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Emulsification capacity of chitosan

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Abstract

Emulsification of sunflower oil by chitosan solutions with deacetylation degrees (DD) between 75 and 95% was studied. Chitosan solutions that allowed a wide range of viscosity were prepared in 0.2, 1.0, 2.0% concentration in 0.1 M HCl. The droplet size distribution, emulsion viscosity, emulsion stability and ageing behavior during 48 h were analyzed as function of the chitosan DD, solution concentration and viscosity. Chitosan produced stable water/oil/water (w/o/w) emulsions. The droplet size distribution was unimodal at low DD and at high DD, for all used concentrations, at intermediate DD, distribution was unimodal only when we used the most concentrated solutions. Meanwhile, droplet size distribution was independent of both chitosan solution viscosity and emulsion viscosity. Emulsion viscosity, emulsion stability and ageing were proportional to chitosan concentration. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Chitosan; Emulsion; Multiple emulsion w/o/w; Deacetylation degree; Viscosity

1. Introduction

Emulsions are metastable colloids made out of two no miscible fluids, one being dispersed in the other, in the presence of surface active agents. They are obtained by shearing two no miscible fluids, leading to the fragmentation of one phase into the other. Because the lifetime of emulsions may become significant (more than a year), they become good candidates for various commercial applications (Bibette, Calderon & Poulin, 1999).

Large surface-active molecules (macromolecules and polyelectrolytes) provide an interesting mean for controlling both stabilization and type of emulsions. An efficient steric stabilization is achieved by adsorbing and controlling the conformation of the molecules at the interface between the disperse and continuous phases. Instead, most low-molecular-weight surface active agents operate only through electrostatic stabilization. Electrosteric stabilization (March & Napper, 1977; Napper, 1983) is the combination of both mechanisms. Non-adsorbing polymers, such as synthetic or natural macromolecules, have the viscosifying effect that influences emulsion stability by decreasing the rate of creaming. Amphiphilic polyelectrolytes like chitosan combine both electrosteric and viscosifying stabilization mechanisms so they appear to be useful emulsifiers. Because of the above considerations, we have previously studied the emulsification properties of chitosan (Schulz, Rodríguez, Del Blanco, Pistonesi & Agulló, 1998; Del Blanco, Rodríguez, Schulz & Agulló, 1999).

Chitosan is a useful emulsifier that yields stable water-in-oil-in-water (w/o/w) multiple emulsions (Schulz et al., 1998). Multiple emulsions were described by Seifritz in 1925. These types of emulsions are a complex system in which droplets of the dispersed phase contain even smaller dispersed droplets of the continuous phase. Multiple emulsions have been prepared by both one-step emulsification and two-step emulsification (Garti, 1994). The most common and better controlled preparation of double emulsions is 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 preparation of the o/w emulsion. However, it was concluded that the classical double emulsion prepared with two sets of monomeric emulsifiers cannot provide long-term stability to the double emulsion, which are thermodynamically unstable (Brodin & Frank, 1978; Garti, Frenkel & Schwartz, 1983). To increase the stability of these multiple emulsions, macromolecules have been used with surfactants (Sela, Magdassi & Garti, 1994).

Chitosan produces w/o/w emulsions without adding any surfactant, because this biopolymer is composed of a mixture of molecules with different degrees of deacetylation (DD). Molecules with high DD promoted the formation of

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o/w emulsions, whereas those of lower DD promoted the emulsification of water inside the oil droplets. For that reason chitosan behaves as a mixture of surfactants with different hydrophile–lipophile balance (HLB) values (Schulz et al. 1998).

In our first work, we studied the emulsification properties of chitosan with a DD of 89% (Schulz et al., 1998). In a second study, we reported the effect of chitosan DD on emulsification properties (Del Blanco et al., 1999). In both, the solutions were prepared containing 1% (w/v) chitosan in 1% (v/v) aqueous acetic acid. We have now examined the behavior of chitosan as emulsifier when hydrochloric acid is used instead of acetic acid in preparation of chitosan solutions and the influence of chitosan DD, chitosan solution viscosity and the concentration of this biopolymer on its emulsification properties in hydrochloric acid medium.

2. Materials and methods

Chitin is produced in our laboratory by deproteinizing and demineralizing cleaned shrimp processing waste as raw material. Seven chitosans of different DD between 75 and 95% were obtained from our chitin under different experimental conditions (varying alkali concentration, temperature and time). Chitosans obtained were powders with less than 3.0% water content and less than 0.2% ash. The DD was measured using a prescribed method (Polish Standard 89/A-86850).

Stock solutions containing 0.2, 1.0 and 2.0% (w/v) chitosan were prepared by adding chitosans to 0.1 M HCl with stirring. After standing for 24 h, undissolved chitosan was separated by filtration through a medium-porosity filter. To determine the chitosan HLB value, the diameter of a toluene drop put on the surface of 0.2, 1 and 2% (v/w) chitosan hydrochloric acid aqueous solutions was compared with those put on solutions of several surfactants with different HLB. The diameter of the drop at rest is related to the HLB value.

To determine the optimal chitosan/oil ratio emulsions were produced by stirring, in a food processor at 9500 rpm for 3 min, the following proportions 98–8, 86–14, 80–20, 70–30, 60–40, 40–60 g chitosan/oil. Formed emulsions were left to rest for one hour and observed if phase separations occurred.

The studies were made with emulsions produced by adding 20 g sunflower oil to 80 g of different concentrations of chitosan in 0.1 M HCl. 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 and observing them by optical microscopy. To determine droplet size distribution, several samples of each emulsion were examined with a microscope (Olympus BH-2-UMA) and photographed with a Sony CCD IRIS/RGB camera. To avoid personal preferences in the selection of droplets for the determination of size distribution, four randomly drawn straight lines were placed, and the diameter of the droplets touching the lines were measured and counted. Approximately 500 droplets were considered in each sample, to ensure confidence statistical data (Dixon & Massey, 1969).

Viscosities of chitosan solutions and emulsions were measured with a Brookfield model DV-IV + viscosimeter. Measurements at different times during 48 h were performed after gentle shaking to homogenize the emulsion in order to ensure the same volume fraction of droplets in all samples.

To determine emulsion stability, samples were left to rest 48 h and the form of separation in layer and elapsed time until the phase separation occurred, were registered.

3. Results and discussion

To demonstrate that chitosan solution in hydrochloric acid has emulsification properties, we did some studies. To evaluate the type of emulsion a drop of a water-soluble dye solution loaded in the chitosan in HCl/oil emulsion dissolved readily in it, whilst a drop of oil soluble dye solution remained on the emulsion surface, indicating that water was the continuous phase. The emulsion was observed by optical microscopy, this procedure revealed that samples were water/oil/water (w/o/w) emulsions (Fig. 1). When 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. The relationship between the diameter of toluene drops laid on aqueous

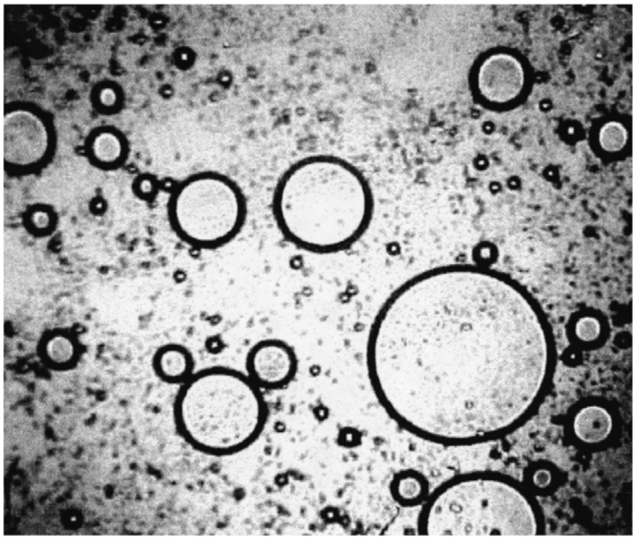

Fig. 1. Photograph of w/o/w emulsion of sunflower oil stabilized with chitosan.

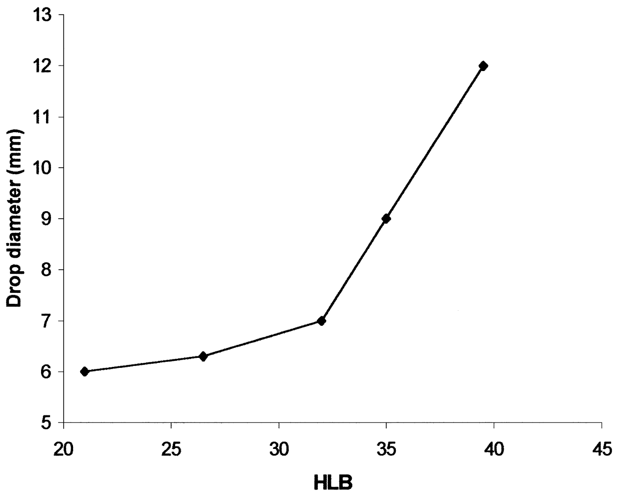

Fig. 2. Diameter of the toluene drops on solutions of 1% (w/v) of surfactants with different HLB values.

surfactant solutions and the surfactant HLB are shown in Fig. 2. The drop over chitosan solution had 8.5 mm diameter, giving an HLB value of about 34. 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.

Going on with our studies in this field we decided to examine the influence of chitosan DD, chitosan solution viscosity and concentration of the biopolymer on emulsification properties using solutions in HCl and sunflower oil.

3.1. Chitosan/oil work ratio

Solutions 0.2, 1 and 2% (w/v) in 0.1 M HCl were prepared with seven chitosans obtained in our laboratory of different DD. These solutions were used to prepare emulsions with sunflower oil. The weight ratio of chitosan solution to oil was varied with a total emulsion weight of 100 g. After leaving emulsions to rest for one hour, we found no phase separation when the chitosan solution/ oil weight ratio was 80/20 in any case, so this proportion was selected to prepare the emulsions.

3.2. Droplet size distribution

The size distribution of its droplets is the most important parameter in characterizing any emulsion. Stability and resistance to creaming, rheology, and chemical reactivity are a few of the phenomena influenced by both relative size and size distribution (Orr, 1985).

In this work, we observed that the found distribution was unimodal at low DD (75%) and at high DD (95%) for all used concentrations (Table 1). At intermediate DD, distribution was unimodal only when we used the most concentrated solutions. On the other hand, droplet size distribution was independent of both chitosan solution viscosity and emulsion viscosity.

The chitosan emulsification properties were proportional to chitosan concentration and the most efficient was 2%,

Table 1
Characteristics of chitosan solutions — sunflower oil emulsions

Chitosan	Deacetylation degree	Concentration (%)	Droplet distribution	η_{CH} (cP)	η_E (cP)	x
Sample 1	75	0.2	Unimodal	3	10	3.33
		1.0	Unimodal	174	362	2.08
		2.0	Unimodal	999	999	1.00
Sample 2	76	0.2	Unimodal	1	6	6.00
		1.0	Polymodal	3	98	3.27
		2.0	Unimodal	999	999	1.00
Sample 3	78	0.2	Polymodal	3	12	4.00
		1.0	Polymodal	38	75	1.97
		2.0	Unimodal	264	242	0.92
Sample 4	85	0.2	Polymodal	2	9	4.50
		1.0	Unimodal	65	105	1.62
		2.0	Unimodal	648	915	1.41
Sample 5	93	0.2	Polymodal	3	24	8.00
		1.0	Polymodal	16	40	2.50
		2.0	Unimodal	63	116	1.84
Sample 6	94	0.2	Unimodal	2	7	3.50
		1.0	Polymodal	26	48	1.85
		2.0	Unimodal	380	415	1.09
Sample 7	95	0.2	Unimodal	1	5	5.00
		1.0	Unimodal	3	8	2.67
		2.0	Unimodal	7	17	2.43

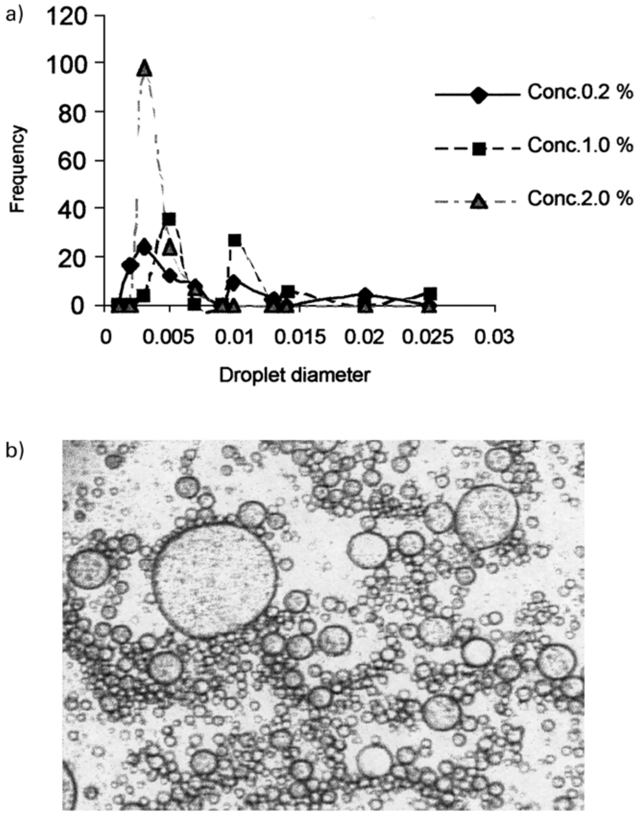

Fig. 3. (a) Droplet size distribution of sample 3 (polymodal); (b) photograph of droplet size distribution (sample 3 at 1% concentration).

because produced unimodal distribution that is coincident with more stability, of the emulsion. Two examples of droplet size distributions and microscopic photographs are shown in Figs. 3(a) and (b) and 4(a) and (b). For all studied distributions the large proportion of droplets had a diameter less than 0.005 cm, so the appearance of the emulsion was 'milky'.

3.3. Emulsion viscosity

Continuous phase viscosity is the most important factor that determines the rheological behavior of an emulsion. The equation $\eta_E = \eta_{CH}x$ expresses a direct proportion between continuous phase viscosity (η_{CH}) and apparent emulsion viscosity (η_E). The factor 'x' represents the addition of other properties that have influenced the emulsion viscosity. Both, chitosan solution and emulsion viscosities were dependent on biopolymer concentration (Table 1). When the biopolymer concentration was 1 and 2%, a direct proportionality between emulsion viscosity and respective chitosan solution viscosity was found, with no dependence on DD, but this relationship was not found when chitosan concentration was 0.2%. Table 2 presents the statistical data, which show this behavior. On the other hand, we verified that when chitosan concentration increased, the x factor decreased for all DD used.

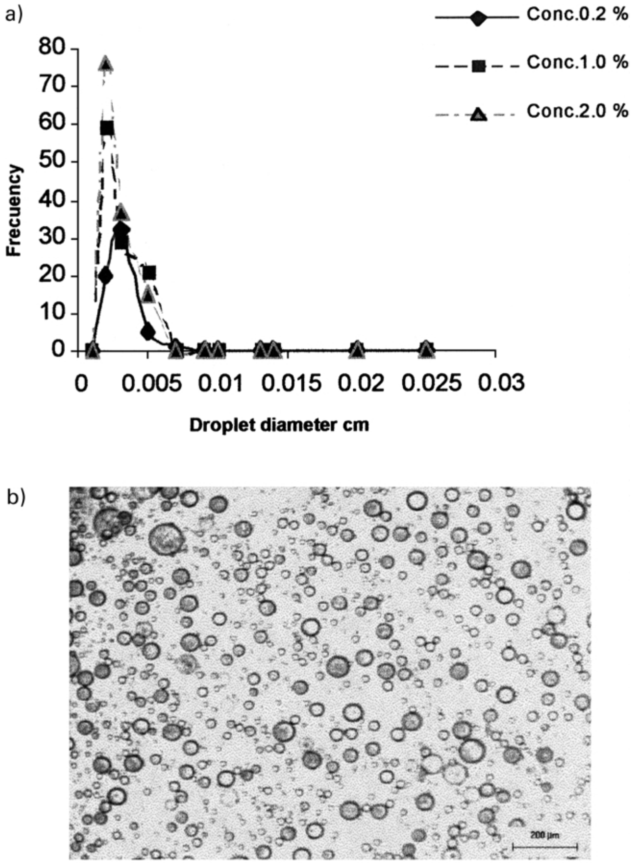

Fig. 4. (a) Droplet size distribution of sample 7 (unimodal); (b) photograph of droplet size distribution (sample 7 at 1% concentration).

3.4. Emulsion ageing

To study the effect of emulsion ageing, viscosity was determined as function of time. Measurements were made at 1, 15 and 48 h after preparing the emulsions. Ageing behavior of emulsions in three concentrations is shown in Fig. 5. The ageing behavior also had the same dependence on chitosan concentration and no dependence on chitosan DD. When chitosan concentration increased, emulsion viscosity as function of time was more stable.

3.5. Emulsion stability

Emulsion stability depends on the chitosan concentration. When chitosan concentration was 0.2%, we observed phase separation after 4–6 h in dilute emulsion at the bottom of

Table 2
Statistics data between chitosan and emulsion viscosities

Chitosan concentration (%)	Regretion equation $\eta_E = a\eta_{CH} + b$	R^2
2	$1.0692x + 29.923$	0.9386
1	$1.8664x + 18.49$	0.9055
0.2	$5.05588x - 0.4118$	0.4978

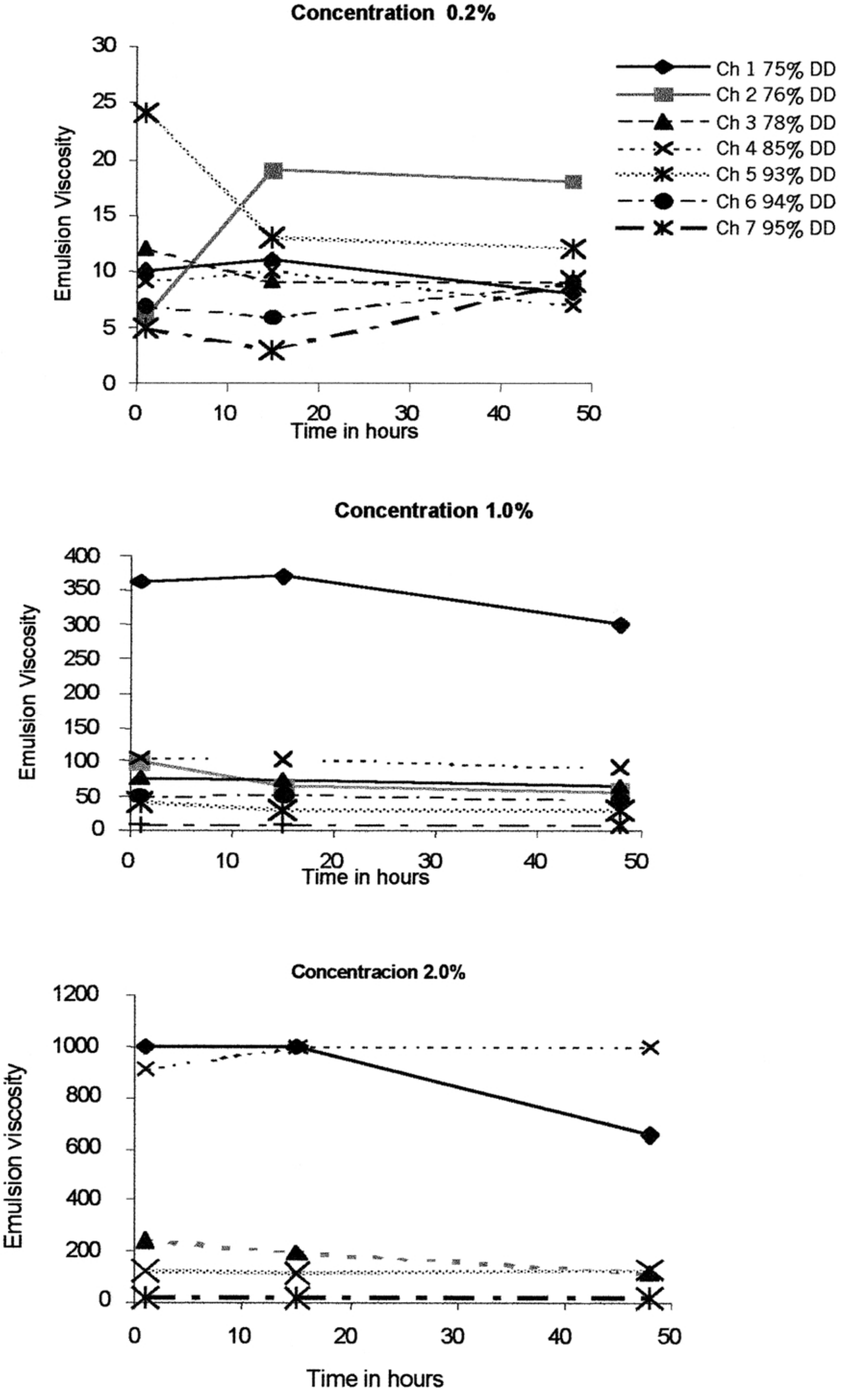

Fig. 5. Ageing behavior of emulsions.

the tube (2/3) and in concentrated emulsion at the top (1/3) that was the same for all DD used in the experiment. Emulsions prepared with 1.0% chitosan showed that time separation was not the same for different DD, but depended on emulsion viscosity. More concentrated emulsions (2.0% chitosan) had good stability, except when the biopolymer viscosity was low. In that case, separation time was accelerated. There were no cases where we detected top oil separation confirming that selected chitosan/oil ratio was correct.

4. Conclusions

With the results obtained in this work we conclude that solutions of chitosan in HCl stabilize double emulsions w/o/w and that the droplet size distribution was unimodal at low DD and at high DD. Meanwhile, droplet size distribution was independent of both chitosan solution viscosity and emulsion viscosity. Emulsion viscosity, emulsion stability and ageing were proportional to chitosan concentration.

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