Colloid Polym Sci (2005) 283 1298–1304
DOI 10.1007/s00396-005-1331-4

ORIGINAL CONTRIBUTION

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Studies on *N*-methylene phosphonic chitosan

Received: 8 November 2004
Accepted: 9 March 2005
Published online: 21 June 2005

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Abstract *N*-methylene phosphonic (NMPC) chitosan was studied by several techniques to determine their properties in aqueous solution and its capacity to emulsify edible oils. The phosphonic groups are all equivalent and their ionization constants were estimated ($pK_{a1} = 6.45$ and $pK_{a2} = 11.75$). The chitosan derivative in pure water starts to aggregate at 0.09% w/v, and the aggregates' structure at 0.5% w/v. Unlike pure chitosan, NMPC chitosan is strongly surface active. Its hydrophile-lipophile balance (HLB) value was estimated in 37, very similar to that of chitosan. As a consequence, it favors the formation of oil in water (o/w) emulsions with scarce water/oil/water (w/o/w) droplets.

Keywords Emulsion · *N*-methylene phosphonic chitosan · HLB · Condutivity · Surface tension

Introduction

Chitosan, 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], which is the main structural component of the cuticles of crustaceous insects and mollusks and the cell walls of microorganisms. Chitosan is a natural positively charged polysaccharide having a potential use in several areas, including applications in cosmetics [2, 3], biotechnology [4–6] and medicine [6–10]. 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, micelles [11] and liposomes [3]. Polysaccharides are promising biodegradable polymers and then are under intensive investigation for industrial applications [12].

The production of stable emulsions is very important in many industrial processes [13, 14]. Processed foods are often colloidal systems such as emulsions, suspensions and foams. Emulsions 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 [15–18]. In such cases an efficient steric stabilization is achieved by adsorpting and

controlling the conformation of the molecules at the interface between the disperse and continuous phases. Natural macromolecular substances, such as gums and proteins, have been used in practice for the stabilization of emulsions since the beginning of civilization, for example, in food and pharmaceutical emulsions [19–22].

The concept of polymeric surfactants is over 40 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 adds up to a large free energy of adsorption. As a result, their adsorption onto surfaces is firmer and therefore they can be used more efficiently in a low concentration. Polymeric surfactants certainly provide an interesting mean 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 [23, 24]. In contrast with small surfactant molecules, very little is known on the effects of polymeric surfactant molecules on emulsions.

Polymeric surfactants offer the advantage of important steric stabilization of dispersions while most of the low molecular surface-active agents operates only through electrostatic stabilization. The combination of both mechanisms is often referred to as electrosteric stabilization [22]. In addition, it is well known that the viscosifying effect of nonadsorbing polymers (synthetic polymers or naturally occuring macromolecules like gums) may influence emulsion stability by decreasing the rate of creaming [25].

In 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 have studied the emulsification properties of chitosan in previous papers [26–28]. Calvo et al. [7] also 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 [29] and biocompatibility [30].

Since chitosan is a cationic polyelectrolyte, we had the idea to explore the use of chitosan derivatives with anionic groups. In previous articles [31, 32], we had described the synthesis and characterization of *N*-methylene phosphonic chitosan (NMPC), a novel soluble derivative of chitosan, which is water-soluble and has negative groups. The goal of this work is to determine solution properties and the emulsifying capacity of NMPC for its possible use in food emulsions. Results may also be used in the preparation of emulsions with applications in pharmacology and cosmetics.

Experimental

Synthesis The synthesis and characterization of NMPC was described elsewhere [32]. Chitosan was prepared from chitin obtained from shrimp shells. The obtained chitosan had a deacetylation degree (DD) of 90%. Two NMPC samples were obtained, one of them by a 7-h reaction with phosphoric acid (NMPC-7) and the other by a 20-h treatment (NMPC-20). Both NMPCs were characterized by atomic absorption, X-ray diffraction, ^{1}H-NMR and FT-IR spectrometries, differential scanning calorimetry and viscosity [32]. The characteristics of both NMPC samples are summarized in Table 1. Substitution degree is defined as the proportion of nitrogen atoms having methylphosphonic groups. Some nitrogen atoms have only one methylphosphonic group whereas others have two. The average molecular weight (M) is also shown in Table 1. Standard solutions of both chitosan derivatives were prepared with a concentration of 1.00% w/v in distilled water. From these solutions, the following dilutions were made: 0.80–0.60–0.50–0.40–0.20–0.10–0.08–0.06–0.05–0.04–0.02 and 0.01% w/v in distilled water.

Conductivity measurements were made on the dilutions with an Altronix CT 1 conductimeter. A volume solution (15.0 ml) of both NMPC samples (one having 0.50% w/v and other 1.00% w/v) was titrated with 0.1084 M NaOH solution and the conductivity measurements were made with the same device. The dilution effect was corrected with the equation:

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

These titrations were also followed by pH determination with pH/ISE meter, Orion 710 A a Broade and James glass electrode (reproducibility ± 0.01 pH units). Surface tension measurements were made on the dilutions with a Du Noüy tensiometer Krüss, which was calibrated with water, hexane and cyclohexane. The hydrophile–lipophile balance (HLB) of both chitosan derivatives was determined by a procedure described elsewhere [26], by the diameter of a toluene drop on the surfactant solution.

Table 1 Properties of *N*-methylene phosphonic (NMPC) samples [32]

Subatance	Substitution degree			*M* (Da)
	N-mono	*N,N* di	Total	
NMPC-7	0.23	0.14	0.37	140,000
NMPC-20	0.10	0.38	0.48	60,000

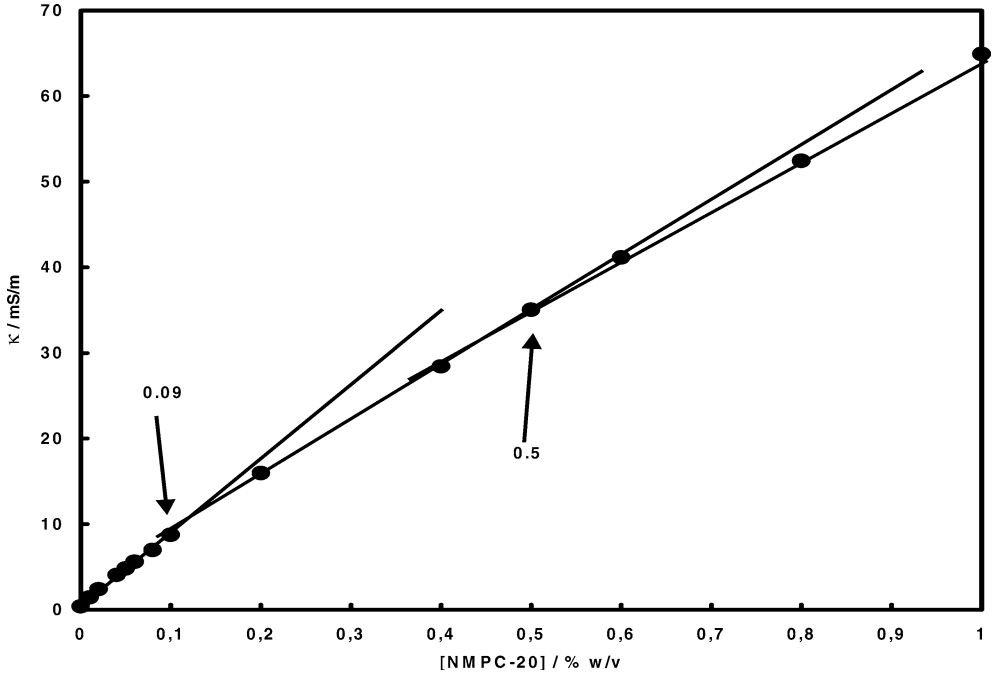

Fig. 1 Specific conductivity of NMPC-20 as a function of concentration. *Arrows* indicate the breaks

To determine the optimal NMPC/oil ratio, emulsions were produced by stirring for 3 min, in a food processor at two different speeds (7,560 and 13,600 rpm). The following in weight proportions of NMPC solution (1% w/w) and oil (sunflower oil): 30–70; 50–50; 80–20; 90–10 were used. All emulsion samples were left to rest for 3 days and phase separation was visually observed. The studies were made with the emulsion that was stable for 72 h, obtained by adding 10 g of sunflower oil to 90 g of NMPC solution (1% w/w), stirring at 13,600 rpm. Microscopic observations were made in an Olympus BH-2-UMA optical microscope equipped with a Sony CCD IRIS/RGB photographic camera. To determine the nature of emulsion, the relative refractive index of different droplets with respect to the surrounding phase was determined by changing the focus depth in the microscopic observation. The stability on time was studied by visual observation of creaming in samples left by 7 days. All measurements were made at 25°C. The stability on freezing was determined by six consecutive cycles on freezing at − 8°C and subsequent thawing at room temperature.

Results and discussion

Solution properties

The specific conductivity as a function of one chitosan derivative concentration is shown in Fig. 1. Both polymeric surfactants show similar behavior. Figure 2 shows the $\Delta\kappa = \kappa_{measured} - \kappa_{extrapolated}$ dependence on concentration for NMPC-20, 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 is a break at 0.09% w/v, which is completely similar to that appearing in common ionic surfactants when micelles are formed. The same break can be seen in the $\Delta\kappa$ versus concentration curve for NMPC-7 (not shown). It may be concluded that at 0.09% w/v the polymeric surfactant molecules aggregate in a micelle-like structure, thus reducing the charge carriers' concentration in solution, in comparison with the added molecules.

The specific conductivity may be represented by the equation [33]:

$$\kappa = \kappa^{\circ} + \Lambda^{\circ} c + c\Phi(c), \tag{2}$$

where κ and κ° are specific conductivities of the solution and the solvent, respectively, and $\Phi(c)$ accounts for the effects of inter-ionic interactions on the conductivity. Thus, at high dilution, a plot of κ versus c should be linear with slope Λ° [34]. According to Vink [33, 35] 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 NMPC-20 at constant concentration of acetic acid (1% v/v) follows a linear relationship:

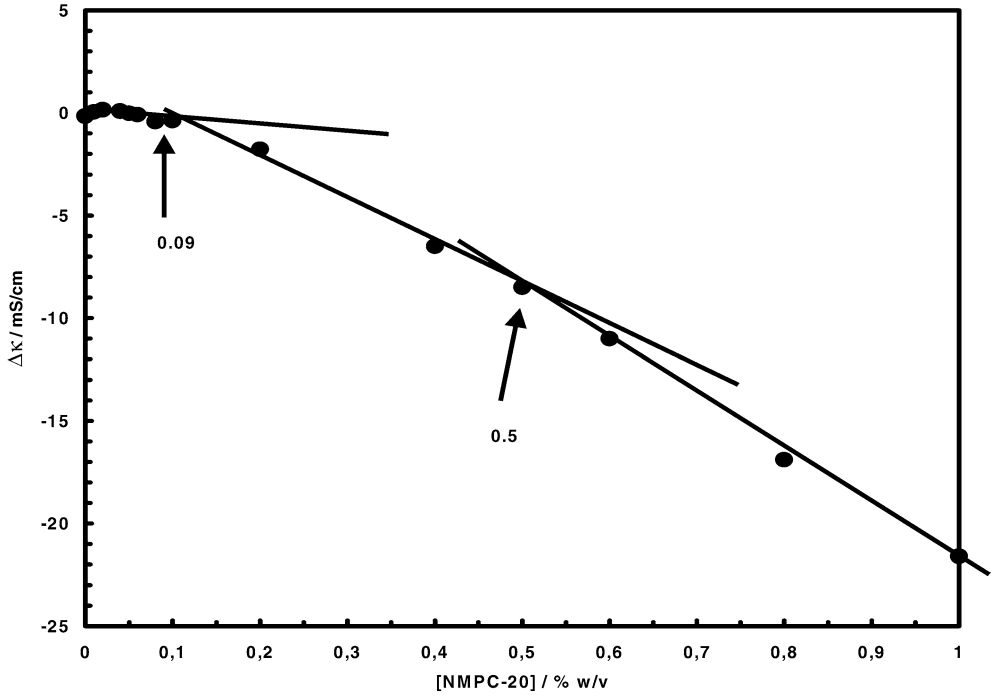

Fig. 2 $\Delta\kappa = \kappa_{measured} - \kappa_{extrapolated}$ dependence on concentration for NMPC-20. *Arrows* indicate the breaks

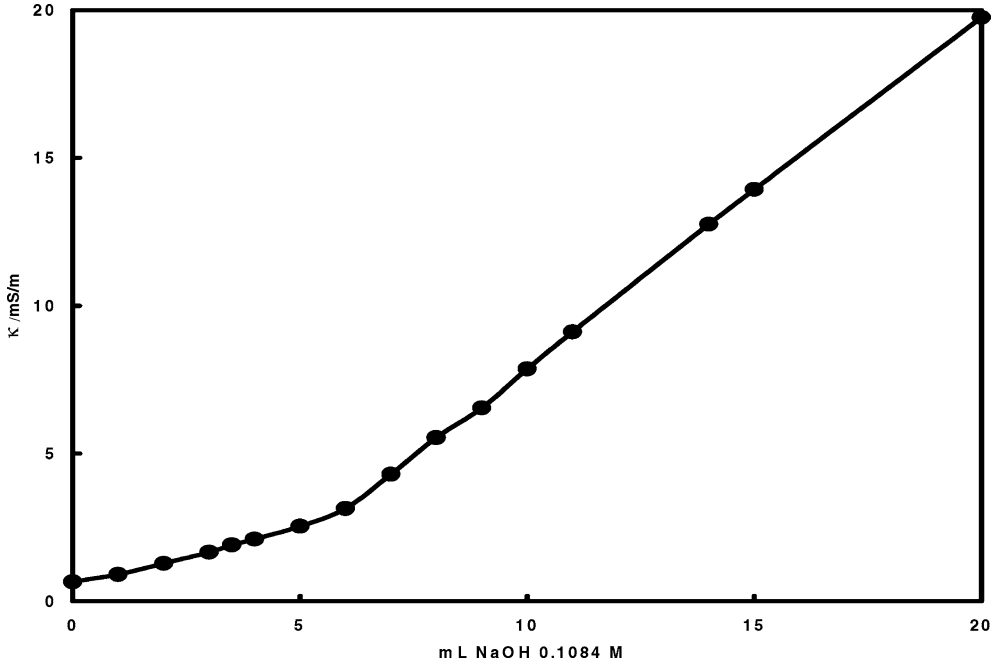

Fig. 3 Conductimetric titration of 15 ml of 1.00 % w/v aqueous NMPC-20 as a function of the volume of added 0.1084 M NaOH solution

$$\kappa(\mu\mathrm{S})/\mathrm{cm}) = 61.9 \pm 7.8 + (8,170 \pm 140)c(\%w/v),$$

$$r = 0.9986.$$

Results of the conductimetric titration of the 20-h reaction derivative (1% initial NMPC concentration) are shown in Fig. 3. The titration of NMPC-7 in both initial concentrations (1.00 and 0.500% w/v) gave an equivalent weight of 238 g. The equivalent weight of NMPC-20 was 222 ± 6 g/equivalent. These results confirm that 13 additional hours of reaction did not significantly increase the degree of substitution.

With the equivalent mass the equivalent conductivity of NMPC-20 was calculated with:

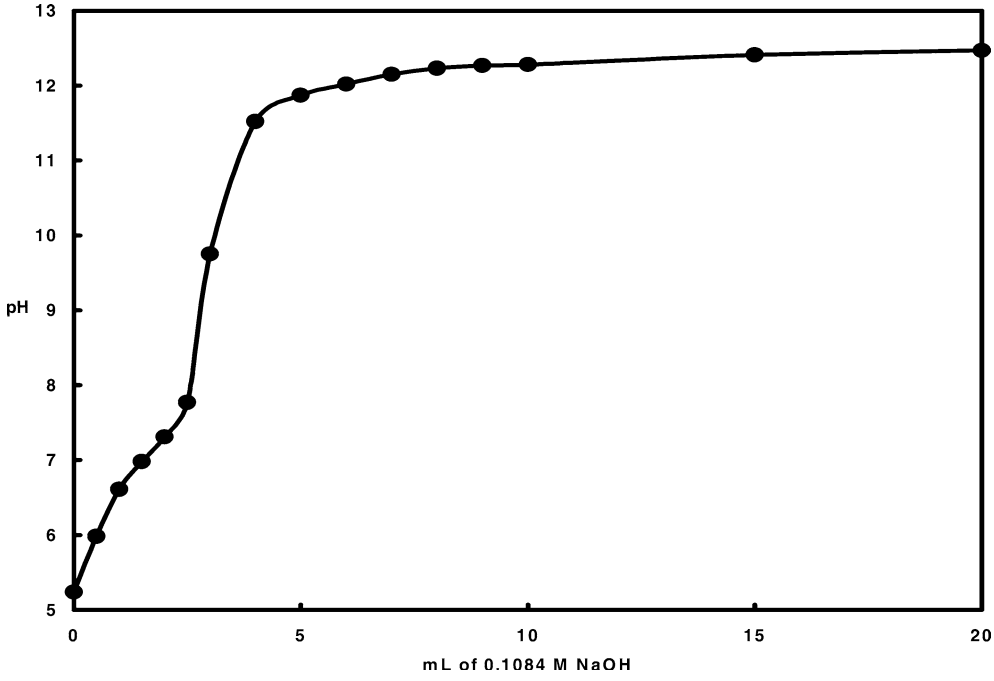

Fig. 4 pH dependence on the volume of added 0.1084 M NaOH solution to 15.0 ml of 1.00 % w/v aqueous solution of NMPC-20

$$\kappa(\mathrm{Scm}^{-1}) = (0.619 \pm 0.078) \times 10^{-4} + (0.1814 \pm 0.0031)c(\mathrm{Equiv\,dm}^{-3})$$

in which the slope is $\Lambda°_P$, that corresponds to equivalent conductivity of the polyelectrolyte. The intercept (6.19×10^{-5} S cm^{-1}) is lower than the solvent conductivity (7.3×10^{-4} S cm^{-1}) and reflects the effect of the polyelectrolyte on $\kappa°$. Similar results have been described for other polyions [33–35]. The NMPC-20 has a very low value of $\Lambda°_P = (0.1814 \pm 0.0031)$ S cm^2 Equiv^{-1} 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}) [34], and that of chitosan in acetic acid ($(2.438 \pm 0.048)\times10^{-2}$ S cm^2 Equiv^{-1}) [26]. This may be a consequence of the low ionization constant of the phosphonic acid groups and the partial internal neutralization with remaining amino groups. Thus the effective charge of the kinetic unit having n phosphonic groups is much less than n. The conductance of the polyelectrolyte solution can be expressed as [34]:

$$\Lambda^{\circ}_{P} = f\left(\lambda^{\circ}_{P} + \lambda^{\circ}_{P}\right), \tag{3}$$

where f is an interaction parameter [35], and $\lambda°_P$ and $\lambda°_{H+}$ refer, respectively, to the limiting equivalent conductance of the counter ion and the polyion. According to Manning's limiting law for the conductance of polyelectrolytes [36], the parameter f is:

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

where ξ is the linear charge density. ξ^{-1} may be interpreted as α, the degree of ionization [35]. In view of the low value of $\Lambda°_P$, it follows that ξ^{-1} must be very small. With the degree of substitution from literature (0.34) [32] and the first ionization constant ($pK_{a1} = 6.45$, see below), the degree of ionization in a 1.0% w/v solution of NMPC-20 may be estimated as $\alpha = \xi^{-1} = (K_{a1}/10\times0.34)^{1/2} = 0.00027$. By application of Eq. 3 and the supposition that $\lambda°_P << \lambda°_{H+}$, $\xi^{-1} = 0.0006$. These values are consequence of the low Ks_{a1} value.

Figure 4 shows one of the pH versus added NaOH volume titration curves. Using the first equivalent point (V_{EP}) determined by conductimetric titration for each sample, and the pH value at 0.5 V_{EP} and 1.5 V_{EP}, the equilibrium constants of the first (K_{a1}) and second (K_{a2}) ionization of the phosphonic acid group (–CH_2-PO_3H_2) were determined. The obtained values were $pK_{a1} = 6.45$ and $pK_{a2} = 11.75$, pK_a values here found are not thermodynamic ones, because the effect of the ionic strength was not computed. These values indicate that the phosphonate groups in NMPC are weaker than in alkane phosphonic acids. As an example, for n-decane phosphonic acid, $pK_{a1} = 3.976 \pm 0.001$ and $pK_{a2} = 7.985 \pm 0.003$ [37]. These values are thermodynamic ones. Since the difference between the activity and the concentration cannot explain the difference between the pK_a values for both substances, the cause may be steric interference between the ionizable groups and the

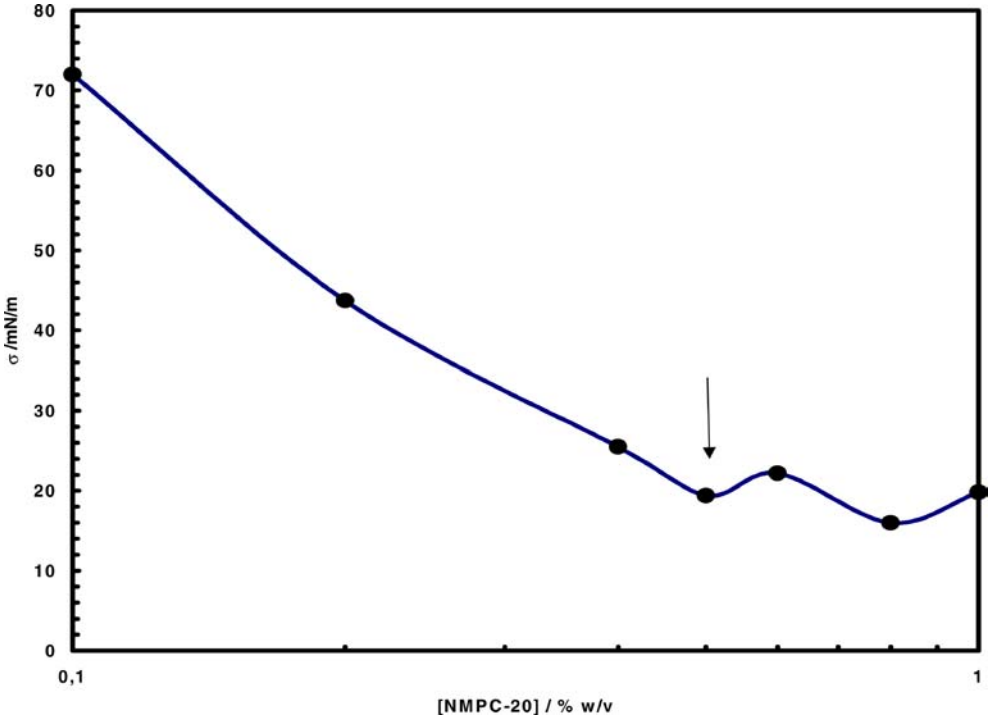

Fig. 5 Dependence of the surface tension on the NMPC-20 concentration in water. *Arrow* indicates the stabilization of the surface tension

chitosan structure, as it was suggested by Hammet [38] and Tafft [39–41] for other organic acids.

The pH versus added V_{NaOH} curves are coherent with the pK_a values found here, because the second equivalent point is undetectable. Moreover, the curves are similar to those of the titration of a single acid, indicating that all the phosphonic groups are equivalent.

Chitosan ammonium group has a $pK_a \asymp 6.3–7$ [42]. Assuming that some amino groups remained unchanged in the chitosan derivative, this substance is an amphoteric one having an isoelectric point $I.P. = (pK_{a\text{-}NH3} + pK_{a1,\text{-}PO3H2})/2 = 6.6$.

The surface tension vs. NMPC-20 concentration is shown in Fig. 5. There is a stabilization in surface tension-*n* at 0.5%, with very low surface tension (˜19 mN/m)(indicated by an arrow). The surface activity $G = -\partial\sigma/\partial C ex_{C=0} = 106$ mN/m%. This behavior is the opposite to that found in chitosan solutions, where the surface tension was 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 [26]. The stabilization of the surface tension indicates that some kind of structural change occurs in the bulk of the solution, such as aggregation. However, conductivity measurements indicate that aggregation starts at 0.09%. Then, the point at 0.5% must indicate a structural change in the aggregates, which compete with the adsorbed state at the air/solution interface. The dependence of κ versus [NMPC] suggests that even above 0.09%, the increase in aggregates' concentration is accompanied by an increase in single dispersed NMPC molecules, because the decrease in specific conductivity is not high. A glance on Fig. 2 shows a change in slope at 0.5%. In common surfactant systems, such change in slope in conductivity curves is associated to changes in aggregates' structure (e.g., the spherical-to-wormlike micelle transformation).

Emulsifying properties

The HLB value of both chitosan derivatives was 37. For chitosan in 1% acetic acid aqueous solution the HLB value is 36.7 [26].

The 1.0% aqueous NMPC/sunflower oil v/v ratios of 30/70 and 50/50 underwent creaming giving a separation in concentrated and diluted emulsion in 15 and 30 min, respectively. The 80/20 ratio was stable until 24 h, and that of 90/10 was stable even at 72 h. No difference was noticed between NMPC-7 and NMPC-20, or between the two stirring rates (7,650 and 13,600 rpm). By determination of the relative refractive indexes, we determined that the continuous phase was water, which was also determined by conductimetry and that the small droplets inside the oil drops were also water. The formation of a mainly oil in water (o/w) emulsion combined with some water/oil/water (w/o/w) droplets, is coherent with the high HLB value estimated in this work. Emulsions were milky and polydisperse, with a

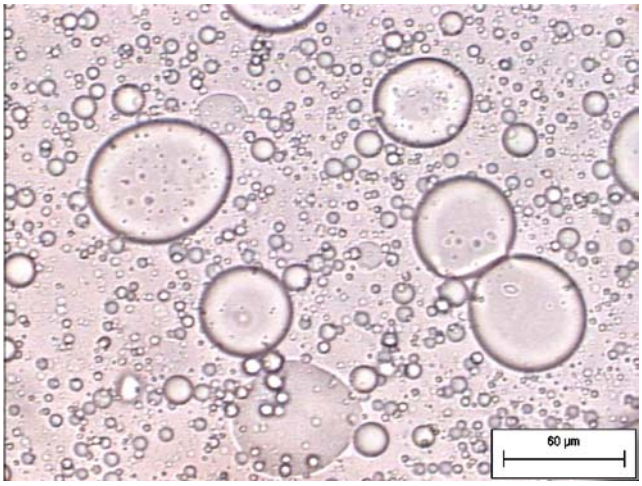

Fig. 6 Microphotography of 72-hour-old emulsion of NMPC-20 showing small droplets of oil dispersed in water and some larger droplets of oil with small water droplets inside

mean droplet diameter of 50 µm. The most stable emulsion in time was that with 1.0% aqueous NMPC/sunflower oil v/v ratio of 90/10. Figure 6 shows a microscopic view of the emulsion obtained with NMPC-20 derivative.

However emulsion was broken after the second cycle of freezing—melting, giving free oil in equilibrium with o/w emulsion, this observation allowed us to conclude that this type of emulsion is not useful in products that need low temperatures for preservation.

Concluding remarks

NMPC phosphonic groups have smaller ionization constants than n-alkane phosphonic acids. All the phosphonic groups are equivalent, and the equivalent weight is about 222 g.NMPC aggregates at about 0.09% w/v, and the aggregates change in structure at 0.5%.The capacity to reduce the surface tension is high ($G = 106$ mN/m%) until the second critical point is reached.NMPC HLB = 37 and the 1.0% aqueous NMPC/sunflower oil v/v ratio of 90/10 forms a stable o/w emulsion with some w/o/w droplets. This emulsion is broken after two freeze-melting cycles.

References

1. Sandfjord P (1989) In: Skjåk-Break G, Anthonsen T, Sandfjord P (eds) Chitin and Chitosan. Elsevier, London, p 543
2. Lang G, Clausen T (1989) In: Skjåk-Break G, Anthonsen T, Sandfjord P (eds) Chitin and Chitosan. Elsevier, London, p 139
3. Onsøyen R (1991) In: Proceedings of the 5th international conference on Chitin and Chitosan, Princeton, pp 17–20
4. Agerkvist I, Eriksson L, Enfors S-O (1990) Enzyme Microb Technol 12:584
5. Agerkvist I (1992) Colloids Surf 69:173
6. Ravi Kumar MN (2000) Reactive Funct Polym 46:1
7. Calvo P, Remuñán-López C, Vila-Jato JL, Alonso MJ (1997) Colloid Polym Sci 275:46
8. Ohya Y, Takei T, Ouchi T (1992) J Bioact Compat Polym 7:243
9. Ohya Y, Takei T, Kobayashi H, Ouchi T (1993) J Microencapsulation 10:1
10. Rao K, Alamelu PS (1992) J Membr Sci 161:71
11. Israelachvili JN, Adams GE (1978) J Chem Soc Faraday Trans 1(74):975
12. Ogawa K, Nakata K, Yamamoto A, Nitta Y, Yui T (1996) Chem Mater 8:2349
13. Becher P (1985) Encyclopedia of Emulsion Technology. Dekker, New York
14. Bravo DC, González RE (1996) J Chem Ed 73:844
15. Sela Y, Magdassi S, Garti N (1994) Colloids Surf A 83:143
16. Cárdenas-Valera AE, Bailey AL, Doroszokowski A (1995) Colloids Surf A 96:53
17. Cárdenas-Valera AE, Bailey AL (1995) Colloids Surf A 97:1
18. Faers MA, Luckham PF (1994) Colloids Surf A 86:317
19. Friberg SR (1976) Food Emulsions. Dekker, New York
20. Schwartz TW (1962) Am Perfum Cosmet 77:85
21. Lankveld JMG, Lyklema J (1972) J Colloid Interface Sci 41:475
22. March GC, Nappler DH (1977) Colloid Interface Sci 61:383
23. Daoud M (1994) Colloids Surf A 86:41
24. Halperin A, Pincus PA (1986) Macromolecules 19:79
25. Satz JL, Ip BK (1986) J Soc Cosmet Chem 37:329
26. Schulz PC, Rodríguez MS, Del Blanco LF, Pistonesi M, Agulló E (1998) Colloid Polym Sci 276:1159
27. Del Blanco LF, Rodríguez MS, Schulz PC, Agulló E (1999) Colloid Polym Sci 277:1087
28. Rodríguez MS, Albertengo LA, Agulló E (2002) Carbohydrate Polym 48:271
29. Lehr CM, Boustra JA, Schacht EH, Junginger HE (1992) Int J Pharm 78:43
30. Hirano S, Seino H, Akiyama Y, Nonaka I (1988) Polym Eng Sci 59:897
31. Heras A, Rodríguez NM, Ramos VM, Agulló E (2001) Carbohydrate Polym 44:1
32. Ramos VM, Rodríguez NM, Díaz MF, Rodríguez MS, Heras A, Agulló E (2003) Carbohydrate Polym 52:39
33. Vink H (1981) J Chem Soc Faraday Trans 1(77):2439
34. Rios HE, Sepúlveda LN, Gamboa CI (1990) J Polym Sci B 28:505
35. Vink H (1982) Macromol Chem 183:2273
36. Manning GS (1972) J Phys Chem 79:262
37. Schulz PC, Lelong ALM (1976) Rev Latinoamer Quím (México) 7:9
38. Hammet (1940) Phys Organic Chem. McGraw Hill, New York, p 184
39. Tafft RW (1952) J Am Chem Soc 74:2729
40. Tafft RW (1952) J Am Chem Soc 74:3130
41. Tafft RW (1953) J Am Chem Soc 57:4231
42. Rinaudo M, Domard A (1989) In: Skjåk-Break G, Anthonsen T, Sandfjord P (eds) Chitin and Chitosan. Elsevier, London, p 71