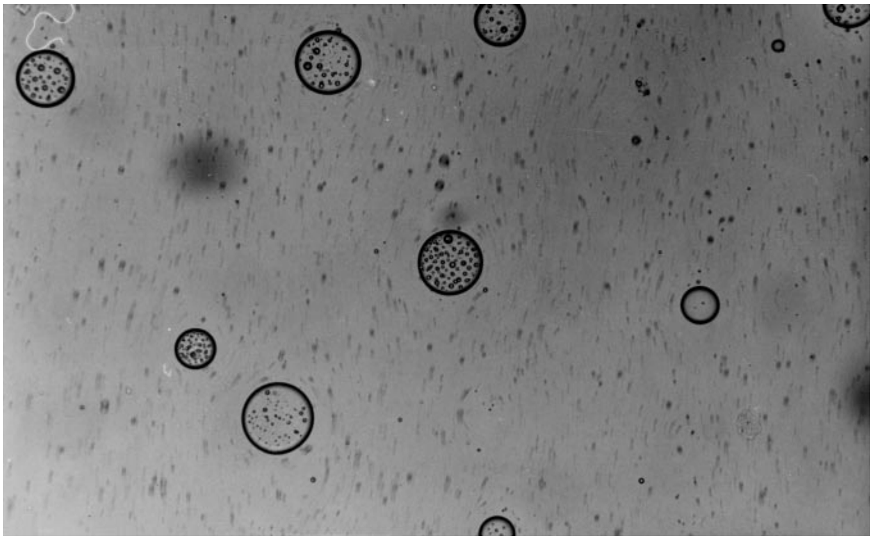
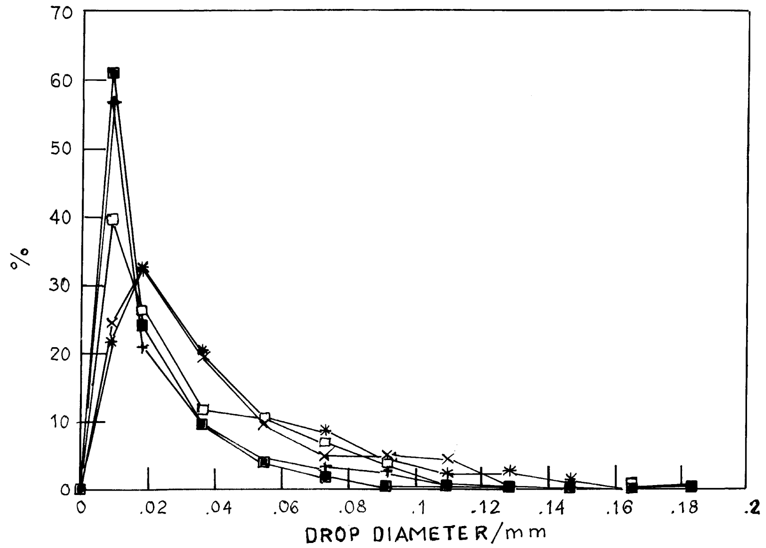


Fig. 6 Droplet diameter distribution at different chitosan/sunflower oil ratios (w/w): (■) 0.16, (+) 0.08, (□) 0.04, (∗) 0.027, (×) 0.02



Chitosan produced a w/o/w double emulsion without adding any surfactant. Because of its high HLB value, chitosan may promote the formation of o/w emulsions. However, chitosan is not a pure compound. It is probably composed of a mixture of molecules with different deacetylation degrees (DD). Because of the high average DD of our chitosan, the amount of molecules with high DD might be much larger than that of low DD. Thus, molecules with high DD promoted the formation of w/o emulsions, whereas those of lower DD (and thus with lower HLB value) promoted the emulsification of water inside the oil droplets. Because of the relative amount of high DD molecules to that of low DD, the continuous phase was water, following Bancroft's rule. Thus, chitosan behaves as a mixture of surfactants with different HLB values.

The optimal oil/chitosan ratio was determined to obtain the most economical and efficient use of the emulsifier. If oil was in excess, three layers appeared in about 3 h. At the optimal oil/chitosan ratio or in excess of chitosan, no phase separation was detected in two months. The optimal oil/chitosan ratio was found by extrapolation to zero volume of the non-emulsified oil layer, giving 0.2 (g of chitosan/g of oil). This optimal emulsion formulation was stable to dilution with aqueous acetic acid. This implies that the emulsifier is preferentially extracted by the oil/water interface and does not redissolve in the aqueous medium, which gives great stability to the emulsion. The double emulsions are intrinsically unstable, mainly due to transport of material (surfactants, solutes, water) between the microdroplets emulsified in the disperse phase and the continuous phase. Several possible mechanisms by which materials can be transported across the oil layer in w/o/w emulsions have been proposed [31, 37]. The most common is the molecular diffusion controlled mechanism of oil soluble substances. In multiple emulsions stabilized with mixed surfactants, mixed reverse micelles can solubilize water and water soluble ingredients and transport them to the other phase [38].

The adsorbed chitosan is probably an interfacial barrier for diffusion-controlled transport of both hydrophobic and hydrophilic substances. Furthermore, the micellar transport via reverse micelles was not possible, because no surfactant was added, thus, migration was very limited inside the oil droplets, thereby increasing the stability.

The affinity between chitosan and water decreases with increasing pH [3]. When the optimal emulsion was made alkaline no changes were seen by the naked eye, but the droplets became more rigid. Microscopically droplets coated by solid, non-bi-refringent chitosan were seen. This means that chitosan remained at the oil/water interface protecting the droplets against coalescence, but not against coagulation.

Figure 6 shows the droplets diameter distribution of different chitosan/oil (Q/O) ratios (w/w). The distributions are almost equal for $Q/O = 0.16$ and 0.08. The maximum is the same ($d \approx 0.01$ mm) for $Q/O = 0.04$, but the distribution is broader, and when the maximum is shifted to higher droplet diameter, the distribution broadens even more for lower Q/O values. When the Q/O ratio decreased, larger droplets appeared (up to 0.4–0.5 mm diameter), but they were of very low proportion in number.

Concluding remarks

- Chitosan acetate macromolecules have probably a low ionization degree in solution, exhibiting a small surface potential and low equivalent conductivity.

- Due to its structure, chitosan is inactive at the air/solution interface, but it can adsorb at the oil/water interface providing both mechanical and electrostatic stability to the droplets.
- Chitosan reacts with SDS to yield an insoluble, elastic film, which can be solubilized with an excess of SDS.
- Chitosan has proved to be an useful emulsifier, which yields stable w/o/w multiple emulsions.
- The droplet size can be controlled by the chitosan/oil ratio.
- The one-step formation of w/o/w double emulsion using chitosan as emulsifier may be due to the inherent heterogeneity of the polyelectrolyte composition. While the major part of the sample is strongly hydrophilic, some molecules may be less deacetylated and hence, be more hydrophobic. These molecules may stabilize the water droplets inside the oil drops, whilst the hydrophilic ones stabilize the oil drops in the multiple emulsion formed.

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