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Physico-chemical studies and emulsifying properties of *N*-propyl-*N*-methylene phosphonic chitosan

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ABSTRACT

Chitosan is a modified, natural carbohydrate polymer derived by deacetylation of chitin. Due to the presence of two functional groups can undergo many chemical modifications. In a previous work we described the synthetic strategy and characterization of a novel soluble derivative: *N*-propyl-*N*-methylene phosphonic chitosan (PNMPC). In the study of some physicochemical properties, results showed that this modified chitosan aggregates in several steps when the concentration is increased. By addition of NaOH the initially coiled molecules stretch exposing more phosphonic acid groups to neutralization and finally give a cooperative reaction with $OH^{(}$. PNMPC has emulsifying properties and gives O/W emulsions with quasi-monodisperse small droplets. Emulsions with 0.18% PNMPC and 30:70 o:w ratio exhibited the best emulsifying properties within the test range. This emulsion ratio showed high stability to long time storage and several successive freeze/thaw and heating/cooling cycles.

1. Introduction

Chitosan, the linear and partly acetylated (1-4)-2-amino-2-deoxy-β-D-glucan, is obtained from marine chitin, the second most abundant carbohydrate polymer on Earth (Muzzarelli, Boudrant, Meyer, Manno, DeMarchis, & Paoletti, 2012). Chitosan is cationic, nontoxic and biodegradable (Muzzarelli, 2010). The use of chitosan in the food industry is related to its functional properties and nutritional and physiological activities. In these applications, chitosan interacts in most cases with biomembrane surfaces, as it is evidenced from literature reports describing the effects of chitosan on lipids and micelles (Dickinson, 2009).

Chitosan has been proved to be a useful emulsifier (Li & Xia, 2011; Payet & Terentjev, 2008; Speiciene, Guilmineau, Kulozic, & Leskauskaite, 2007). It has been widely employed in food, pharmaceuticals, paper and other industries primarily due to its excellent emulsification and thickening properties (Ogawa, Nakata, Yamamoto, Nitta, & Yui, 1996).

The interactions between proteins and the carbohydrates such as chitosan can also help to stabilize the emulsions. Several polysaccharides have been used together with proteins to enhance the emulsion stability (Chuah, Kuroiwa, Kobayashi, & Nakajima, 2009; Jourdain, Leser, Schmitt, Michel, & Dickinson, 2007; Wang, B., Wang, L.J., Li, Adhikari, & Shi, 2011).

Due to the presence of two functional groups chitosan can undergo many chemical modifications which render it a very attractive material with tremendous applications in various fields. In a previous work we described the synthetic strategy and characterization of a novel soluble derivative: *N*-propyl-*N*-methylene phosphonic chitosan (PNMPC) (Zuñiga, Debbaudt, Albertengo, & Rodriguez, 2010). PNMPC has a predominant monoalkylated form, though it may be detected the dialkylated one. The introduction of the short chain slightly enhanced its water solubility as a consequence of the formation of the dialkyl moiety which increases the carbon contribution. Furthermore derivative molecular weight is about 60×10^3, X-ray diffraction and SEM showed certain degree of crystallinity and homogeneous surface with a rather packed structure.

The goal of this work is to determine solution properties and emulsifying capacity of PNMPC for its possible use in food emulsions. Results could be also used in the preparation of emulsions with applications in pharmacology and cosmetics.

2. Experimental

2.1. Synthesis of N-propyl-N-methylene phosphonic chitosan (PNMPC)

NMPC (2.11 g) was suspended in 200 mL of 1:1 (*v*/*v*) distilled water-methanol (Anedra, Buenos Aires, Argentina), 3.0 g propyl

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aldehyde (Sigma–Aldrich, St. Louis, MO) was added and stirred for 30 min. Reduction was carried out with a sodium borohydride (Aldrich, Milwaukee, WI) solution (0.5 g dissolved in 10 mL of water) added in small portions for 2 h with mechanical stirring. The mixture was stirred at room temperature overnight. PNMPC sodium salt was obtained by dialyzing the reaction mixture (dialysis tubing with a cut-off value of 12,400 Da, Sigma–Aldrich, St Louis, MO) against demineralized water until water pH of 6.8. Finally the solution was freeze dried. All reagents used were of analytical grade or better.

PNMPC was characterized by X-ray diffraction spectrometry, FT-IR, ^{1}H, ^{13}C and ^{31}P NMR spectroscopy, molecular weight determination and elemental analysis (Zuñiga et al., 2010).

Conductivity measurements were made on the dilutions with an Altronix CT 1 conductimeter. A sample of 15.00 mL 0.18% (w/v) of PNMPC was titrated with 0.0925 M NaOH (Anedra, Buenos Aires, Argentina) solution and the conductivity was measured after each addition. The dilution effect was corrected with the equation:

$$\kappa_{\text{corrected}} = \kappa_{\text{measured}} \times \frac{(V_{\text{initial}} + V_{\text{added}})}{V_{\text{added}}} \quad (1)$$

This titration was also followed by pH determination with pH/ISE meter, Orion 710 with a Broade and Jame glass electrode (reproducibility ±0.01 pH units).

2.2. Emulsion preparation

Emulsions were prepared with solutions of *N*-propyl-*N*-methylene phosphonic chitosan (PNMPC) as emulsifier, and sunflower oil (100%) purchased from a local supermarket, by stirring at 13,600 rpm with a Braun Food Processor MR 5550 CA during 4 min. Average saponification index and acid value of the sunflower oil were 190 and 0.19 mg KOH/g respectively. Brookfield viscometer (Model DV-II+, Brookfield Engineering Inc., USA) was used to measure the viscosity.

2.3. Determination of PNMPC concentration

To determine minimum PNMPC concentration to obtain good emulsification, emulsions were prepared by adding 20.0 g sunflower oil to 80.0 g of PNMPC dissolved in water in different concentrations (0.10%, 0.15%, 0.18% 0.20%).

2.4. Emulsion type

Microscopic observations were made in an Olympus BH-2-UMA optical microscope equipped with a Sony CCD IRIS/RGB photographic camera.

2.5. Optimal oil/PNMPC solution ratio

To determine the optimal oil/PNMPC solution ratio for good emulsification and stability, emulsions were made with a constant amount (40 g) of PNMPC 0.18% and different amounts of sunflower oil (5.0. 10.0, 15.0, 20.0, 30.0, 40.0, 50.0 g). Emulsions were prepared as previously described and were stored in 100 mL graduated glass cylinders covered with stoppers for 30 days at room temperature. The creaming behavior of emulsion samples was monitored by visually observation, and measuring the height of the layers formed at regular time intervals and all over the study period. The extent of creaming was characterized by creaming index using the following equation (Krstonosic, Dokic, L., Dokic, P., & Dapcevic, 2009):

$$\text{Creaming Index} = 100 \times \frac{H_C}{H_E} \quad (2)$$

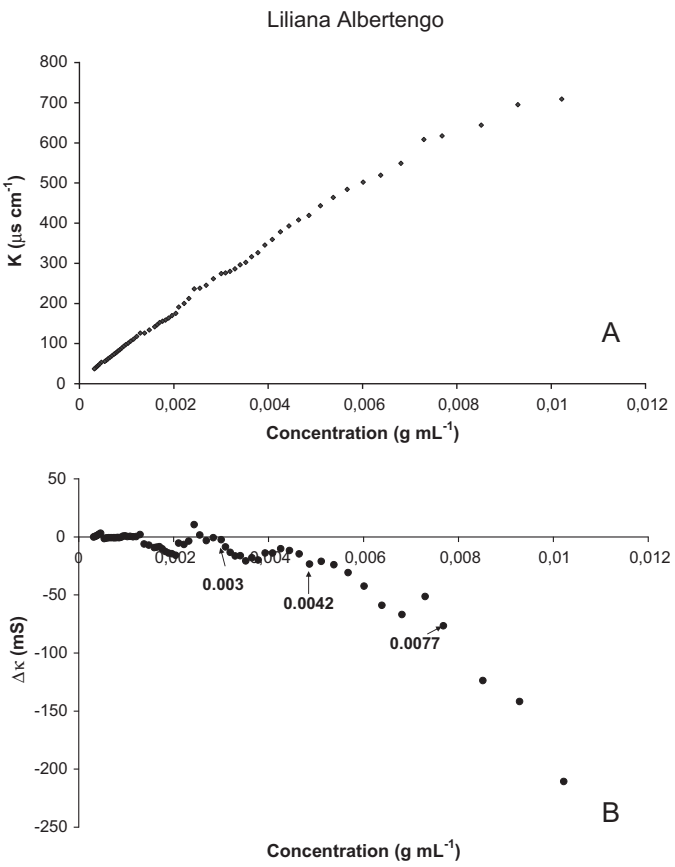

Fig. 1. Relationship between PNMPC concentration and (A) specific conductivity and (B) (K. Arrows indicate the breaks.

where H_C is the volume of dilute emulsion layer at the bottom of the cylinder and H_E is the total volume of emulsion.

2.6. Emulsions stability

To perform studies on the stability of emulsions along time and temperature changes, emulsions with the following o/w ratios 30/70, 40/60 and 50/50 were made. For time dependence of stability testing samples of these emulsions were transferred into sealed glass tubes and stored at room temperature (20 °C). The creaming stability of the emulsion samples was monitored by visual and microscopic observation over a period of 60 days.

The stability of emulsions to temperature changes was determined by six consecutive cycles of freezing at −18 °C followed by

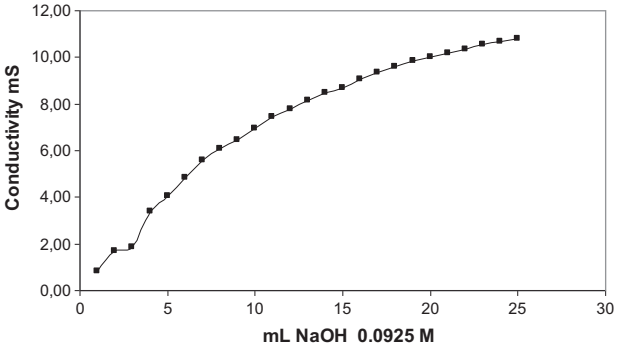

Fig. 2. Conductimetric titration curve of 15 mL of 0.18% PNMPC solution as a function of the volume of added 0.0925 M NaOH solution.

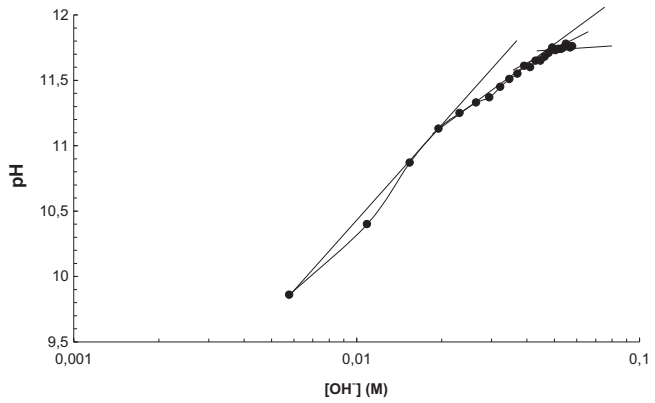

Fig. 3. Evolution of the pH in the 15 mL of 0.18% PNMPC solution as a function of the OH^- concentration.

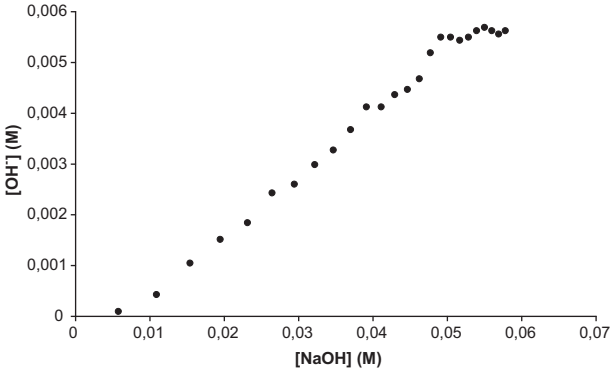

Fig. 4. Molar concentration of OH^- as a function of molar concentration of NaOH.

thawing at room temperature and six cycles of heating at 100 °C and cooling at room temperature.

3. Results and discussion

3.1. Physicochemical characteristics of PNMPC. Solution properties

3.1.1. Specific conductivity

The specific conductivity as a function of a chitosan derivative concentration is shown in Fig. 1A. As can be seen, there is a good linear relationship between the conductivity and the PNMPC concentration at low concentration, but there are changes when the concentration of the modified chitosan is incremented.

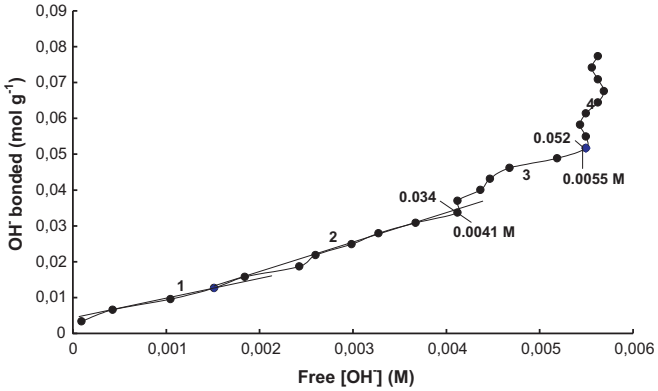

Fig. 5. Correlation between OH^- ions which reacted with phosphonic groups and free OH^- ions.

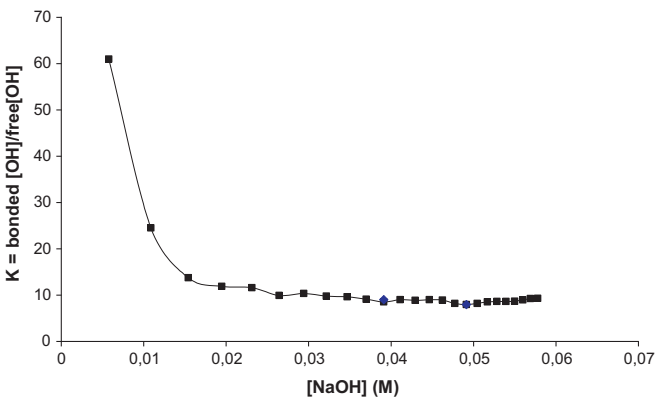

Fig. 6. Partition constant K that relates the concentrations of polymer-bonded and free hydroxyl ions versus the NaOH concentration.

Fig. 1B shows the $\Delta\kappa = \kappa_{measured} - \kappa_{extrapolated}$ dependence on concentration for PNMPC, where $\kappa_{measured}$ is the specific conductivity measured and $\kappa_{extrapolated}$ is that extrapolated from the low concentration points. This representation magnifies the differences in slope, facilitating the detection of breaks. It is easily seen that there are three points of aggregation in greater colloidal systems at 0.003; 0.0042 and 0.0077 g/mL. This behavior is similar to that of self-aggregating substances such as surfactants. It may be assumed that at low concentration probably the molecules of the modified carbohydrate polymer are unaggregated and then the conductivity increases almost linearly with concentration, while at 0.003 g/mL there is an aggregation of molecules. At the other points these aggregates probably change in size and shape, giving rise to less conducting species.

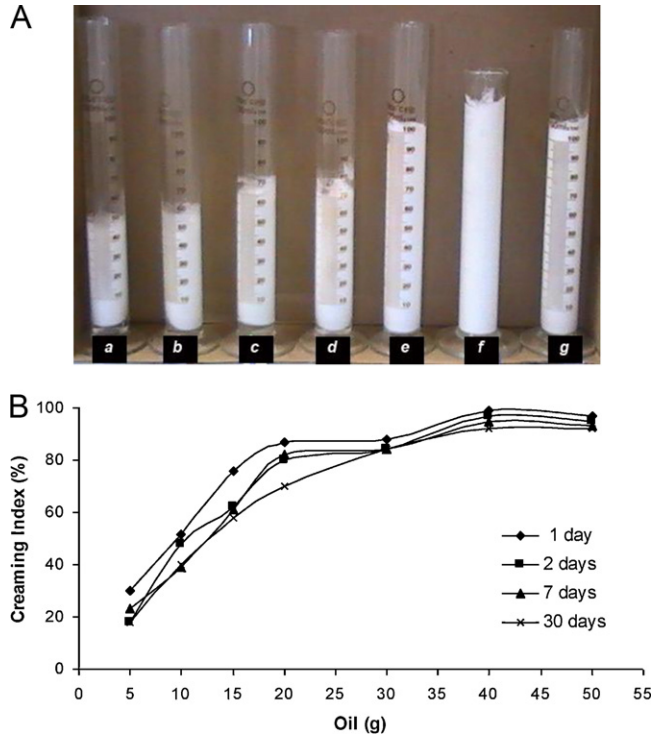

Fig. 7. (A) Emulsions prepared with constant amounts of PNMPC (40 g, 0.18%) and increasing amounts of oil: (a) 5 g, (b) 10 g, (c) 15 g, (d) 20 g, (e) 30 g, (f) 40 g, (g) 50 g and (B) his influence on creaming stability during storage time at room temperature.

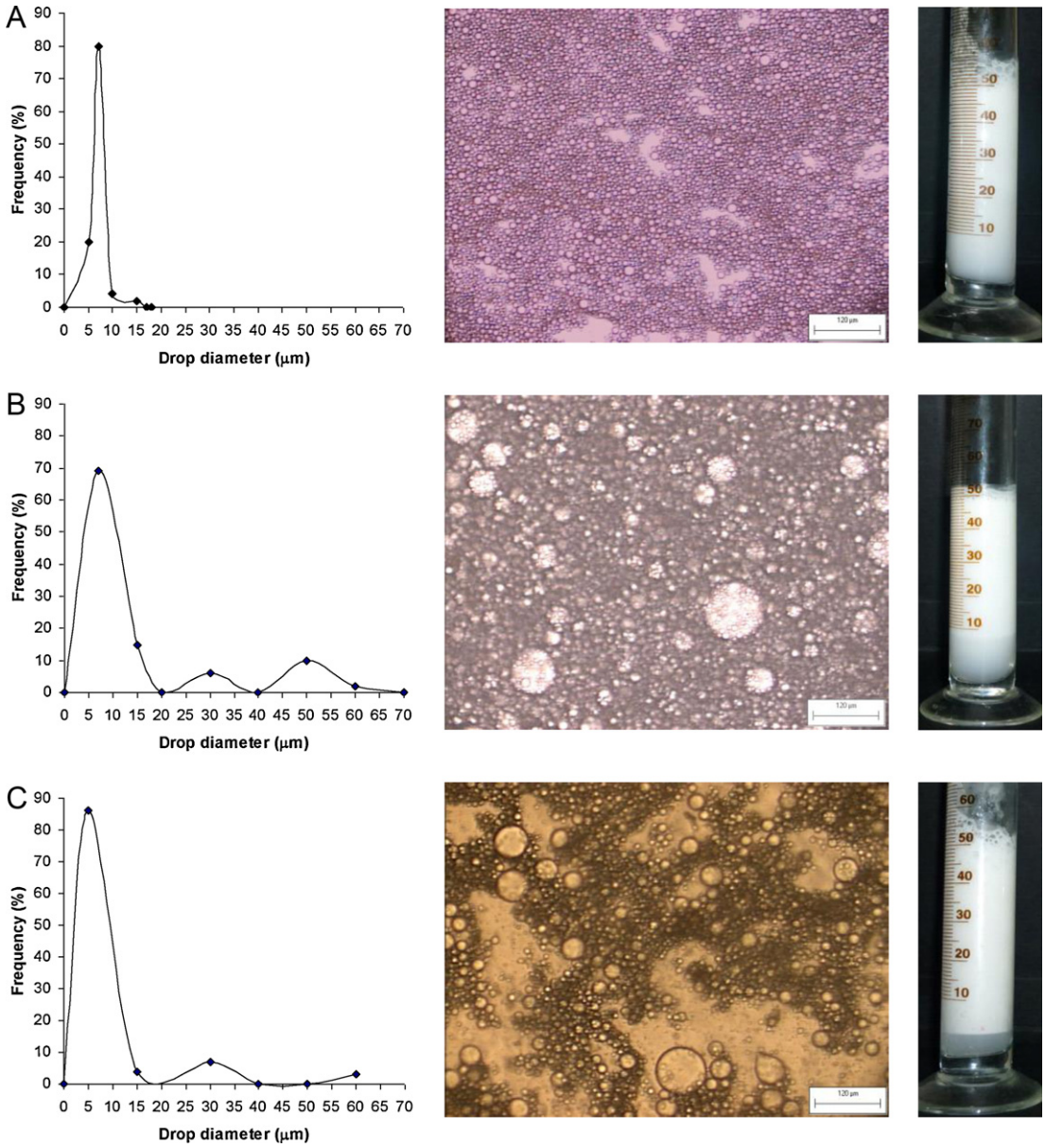

Fig. 8. Particle size distribution, microscopic views and visual appearance of emulsions at different o:w ratios: (A) 30:70; (B) 40:60 and (C) 50:50.

3.1.2. Conductimetric titration

Results of the conductimetric titration of the PNMPC (0.18% initial concentration) are shown in Fig. 2. Two behaviors can be observed. A conformational change is revealed and the phosphonic groups are more exposed when the titration advanced.

The interpretation of this behavior can be facilitated with the determination of pH in the titration (Fig. 3). Almost four linear zones with different slopes can be visualized, indicating changes in the interaction of the polyelectrolyte with hydroxide ions.

As it can be observed in Fig. 4, as further NaOH is added, it reacts with PNMPC phosphonic groups giving charged phosphonate ones, and this union becomes cooperative at higher concentrations of OH^-. These results can be explained by postulating that at higher concentrations of NaOH, the PNMPC structure is opened because of the repulsion between the charged phosphonate groups, and then more acid groups, which initially were screened by the crowded structure of the polymer molecule, are exposed to interact with OH^- ions.

To a better visualization of this phenomenon Fig. 5 shows the correlation between hydroxyl ions which reacted with phosphonic groups and free OH^- ions. Four zones corresponding to structural changes are observed. The first segment is lineal because of proportionality between bonded and free hydroxyl groups. In the second zone, the chitosan derivative structure begins to swell allowing more PO_3H_2 groups available to neutralization. In the third segment it can be observed that the structure is more open with a higher exposition of phosphonic groups which were located more deeply in the polymeric structure. Finally, in the fourth zone, the structure is completely open and the resulting situation is a massive neutralization of internal $H_2PO_3^-$ groups in an apparent cooperative process.

Fig. 6 represents the partition constant K (defined as the ratio of the OH^- concentration in the PNMPC solution and water), that relates the concentrations of polymer-bonded and free hydroxyl ions versus the NaOH concentration. It can be observed that K is a constant in the NaOH concentration range from 0.039 to 0.049 M and this is related to the acid constant of orthophosphoric acid.

The previously identified points indicate changes in the interaction between the hydroxide ion and the polyelectrolyte, due to the above mentioned structural changes, since the exposition of acid groups vary their availability to neutralization. The average values in the linear region should be an estimation of the real partition constant ($K = 8.64$).

3.2. Emulsifying properties of PNMPC

3.2.1. Optimum chitosan derivative concentration for emulsification

The study of the optimum concentration of the carbohydrate derivative was performed by preparing emulsions in a ratio of 80 g aqueous PNMPC solution (concentrations 0.10; 0.15; 0.18 and 0.20%) with 20 g of sunflower oil which, in previous studies with chitosan and their derivatives, have been proved to be the most stable oil phase (Rodriguez, Albertengo, Etcheverry, & Schulz, 2005; Schulz, Rodriguez, Del Blanco, Pistonesi, & Agullo, 1998).

We found that the lower PNMPC concentration with higher stability was 0.18% at room temperature. Emulsion viscosity was 30 mPa s, at 25 °C and 50 rpm.

3.2.2. Optimal oil/PNMPC ratio

To determine the optimal ratio between the PNMPC solution and oil, emulsions were prepared with constant amounts of the emulsifier (40 g, 0.18%) and increasing amounts of oil (from 5 up to 50 g). The creaming index was measured by direct observation of emulsions using graduated cylinders. Creaming is one of the emulsion instabilities, caused by gravity (Tadros, 2004). The emulsions were separated into two phases: an optically opaque "cream" layer at the top (oil-rich) and a transparent "serum" layer at the bottom (Fig. 7A).

Changes during storage of emulsions formed with different oil amounts are shown in Fig. 7B. A larger value of the creaming index is an indication of a more stable emulsion.

Fig. 7B shows that at low oil concentrations (5 and 10 g), corresponding to an o:w ratio of 10:90 and 20:80, the creaming indexes were low at 24 h (30–50%), and these percentages remained stable over the time of study (30 days). However, creaming percentages increased as the amount of oil increases, remaining over 80% since o:w 30:70 onwards and showing a great stability. Based on these results, we have selected the oil-to-water ratios (30:70, 40:60 and 50:50) to study the emulsion stability.

From visual observation over 60 days, we concluded that all these emulsions resulted stable over long periods of time. The milky white appearance of emulsions indicates that the droplet size is smaller than 25 (m.

The microscopic observation shows that the studied o:w ratios form emulsions with individual droplets evenly distributed, except for the 50:50 o:w ratio that presented signs of coalescence throughout the test period. However rupture (visible droplets of oil dispersed in the emulsion) was not observed in emulsions. Besides, there was evidence of w/o/w multiple emulsions in freshly prepared 40:60 o:w sample.

Fig. 8 shows the particle size distribution for the different systems studied (o:w, 30:70, 40:60 and 50:50). All the systems show the maximum proportion of particles with diameter of 5 (m. However, the 30:70 sample has a unimodal, very narrow distribution, whilst the 40:60 and 50:50 have broader peaks around 5 (m and the distribution is multimodal, in agreement with optical microscopy evidence. The combination of small droplets and high viscosity caused by PNMPC augments the stability of emulsions to creaming.

In the temperature changes stability study, emulsions characteristics were not changed under repeated cycles of freezing to −18 °C followed by thawing at room temperature and heating at 100 °C for 10 min followed by cooling at room temperature.

The fact that chitosan-containing emulsion is more resistant to coalescence during freezing–thawing tests can probably be attributed to the formation of thicker interfacial layers surrounding the emulsion droplets (Chuah et al., 2009; McClements & Decker, 2005).

Emulsions with 0.18% PNMPC and 30:70 o:w ratio exhibited the best emulsifying properties within the test range. Increasing the oil content the distribution of sizes becomes multimodal, which indicates that the modified carbohydrate amount does not suffice to produce enough area of interfacial layer to stabilize small, quasi-monodisperse droplets.

4. Conclusions

The physicochemical studies on the PNMPC solutions indicate that at low concentration the solution is probably formed by free molecules, folded because of the hydrophobic part of the polymer, which maintain part of the phosphonic acid groups protected against the attack of hydroxide ions. Increasing the concentration these molecules agglomerate and the aggregates undergo changes at higher concentrations, probably in size and shape.

The neutralization of PNMPC acid groups probably leads to a stretching of the initially folded, thus exposing more phosphonic acid groups to neutralization. At some point, all groups are exposed and the union of hydroxyl groups with the polyelectrolyte becomes cooperative.

PNMPC shows good emulsifying properties, giving O/W emulsions with high stability in time, and to several freezing/thawing and heating/cooling cycles. The best stability was obtained with 30:70 oil-to-PNMPC aqueous solution (0.18% *w*/*v*) ratio, giving a quasi-monodisperse of small droplet. This kind of droplets, together with the high viscosity of the inter-droplets aqueous solution of PNMPC makes the system very stable.

The results of this work suggest that *N*-propyl-*N*-methylene phosphonic chitosan may be a useful emulsifier in a wide range of food, pharmaceutical and cosmetic products.

References

Chuah, A. M., Kuroiwa, T., Kobayashi, I., & Nakajima, M. (2009). Effect of chitosan on the stability and properties of modified lecithin stabilized oil-in-water monodisperse emulsion prepared by microchannel emulsification. *Food Hydrocolloids*, *23*, 600–610.

Dickinson, E. (2009). Hydrocolloids as emulsifiers and emulsion stabilizers. *Food Hydrocolloids*, *23*, 1473–1482.

Jourdain, L., Leser, M. E., Schmitt, C., Michel, M., & Dickinson, E. (2007). Stability of emulsions containing sodium caseinate and dextran sulfate: relationship to complexation in solution. *Food Hydrocolloids*, *22*, 647–659.

Krstonosic, V., Dokic, L., Dokic, P., & Dapcevic, T. (2009). Effects of xhantan gums on physicochemical properties and stability of corn oil-in-water emulsions stabilized by polyoxyethylene (20) sorbitan monooleate. *Food Hydrocolloids*, *23*, 2212–2218.

Li, X. K., & Xia, W. S. (2011). Effects of concentration, degree of deacetylation and molecular weight on emulsifying properties of chitosan. *International Journal of Biological Macromolecules*, *48*, 768–772.

McClements, D.J., & Decker, E.A. (2005). Biopolymer encapsulation and stabilization of lipid systems and methods for utilization thereof. US Patent 2005/0202149A1.

Muzzarelli, R. A. A. (2010). Chitins and chitosan as immunoadjuvants and non-allergenic drug carriers. *Marine Drugs*, *8*, 292–312.

Muzzarelli, R. A. A., Boudrant, J., Meyer, D., Manno, N., DeMarchis, M., & Paoletti, M. G. (2012). Current views on fungal chitin/chitosan, human chitinases, food preservation, glucans, pectins and inulin: A tribute to Henri Braconnot, precursor of the carbohydrate polymers science, on the chitin bicentennial. *Carbohydrate Polymers*, *87*, 995–1012.

Ogawa, K., Nakata, K., Yamamoto, A., Nitta, Y., & Yui, T. (1996). X-ray study of chitosan L- and D-ascorbates. *Chemistry of Materials*, *8*, 2349–2351.

Payet, L., & Terentjev, E. M. (2008). Emulsification and stabilization mechanisms of O/W emulsions in the presence of chitosan. *Langmuir*, *24*, 12247–12252.

Rodriguez, M. S., Albertengo, L., Etcheverry, M., & Schulz, P. C. (2005). Studies on N-methylene phosphonic chitosan. *Colloid and Polymer Science*, *283*, 1298–1304.

Schulz, P. C., Rodriguez, M. S., Del Blanco, L. F., Pistonesi, M., & Agullo, E. (1998). Emulsification properties of chitosan. *Colloid and Polymer Science*, *276*, 1159–1165.

Speiciene, V., Guilmineau, F., Kulozic, U., & Leskauskaite, D. (2007). The effect of chitosan on the properties of emulsions stabilized by whey proteins. *Food Chemistry*, *102*, 1048–1054.

Tadros, T. (2004). Application of rheology for assessment and prediction of the longterm physical stability of emulsions. *Advances in Colloid and Interface Science*, *108–109*, 227–258.

Wang, B., Wang, L. J., Li, D., Adhikari, B., & Shi, J. (2011). Effect of gum Arabic on stability of oil-in-water emulsion stabilized by flaxseed and soybean protein. *Carbohydrate Polymers*, *86*, 343–351.

Zuñiga, A., Debbaudt, A., Albertengo, L., & Rodriguez, M. S. (2010). Synthesis and characterization of N-propyl-N-methylene phosphonic chitosan derivative. *Carbohydrate Polymers*, *79*, 475–480.