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SHORT COMMUNICATION

L.F. Del Blanco
M.S. Rodriguez
P.C. Schulz
E. Agulló

Influence of the deacetylation degree on chitosan emulsification properties

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L.F. Del Blanco · M.S. Rodriguez
P.C. Schulz (✉) · E Agulló
Departamento de Química e Ingeniería
Quimica, Universidad Nacional del Sur
Avda. Alem 1253, 8000 Bahía Blanca
(Bs. As.) Argentina

Abstract The emulsification of sunflower oil by chitosan solutions with deacetylation degrees (DD) between 73 and 95% was studied using different techniques. The droplet size distributions, conductivity, ageing behavior and viscosity of emulsions were studied as functions of the chitosan DD. All DD gave stable polydisperse water-in-oil-in-water emulsions with different viscosities. Two optimum DD values were found, 81 and 88%, giving complete emulsification without residual oil or sedimentation. Chitosans with intermediate DD were less effective emulsifiers. Chitosans with higher DD gave poor emulsification.

Key words Chitosan · Emulsion · Deacetylation degree · Emulsion stability

Introduction

The production of stable emulsions is very important in many industrial processes [1, 2]. Processed foods are often colloidal systems such as emulsions, suspensions and foams. Emulsion formation may also be an intermediate step in food processing, as is the case with semimoist foods, coffee whiteners, cake mixes, etc.

In some applications the use of large surface-active molecules (macromolecules and polyelectrolytes) has proved to be useful [3–6]. In such cases efficient steric stabilization is achieved by adsorbing and controlling the conformation of the molecules at the interface between the disperse and continuous phases. Naturally occurring macromolecular substances, such as gums and proteins, have been used since the beginning of civilization for the stabilization of emulsions, for example, in food and pharmaceutical emulsions [7–10].

The concept of polymeric surfactants is over 35 years old. In comparison with low-molecular-weight surfactants, macromolecular surfactants have the advantage of attaching to a surface via several segments and even if the free energy of adsorption is low per segment, the attachment of several segments results in a large free energy of adsorption. As a result their adsorption onto surfaces is firmer and, therefore, they can be used more efficiently in low concentrations. Polymeric surfactants certainly provide an interesting means for controlling both the stabilization and the type of emulsions. From theoretical considerations, copolymers and more surprisingly homopolymers were found to adsorb at liquid–liquid interfaces [11, 12]. In contrast to small surfactant molecules, very little is known of the effects of polymeric surfactant molecules on emulsions.

Polymeric surfactants offer the advantage of important steric stabilization of dispersions while most low-molecular-weight surface-active agents operate only through electrostatic stabilization. The combination of both mechanisms is often referred to as electrosteric stabilization [10, 13]. In addition, it is well known that the viscosifying effect of nonadsorbing polymers (synthetic polymers or naturally occurring macromolecules such as gums) may influence emulsion stability by decreasing the rate of creaming [14].

In the light of the above considerations, amphiphilic polyelectrolytes appear to be promising emulsifiers since they offer the opportunity to combine both electrosteric and viscosifying stabilization mechanisms. Following this idea, we reported the emulsification properties of chitosan in a previous paper [15].

Chitosan, β-(1-4)-linked 2-amino-2-deoxy-D-glucose, is a linear polymer prepared by partial alkaline deacetylation of chitin, poly(*N*-acetyl-D-glycosamine), a main structural component of the cuticles of insects, mollusks and crustacea, and the cell membranes of microorganisms [16]. Chitosan is used in several technologies such as biotechnology [17–19], cosmetics [20, 21] and medicine [22–25]. The lack of toxicity of chitosan upon oral and nasal administration, [26, 27] the low toxicity an intravenous administration [28] and its biocompatibility [29] make this compound ideal for uses related to food industries.

Chitosan has proved to be a useful emulsifier [15]. It yields stable water-in-oil-in-water (w/o/w) multiple emulsions. The previous study was made on chitosan with a single deacetylation degree (DD) of 89.0%. In this paper we report the effect of chitosan DD on the chitosan emulsification properties. To compare the new results with that of the previous paper, we used sunflower oil to prepare the emulsions. This is a consequence of our interest in studying the use of chitosan in food emulsions.

Experimental

Chitosan of different (DD), between 73 and 94%, was obtained by treating chitin from shrimp outer shells under different conditions (varying NaOH concentration, temperature and time). The chitosans obtained were powders with less than 3.0% water content and less than 0.20% ash. The DD was measured using a method described in the literature [30].

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

Emulsions were produced by adding 20 g sunflower oil to 80 g 1% w/v chitosan in 1% v/v aqueous acetic acid (pH 3.05). This was the optimum proportion found previously [15]. Samples were stirred in a food processor for 3 min. Double-distilled water was employed. The final pH of the chitosan solutions was 3.87, and the ionic strength was 3×10^{-3} mol dm^{-3}. Three independent series of emulsions were prepared.

Emulsion samples were placed in graduated tubes sealed with a polyethylene membrane and were put in a fridge to freeze. Afterwards the frozen emulsions were allowed to melt at room temperature. The freeze-melting cycle was repeated up to six times. In each cycle, phase separation was controlled by visual observation, and the distribution of droplet diameters by microscopy. Emulsion samples were put on a heated stage at 100 °C and observed microscopically to detect changes.

The viscosities of the chitosan solutions and the emulsions were measured with a Brookfield model DV-II+ viscosimeter. The shear rate was 64.5 s^{-1}, whereas the shear stress was dependent on the sample viscosity [shear stress (N m^{-2}) = 0.465η (Pa s)]. Measurements on aged emulsions were performed after gentle shaking to homogenize the emulsion in order to ensure the same volume fraction of droplets in all samples.

Emulsion conductivity was measured with a CRIBABB digital conductimeter and an immersion cell at 25 °C.

Samples were centrifuged in graduated tubes. Then the volumes of remaining oil, concentrate and dilute emulsions were recorded.

To determine the droplet size distribution several samples of each emulsion were placed between slides and photographed in a microscope. A fibre of known thickness was put in the samples and was used as an internal standard to measure the diameter of the droplets in the photographs. To avoid personal preferences in the selection of droplets for the determination of droplets size distributions, a transparent sheet with four randomly drawn straight lines was placed on each photograph, and the diameters of the droplets touching the lines were measured. These diameters were classified in categories from 0 to 5 μm, 6 to 10 μm, etc. About 500 droplets were measured in each sample, giving a confidence level of about 7% [30].

Results

Freeze–melting stability

Up to six successive freeze–melting runs were made on the different emulsions, without any modification from the initial state.

Heating stability

Emulsions were heated to 100 °C without any modification from the initial state. The droplet size did not change. Coalescence was not observed.

Droplet size distribution

The droplet size distribution of the different emulsions was determined by microscopic observation of freshly prepared samples. Before examining emulsions which had been prepared several days previously the emulsions were homogenized by gentle stirring. No significant changes occurred with time.

The general facts were that at high DD (≥89%) the distributions were unimodal, whereas they were bi- or polymodal at lower DD. In samples with DD ≤80% the distributions were in general broader, with two or three peaks of similar importance. Some droplet size distributions are shown in Fig. 1. The positions of the maxima in the droplet size distributions as functions of the DD are shown in Fig. 2. The two smallest mean diameters (14 ± 1 and 38 ± 4 μm) belonged to the larger proportion of droplets, mainly the first one. The other maxima corresponded to minor proportions.

Emulsion conductivity

The specific conductivity of freshly prepared emulsions slowly increased with DD following the linear equation $\kappa(\mathrm{mS\ cm^{-1}}) = 4.1_3 \times (\%\ \mathrm{DD}) + 1.3_2 \times 10^3$; r the correlation coefficient being 0.157.

Emulsion viscosity

The relationship between the viscosities (η_e) of freshly prepared emulsions and the respective chitosan solution

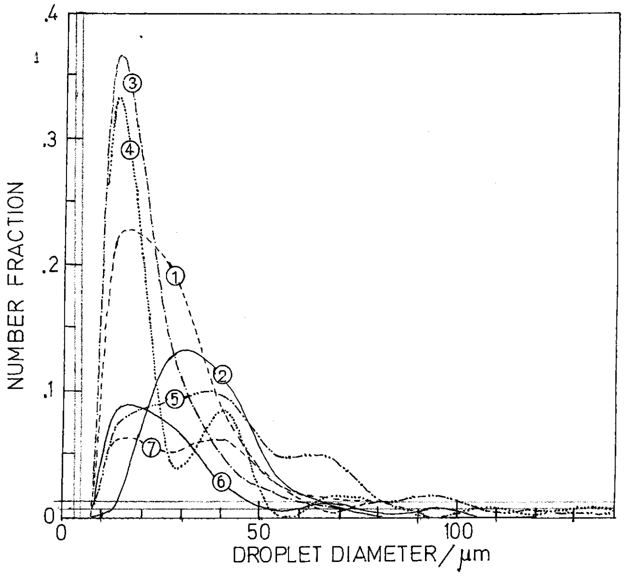

Fig. 1 Droplet diameter distribution of some emulsions. *1*: Deacetylation degree (DD) = 94%; *2*: DD = 92.3%; *3*: DD = 89%; *4*: DD = 86%; *5*: DD = 78%; *6*: DD = 75%; *7*: DD = 73%

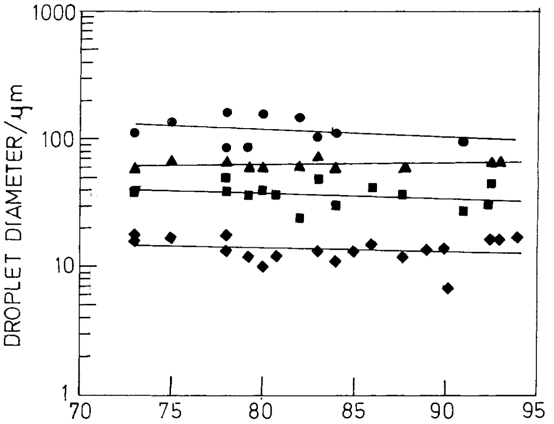

Fig. 2 Droplet diameter maxima versus., chitosan DD. The *lines* are the least-squares fits. All slopes did not significantly differ from zero. The position of the maxima were 0.014 ± 0.001 mm (◆); 0.038 ± 0.004 mm (■); 0.064 ± 0.003 mm (▲) and 0.12 ± 0.02 mm (●)

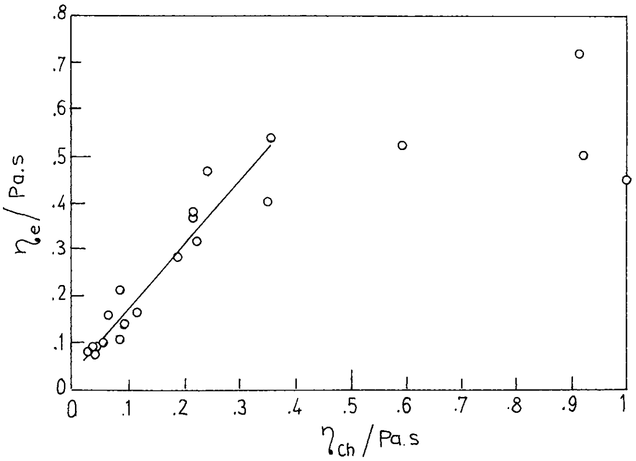

Fig. 3 Viscosity of freshly prepared emulsions versus chitosan solution viscosity

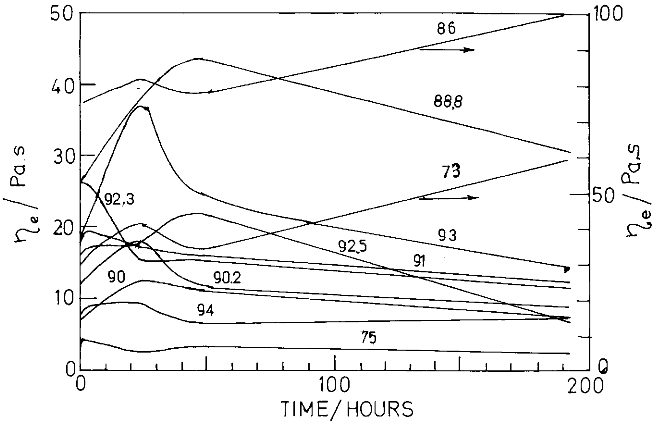

Fig. 4 Emulsion viscosity change with time. The *numbers* on the curves denote chitosan DD

viscosities (η_{ch}) is shown in Fig. 3. The emulsions had the same viscosities as the chitosan solutions without oil up to 0.4 Pa s. Above this value the emulsion viscosity became almost constant. All emulsions had the same volume fraction of dispersed phase ($\Phi = 0.2$), but the droplet size distributions were not the same.

To study the effect of ageing the emulsions, the viscosity was determined as a function of time. Viscosity measurements were made at 0, 1, 24, 72 and 168 h after preparing the emulsions. The ageing behaviour of the emulsions as a function of chitosan DD is shown in Fig. 4. It can be seen that there were several DD in which the viscosity did not change with time: 81 and 88% with high η_e, and 80, 83 and 86% with low η_e. The other DD gave emulsions whose viscosity changed with time, as can also be seen in Fig. 4. In general emulsions made with chitosan of low DD (< 89%) showed a viscosity increase with time, whereas at higher DD there was an initial increase followed by a slight decrease with time which was accompanied by the appearance of free oil. This may be due to the initial formation of unstable droplets which coalesced with time to form free oil.

Centrifugation behavior

When centrifuged, the emulsions in general formed three layers: free oil, concentrated w/o/w emulsion and very dilute w/o/w emulsion. The amounts of these layers can be seen in Fig. 5, on a volume percent basis. Free oil

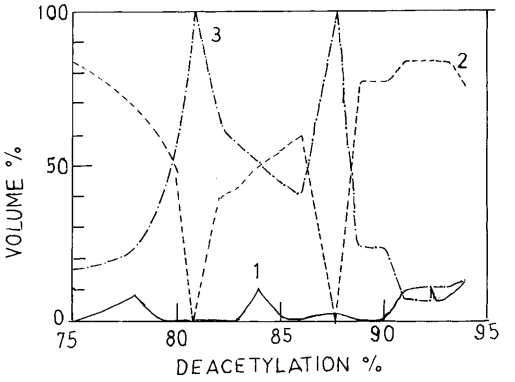

Fig. 5 Volume percent of free oil (**1**), dilute emulsion (**2**) and concentrated emulsion (**3**) versus chitosan DD

mainly appeared at DD ≥ 91%, together with a little concentrated emulsion. There were two optimum DD to produce emulsification of the oil: 80 and 88%, which emulsified all the system into a unique emulsion. Between 82 and 86% DD the oil was almost completely emulsified, but two layers formed: dilute and concentrated emulsions. In addition, some free oil appeared when DD = 84. When DD < 80%, the emulsification capacity of chitosan decreased with decreasing DD.

Discussion

The heating and freeze–thaw stability of the emulsions was high. No dependence of these properties on DD was detected. These experiments confirmed the stability found previously [15].

Chitosan emulsions were highly stable on ageing. No changes in droplet size distribution were detected, or in the relative volumes of emulsion and free (nonemulsified) oil.

The emulsion conductivity had a slight dependence on DD. Since the volume fraction of droplets was always the same, the change must be related to the conductivity of the interdroplet solution. This situation reflected two different facts:

- The chitosan conductivity itself increases, because it is a charged polyelectrolyte whose charge per kinetic unit increases with increasing DD.
- The increasing proportion of chitosan which remained in the aqueous solution, because of the reduction in hydrophobicity with increasing DD.

The effect of the increasing charge in the kinetic unit was probably reduced because the more charged the polyelectrolyte is, the more the linear chain is extended by the electrostatic repulsion between the charges. It is known [31, 32] that the charge density along the chain increases with an increase in the DD. The chitosan chain is extended in aqueous solution more than noncharged polymers in a good solvent. This is mainly due to its backbone and polyelectrolyte structure [33]. This increases the drag and reduces the electrical mobility. Both effects probably cancel each other in some extensions.

The droplet conductivity was relatively low because of the low volume fraction and the high conductivity of the interdroplet solution, an aqueous solution of acetic acid and chitosan.

Like disordered proteins, chitosan can be regarded as a copolymeric highly hydrated [34] polyelectrolyte. Its structure is probably an aleatory succession of blocks of β-(1-4)-linked 2-amino-2-deoxy-D-glucose (the hydrophilic block) and poly (*N*-acetyl-D-glucosamine), the hydrophobic block. As with other polyelectrolytes, adsorbed layers of chitosan offer the advantage of combining steric and electrostatic emulsion stabilization [10, 13]. Because of its polar groups, chitosan also provides additional stabilization due to hydration forces [35]. Even in dilute solution chitosan still forms a small number of larger-sized aggregates [33]. In the highly concentrated oil/water interface, the formation of these aggregates would enhance the interface elasticity.

In Fig. 3 it can be seen that the emulsions had the same viscosity as the chitosan solutions without added oil up to 0.4 Pa s. Above this value the emulsion viscosity became almost constant. All emulsions had the same volume fraction ($\Phi = 0.2$) of spherical droplets, but the droplet size distributions were not the same for different samples.

For hard-sphere suspensions, the relative viscosity follows a virial equation depending only on the volume fraction of dispersed spheres and the viscosity of the continuous phase [36–39], which was found to be valid up to $\Phi \approx 0.35$ [37, 38]. The viscosity of a dilute suspension of spherical particles is insensitive to the particle size distribution, provided Φ is constant. This is not true in concentrated emulsions ($\Phi \geq 0.5$) [40]. Emulsions in which droplets are enveloped by an elastic or viscoelastic film of adsorbed molecules (as is the case in chitosan-stabilized emulsions) behave as hard sphere suspensions (see Ref. 1, vol. 1, p. 415).

Since the pH and ionic strength were maintained at constant levels in the different samples, the electroviscous effect might also remain constant [41, 42].

The above considerations lead to interpreting the changes in the emulsion viscosity as being mainly caused by changes in the viscosity of the interdroplet aqueous solution. As already pointed out, chitosan in solution is an extended linear polyelectrolyte, and the longer the polyelectrolyte molecule, the greater the viscosity of the solution. The reduction in the emulsion viscosity was therefore probably due to adsorption of the longer chitosan molecules at the oil/water interface. This effect must be important in high-viscosity chitosan solutions, in which longer molecules must exist, since all solutions had the same chitosan concentration by weight.

The changes in viscosity on ageing may be attributed to changes in the composition of the continuous phase. Some initially adsorbed chitosan molecules were released into the aqueous phase, producing an increase in viscosity. Initially nonadsorbed chitosan molecules were taken up by the oil/water interphase, resulting in a reduction in the viscosity.

In agreement with theories of depletion flocculation by nonadsorbing polymers [43, 44], the presence of an excess of chitosan or some nonadsorbing chitosan fractions of high DD may induce a substantial increase in creaming and flocculation stability; however, if the ratio of hydrophilic to hydrophobic groups is too high, the molecules become more soluble in the aqueous phase. Hence, chitosan molecules with a very short anchoring group were found to be poor stabilizers.

Chitosan is a mixture of several fractions of molecules with different DD [33]. Even if the DD is the same, chitosans of different origin have different properties. The molecular-weight distribution of chitosans from different batches with the same DD are broad and have different maxima [33]. The differences depend on the chitin origin and the season of the year, and vary from one batch to another even if the same chitin is used in hydrolysis and the same DD is obtained. This inhomogeneity was reflected in the droplet size distributions: the higher the DD, the less diverse the fractions. In the extreme case of DD = 100%, only one kind of chitosan will exist. This may explain the predominance of unimodal droplet size distributions when chitosans with high DD were employed. Chitosans with lower DD probably had several fractions, some of them with high and others with low DD, giving an average value which was the nominal one. This situation produced polymodal droplet size distributions, each peak being produced by a particular fraction of chitosan. Nevertheless, in Fig. 2 it can be seen that irrespective of the nominal DD, there were preferential droplet diameters. The smallest droplets were predominant. Figure 1 shows the significance of droplet size: the maxima dropped sharply with increasing diameter. This may mean that chitin hydrolysis follows some preferential mechanisms, i.e., it may not be completely aleatory. Figure 2 also shows the general trend that the lower the DD, the more polymodal the droplet size distribution.

The size distribution of its droplets is a most important parameter in characterizing any emulsion. Two emulsions may have the same average droplet diameter and yet exhibit quite dissimilar behavior because of differences in their diameter distributions. Stability and resistance to creaming, rheology, chemical reactivity, and physiological efficiency are but a few of the phenomena influenced by both relative size and size distribution [45].

Figure 5 shows that the best DD for emulsification lay between about 80 and 89%, with two optimum values, 81 and 88%. The droplet size distribution was trimodal in emulsions with DD = 88%, having droplet diameter maxima at 12, 37 and 61 μm each peak having about the same area. The emulsion with DD = 81% was bimodal, with maxima at 12 and 37 μm, having about the same area. The emulsion with DD = 88% had a low viscosity which had only a slight dependence on time. The emulsion with DD = 81% had a higher viscosity and this increased with time.

To ensure good emulsion stability, the copolymer should have an optimum ratio between the stabilizing moiety and the backbone [4]. In view of the inhomogeneous nature of chitosan, this optimum ratio was probably obtained by blending different fractions. In this case, more than one composition may produce stable emulsions, giving the above results.

Concluding remarks

- All chitosan emulsions were highly stable under temperature changes and ageing. They were also stable to flocculation and coalescence. This may be due to the formation of a highly hydrated steric–mechanical barrier on the surface of the droplet, which was also positively charged. This barrier prevented oil droplet contact by both mechanical and electrical constraints. These properties do not depend on the chitosan DD, but highly deacetylated chitosan was a less effective emulsifier.
- The best chitosan DD for sunflower oil emulsification lies between about 80 and 89%, with two optimum values, 81 and 88%, in which the system was a homogeneous emulsion without free oil or dilute emulsion separation. The first chitosan DD gives high viscosity emulsions, whilst the second gives a low viscosity one. These characteristics may be useful for the design of food (or cosmetic) emulsions with different uses (i.e., stable emulsions with different viscosities).
- It must be noted that all emulsions were made with sunflower oil, and changes in the nature of the oil may modify the emulsion behavior. Other kinds of oil may require different DD to obtain optimum emulsions.

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