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On the stiffness of chitosan hydrochloride in acid-free aqueous solutions

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Abstract

Chitosan hydrochlorides ($F_A = 0.226$, i.e. DA = 22.6%) were randomly degraded by ultrasonication and characterized by viscosity measurements in aqueous acid-free solutions. It is shown that acid-free aqueous solutions of chitosan hydrochloride of variable ionic strengths ($0.06\ M \leq \mu \leq 0.3\ M$) are free of aggregation as evaluated by the values of the Huggins constants ($0.31 \leq k \leq 0.63$). These solutions were employed to study the solution properties of chitosan hydrochloride at different ionic strengths, which allowed the determination of its salt tolerance as well as its characteristic stiffness parameter. Following Smidsrod's approach chitosan hydrochloride gave a stiffness parameter $B = 0.06$, in agreement with the value reported by Rinaudo et al. It is suggested that the higher values of B reported for chitosan in the literature may be attributed to aggregation.

Keywords: Chitosan hydrochloride; Acid-free aqueous solutions; Stiffness parameter; Huggins constant

1. Introduction

Chitosan is a cationic polyelectrolyte when dissolved in dilute acid solutions. It is usually prepared by the deacetylation of chitin, an abundant naturally occurring polysaccharide (Muzarelli, 1978). Ideally, the primary structures of chitin and chitosan correspond to those of poly[β(1 → 4)-2-acetamido-2-deoxy-D-glucopyranose] and poly[β(1 → 4)-2-amino-2-deoxy-D-glucopyranose], respectively.

Chitin and chitosan usually exist in nature as components of complex composites. In these composites, such as the cell walls of fungi and the exoskeleta of sea animals and insects, chitin and chitosan are combined with other substances such as proteins, polysaccharides, lipids, pigments and inorganic material (Roberts, 1992a).

Chitin usually contains some 2-amino-2-deoxy-D-glucopyranose units. It is not known if these are present in the native chitin or if they occur as a consequence of the hydrolysis of acetamido groups, since isolation from these composites generally requires strong alkaline media, the exact conditions depending on the source and purity of the final product (Roberts, 1992a).

Chitosan generally also contains 2-acetamido-2-deoxy-D-glucopyranose in addition to 2-amino-2-deoxy-D-glucopyranose units since fully deacetylated chitosan is rarely prepared due to the occurrence of severe depolymerization (Domard & Rinaudo, 1983). Nevertheless, the use of mild reaction conditions and the presence of oxygen scavengers in the reaction medium may prevent chain depolymerization. Recently, a new method for the deacetylation of chitosan using aqueous sodium hydroxide (5–10%) was patented, which allows the preparation of extensively deacetylated chitosan with little polymer degradation (Rinaudo, Le Dung & Milas, 1993a).

Although another nomenclature system has been proposed (Roberts, 1992b; Roberts, 1997), chitin and chitosan are terms of long standing use and the most practical way to distinguish between them is based on the proportion of 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose units present in their chains, expressed as the average degree of acetylation (DA) or as the mole fraction of acetylated glycosamine units (F_A), and also on their solubilities.

Chitin has mostly acetylated units and the literature reports that it can be dissolved in a limited number of solvents such as aqueous solutions of neutral salts, concentrated solutions of strong acids and in some organic solvents (Roberts, 1992c). The best solvents for highly acetylated α-chitin are DMAc/LiCl and *N*-methylpyrrolidone/LiCl since they are non-degrading solvents and also they allow the dissolution of a substantial amount of chitin. Other solvents are not so good since they cause degradation and

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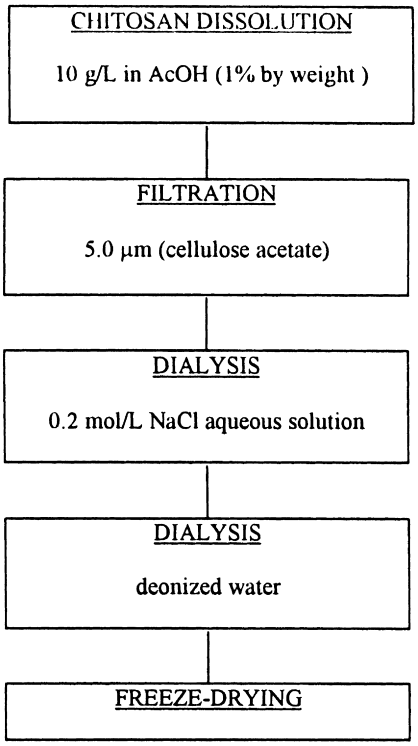

Fig. 1. Schematic experimental procedure used to prepare chitosan hydrochloride.

other chemical modifications at the same time as dissolving the chitin.

When the deacetylated units predominate the term, chitosan is used to identify the polymer. Due to the protonation of the nitrogen atoms of its amino groups, chitosan is soluble in dilute acid solutions, acetic acid and hydrochloric acid being the most common acids used for its dissolution (Roberts, 1992d). In these solvents chitosan has the typical behaviour of polyelectrolytes as a consequence of the presence of positive charges along its chains. However, as it is usually dissolved in the presence of excess acid, the occurrence of aggregation of the polymeric chains may complicate the study of its solution properties (Rinaudo, Milas & Le Dung, 1993b). Moreover, according to the strength of the acid, the precipitation of chitosan can occur even in relatively dilute acid solutions (Rinaudo, Pavlov & Desbrieres, 1999a,b). It is also difficult to compare chitosan with anionic polyelectrolytes since these are usually studied in acid-free aqueous solutions while non-stoichiometric amounts of acid equivalents are generally used to dissolve chitosan.

The chain flexibility and the conformation of polyelectrolytes in solution determine most of its hydrodynamic properties (Milas, Rinaudo, Knipper & Schuppiser, 1990). The salt concentration can drastically influence the intrinsic viscosity, particularly at low salt levels. This dependence provides information on the intrinsic chain flexibility.

An empirical parameter, *B*, has been proposed as a practical measure of the relative rigidity of polyelectrolytes in solutions (Smidsrod & Haug, 1971). This parameter has been widely used for its simplicity. It gives good qualitative agreement with estimates of flexibility derived from more complex approaches, does not require a knowledge of molecular weight and can be used over a broad range of charge densities.

Most of the studies aiming to evaluate the stiffness of chitosan by employing the Smidsrod approach were done with aqueous solutions containing excess of low-molecular weight acid (Wang, Bo, Li & Qin, 1991; Anthonsen, Varum & Smidsrod, 1993; Rinaudo et al., 1993b; Tsaih & Chen, 1997) since its presence was necessary to assure the complete dissolution of the chitosan samples.

The study of chitosan in acid-free aqueous solutions has not been reported so far, but in a recent work it was shown that solutions of chitosan hydrochlorides in aqueous NaCl (0.1 and 0.2 mol/l) are appropriate to such studies since they were free of aggregation as estimated by the values of the Huggins constants, k', in these solvents (Signini & Campana, 1999).

This work deals with the use of acid-free aqueous solutions of purified chitosan hydrochloride to determine its salt tolerance and the stiffness of the polyelectrolyte chain by employing the Smidsrod approach.

2. Experimental

2.1. Purification of chitosan hydrochloride

The sample of chitosan employed in this study was a commercial product from Fluka-Biochimika/Switzerland and its purification as water soluble hydrochloride was done by following the experimental procedure depicted in Fig. 1 which has been fully presented elsewhere (Signini & Campana, 1999). Following this procedure white flakes of water soluble chitosan hydrochloride were obtained.

To obtain lower molecular weight samples of chitosan, the commercial product (1 g) was dissolved in 100 ml of dilute acetic acid (1% by weight), the resulting solution was filtered to exclude aggregates and insoluble matter and it was then submitted to ultrasonic degradation. A Branson Sonifier 450 was used in these experiments and the chitosan solution was sonicated for a variable time at room temperature employing in all cases a pulsed power of 105 W. Following this treatment, the solution was centrifuged for 20 min at 10,000*g* and the purified chitosan hydrochloride was obtained as depicted in Fig. 1.

2.2. Characterization of chitosan hydrochloride

The average degrees of acetylation were determined by ^{1}H nmr spectroscopy of the solutions of purified chitosans in D_2O/HCl (100:1 v/v) at 80°C by using a 200 MHz spectrometer from Bruker according to a procedure previously described (Desbrieres, Martinez & Rinaudo, 1996). The

Table 1
Average degrees of acetylation (DA) of chitosan hydrochlorides determined by ^{1}H NMR spectroscopy in D_2O/HCl (100:1 v/v), intrinsic viscosities ($[\eta]_{0.1}$) and Huggins constants (k') determined in aqueous 0.1 mol/l NaCl

Sample[a]	%DA	$[\eta]_{0.1}$ (dl/g)	k'
A	22.7	10.03	0.47
4A	22.2	8.73	0.46
7A	23.3	8.20	0.31
10A	22.3	7.50	0.43

[a] The numbers 4, 7 and 10 which precede the letter A, used to identify the original purified sample, represent the time (in min) of ultrasonication before the purification of the samples as hydrochlorides.

calculations were done by using the expressions presented below and the degrees of acetylation were expressed as the values averaged from the use of both expressions.

$$\%DA = [(I_{Met}/3)/(I_{H2})] \times 100 \quad (1)$$

$$\%DA = [(I_{Met}/3)/(I_{H2/6}/6)] \times 100 \quad (2)$$

where I_{Met} is the integral intensity of the signal from the methyl protons of acetamide groups; I_{H2} the integral intensity of the signal from the H atom bonded to the carbon 2 of the glycosidic ring; $I_{H2/6}$ the sum of the integral intensities of the signals from the H atoms bonded to carbons 2, 3, 4, 5 and 6 of the glycosidic unit.

The intrinsic viscosities were determined by capillary viscometry at $25 \pm 0.01°C$ of aqueous solutions previously filtered through 0.22 μm membranes. The purified chitosan hydrochlorides were studied in aqueous solutions of NaCl of variable concentration ($0.06 \text{ mol/l} \leq [\text{NaCl}] \leq 0.3 \text{ mol/l}$) and the polymer concentrations were low enough to provide relative viscosities in the range $2.0 > \eta_{rel.} > 1.2$, from the initial solution toward the final diluted solution.

The AVS-350 viscometer coupled to the AVS-20 automatic burette, both from Schott-Geräte, was used in these determinations. A glass capillary ($\phi = 0.53$ mm) was employed and the solutions of the purified chitosans were sequentially diluted directly by the addition of previously programmed volumes of the appropriate solvent.

3. Results and discussions

The procedure used to prepare chitosan hydrochloride resulted in a pure form of the commercial product which was completely soluble in pure water and in dilute acid as evaluated by the absence of insoluble matter and through viscosity measurements before and after filtration by membranes of decreasing porosity (down to 0.22 μm) (Signini, 1998). The purified sample was also soluble in aqueous solutions of NaCl resulting in aggregate-free solutions as evaluated by the values of the Huggins constants (Signini & Campana, 1999).

By comparing purified chitosans, as hydrochloride and in the uncharged form, it was also observed that their average degrees of acetylation are very similar, independently of determining them by conductometric titrations or by ^{1}H NMR spectroscopy (Signini & Campana, 1999).

The average DA of the chitosan hydrochloride obtained from the commercial product and of those samples prepared by ultra-sound depolymerization are also very similar (Table 1), in agreement with the assumption that the ultra-sound treatment provokes no changes in the degree of acetylation. Similar results were observed on the sonication of chitin and chitosan in aqueous acid media (Takahashi, 1997). Thus, an average value of DA = 22.6% (F_A = 0.226) has been taken as representative for the original sample and for those degraded by the ultra-sound treatment.

From the data in Table 1 it may also be concluded that the ultra-sound treatment resulted in chain depolymerization as evaluated by the decrease in the intrinsic viscosities of the aqueous NaCl solutions of purified chitosan hydrochlorides as a function of the duration of the treatment.

A linear decrease in intrinsic viscosity of the chitosan

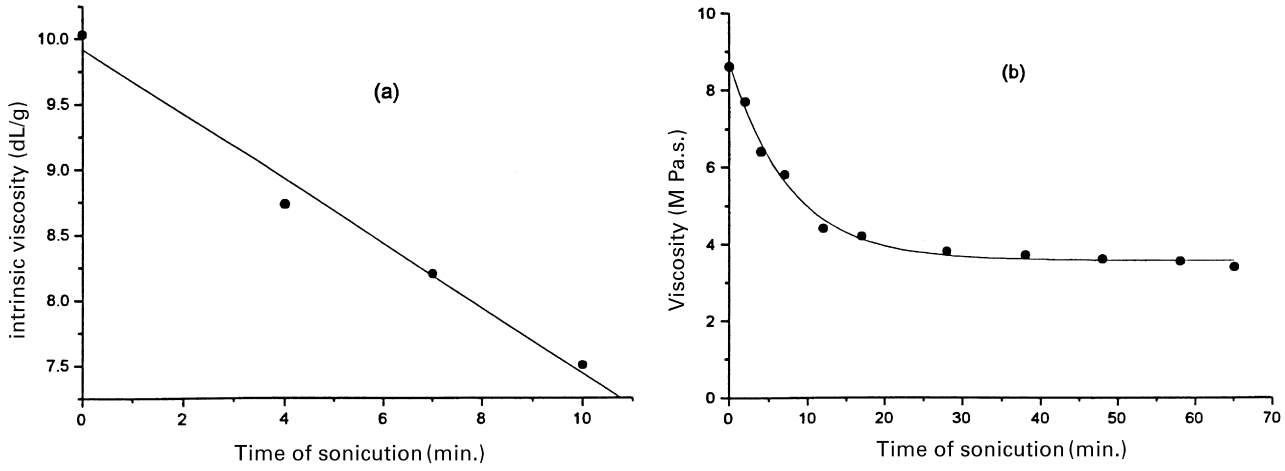

Fig. 2. Decrease of intrinsic viscosity of chitosan hydrochloride in aqueous NaCl 0.1 M as a function of the time of sonolysis (a) and dependence of viscosity of solutions of chitosan in acetic acid, measured in a Brookfield rheometer LVDV-II with UL adapter at 60 rpm, on the time of sonication (b).

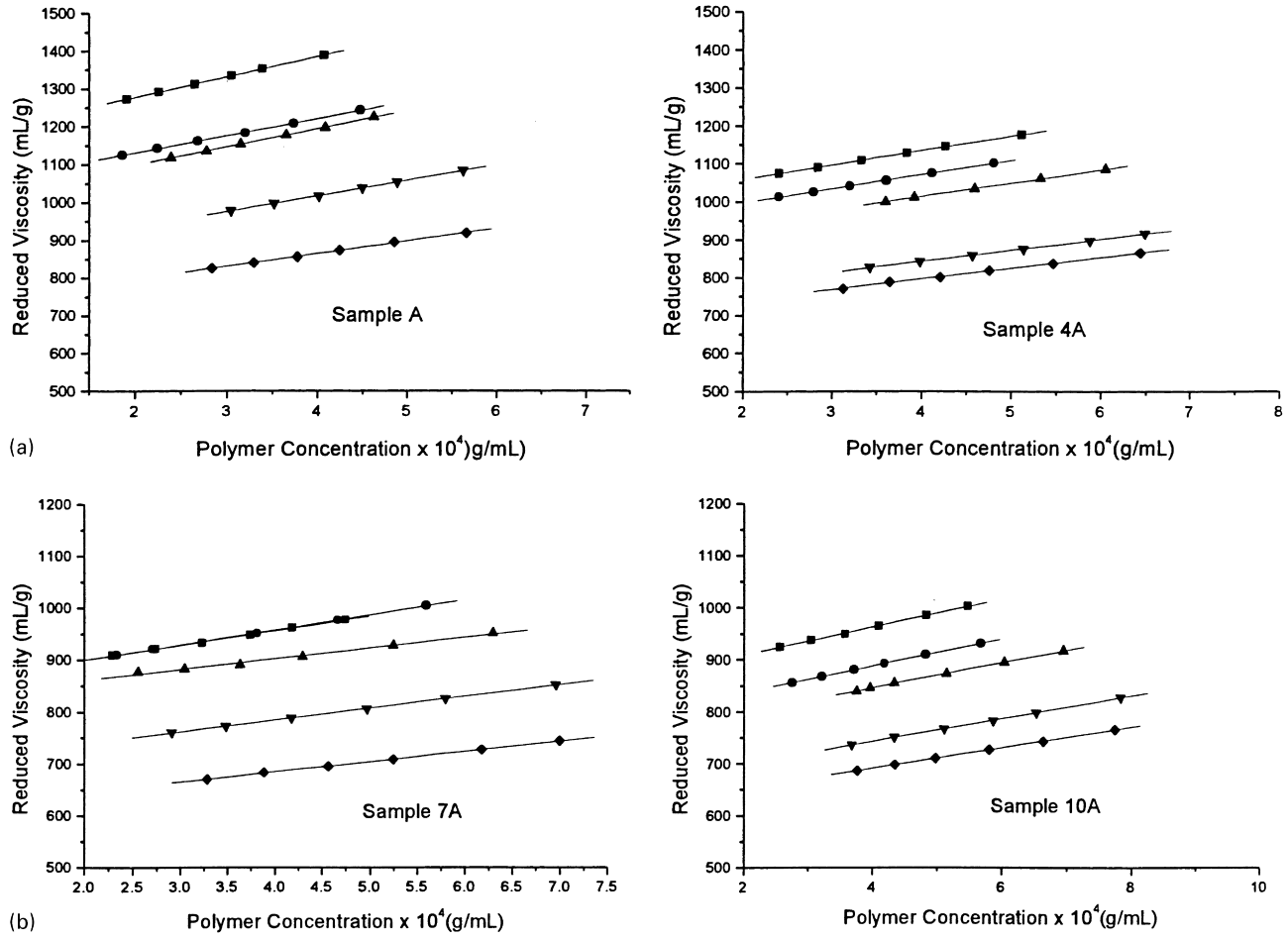

Fig. 3. (a) Curves of reduced viscosity versus chitosan concentration for samples A and 4A at different concentrations of NaCl: (■) 0.06 M; (●) 0.08 M; (▲) 0.1 M; (▼) 0.2 M and (◆) 0.3 M. (b) Curves of reduced viscosity versus chitosan concentration for samples 7A and 10A at different concentrations of NaCl: (■) 0.06 M; (●) 0.08 M; (▲) 0.1 M; (▼) 0.2 M and (◆) 0.3 M.

hydrochlorides determined in aqueous 0.1 mol/l NaCl was found as a function of the duration of the ultra-sound treatment (Fig. 2a). This is due to the fact that for short times of depolymerization one is far from the levelling-off intrinsic viscosity and a relatively sharp linear decrease of the solution viscosity is observed (Fig. 2b).

It has long been known that the ultrasonic treatment of polymer solutions results in a decrease of the solution viscosity and, as early investigations concluded, it must be associated to the scission of polymer chains. Generally, the scissions occur more frequently at the middle of the polymer chains and the rate of depolymerisation decreases with decreasing molecular weight (Price, 1996). Thus, the ultrasonic depolymerisation is accompanied by changes in the molecular weight distribution, as a consequence of a non-random scission process (Peters, 1996). Many other factors, including the ultrasonic parameters (frequency and intensity), solution parameters (solvent, dissolved gases, polymer nature and its concentration) and experimental conditions (duration of the treatment, external temperature and pressure), affect the degradation provoked by the ultrasound treatment (Mason, 1990; Peters, 1996; Price, 1996). According to a recent investigation on the ultrasonic degradation of water-soluble polymers, the more rigid polymeric chains are less scissile and, also, short irradiation times have little effect on the molecular weight distribution of such polymers (Koda, Mori, Matsumoto & Nomura, 1994). This seems to be the case of chitosan, which is considered to be a semi-rigid chain (Rinaudo et al., 1993b) and, as already mentioned, the sonication of chitosan hydrochloride sample studied in this work was carried out for short times (up to 10 min). Thus, it is assumed that ultrasonic treated chitosan hydrochloride was randomly degraded and that negligible changes of molecular weight distribution occurred after sonication.

By dissolving the chitosan hydrochlorides in the appropriate aqueous NaCl solutions it was also possible to study the effect of the ionic strength on the chain conformation by carrying out the viscosity measurements on these solutions. The treatment of the experimental data through the Huggins equation (Huggins, 1942) resulted in straight lines with good linearity ($r \geq 0.98$) in the range of ionic strength studied in this work (Fig. 3).

The values of the Huggins constants determined from

Table 2
Values of intrinsic viscosities at 0.1 mol/l NaCl ($[\eta]_{0.1}$) and of salt tolerances (S) for chitosan hydrochlorides

Sample	$[\eta]_{0.1}$ (dl/g)	S (dl $M^{1/2}$/g)
A	10.03	1.675
4A	8.73	1.377
7A	8.20	1.382
10A	7.50	1.064

these curves are in the range $0.31 \leq k' \leq 0.63$ (Table 1), suggesting that aggregate-free solutions were obtained in all cases since they are small in the range expected for good polyelectrolyte solutions (Rinaudo et al., 1993b). This conclusion confirms the recent finding that these solutions are free of aggregates and therefore appropriate for the study of the hydrodynamic properties of this polyelectrolyte in solution (Signini & Campana, 1999). However, it may be added that the higher the values of k' found the higher the ionic strengths, indicating the concomitant decrease of the solvent quality as a function of increasing external salt concentration.

For viscosimetric studies of polyelectrolytes, it is also important to ensure a constant charge content along the polymer chain. When anionic polyelectrolytes are isolated/purified in the salt form, they may be directly dissolved in pure water or in salt-containing aqueous solutions of defined ionic strength. In the case of chitosan purified in the uncharged form, the polymer is usually dissolved in acids or buffer solutions of pH $\cong$ 4.5. In these cases, since the pK_0 of chitosan is close to 6.5, it is to be expected that available amino groups are protonated and the polysaccharide chains are fully charged. After properly isolating/purifying chitosan hydrochloride, since all amino groups along the polysaccharide's chains are protonated, it is also completely soluble in pure water. This is the case for the samples studied in this work which, as already mentioned, are fully soluble in pure water and in aqueous NaCl solutions. The values of average degrees of acetylation of chitosan samples, determined by conductometric titrations or by ^{1}H NMR spectroscopy, are also nearly the same independent of purifying it as hydrochloride or in the uncharged form. These latter results have been taken as evidence that chitosan hydrochloride is fully charged. In fact, by applying the first analytical method the content of protonated (charged) amino groups is determined while the latter method allows the determination of acetylated amino groups and, indirectly, the content of deacetylated (charged + uncharged) amino groups is also known. After dissolving chitosan hydrochloride, the occurrence of hydrolysis of protonated amino groups should be a concern, but as recently reported, less than 0.02% of its protonated amino groups undergo hydrolysis in aqueous dilute solutions (Rinaudo et al., 1999a,b). Thus, one may assume that the charge content of chitosan hydrochloride in aqueous acid-free solutions does not change as long as the pH of the solution is constant.

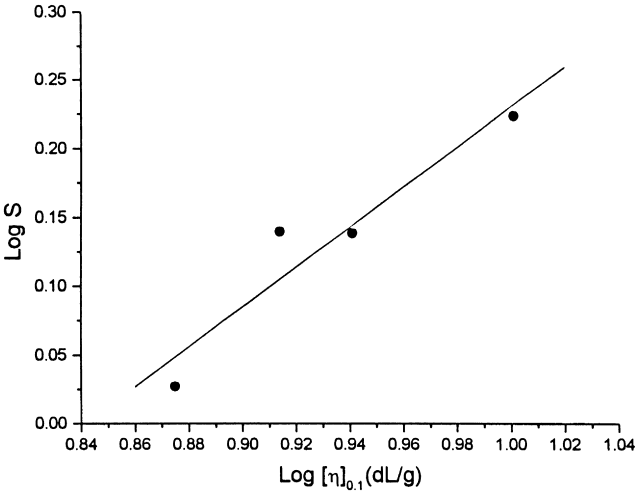

Fig. 4. Plot of the logarithm of the salt tolerance factor, log S, versus the logarithm of the intrinsic viscosity at 0.1 M NaCl, $\log[\eta]_{0.1}$, for chitosan hydrochloride.

As seen in Table 1, all chitosan hydrochlorides presented the characteristic polyelectrolyte behaviour since their intrinsic viscosities decreased as the ionic strength of their solutions was increased and, as it is usually found with polyelectrolytes, these decreases were well described by the expression below.

$$[\eta]_\mu = [\eta]_\infty + S\,\mu^{-1/2} \quad (3)$$

where $[\eta]_\mu$ is the intrinsic viscosity at ionic strength μ, $[\eta]_\infty$ the intrinsic viscosity extrapolated to infinite ionic strength, S the salt tolerance.

From the values of the tolerances of the samples to the salt concentration, S, and from their intrinsic viscosities at 0.1 mol/l NaCl (Table 2), the stiffness parameter (Smidsrod & Haug, 1971) was determined by extrapolating the logarithm form of the expression shown below to $[\eta]_{0.1} =$ 1 dl/g.

$$S = B([\eta]_{0.1})^{\nu} \quad (4)$$

Using this treatment a straight line is obtained (Fig. 4; $r =$ 0.95399 and $\nu = 1.45$) whose extrapolation results in $B \cong$ 0.06 as the stiffness parameter of chitosan hydrochloride in acid-free aqueous solution. This value is in close agreement with that reported in the literature for chitosans of different degrees of acetylation (2.0% $\leq$ DA $\leq$ 21.0%) studied in acetate buffer (Rinaudo et al., 1993b). However, at first glance, it does not agree with those determined by Anthonsen et al. (1993) and by Tsaih and Chen (1997), who studied chitosan hydrochloride in acetate buffer and neutral chitosan dissolved in dilute HCl/NaCl aqueous solutions, respectively. A comparison of the values of the stiffness parameter, B, determined for chitosan in this work and in previous studies is shown in Table 3.

The higher values of B are obtained by Anthonsen et al. (1993) and by Tsaih and Chen (1997) while the lower ones

Table 3
Comparison of characteristic parameters of chitosans for the treatment of Smidsrod at ionic strength 0.1 M

Reference	%DA	$[\eta]_{0.1}$ (dl/g)	S (dl $M^{1/2}$/g)	B	ν
Anthonsen et al., 1993					
$M_n = 125,000$	15	5.67	0.883	0.10	1.217
$M_n = 245,000$	15	7.80	1.070	0.10	1.217
Tsaih & Chen, 1997					
$M_w = 120,000$	17	2.231	0.174	0.138	1.217
$M_w = 280,000$	17	1.216	0.390	0.149	1.217
Rinaudo et al., 1993b					
$M_v = 127,000$	21	4.90	0.60	0.065	1.3
This work					
A	22.6	10.03	1.675	0.06	1.45
4A	22.6	8.73	1.377	0.06	1.45
7A	22.6	8.20	1.382	0.06	1.45
10A	22.6	7.50	1.064	0.06	1.45

were found by Rinaudo et al. (1993b) and in this study. Also, Tsaih and Chen claimed that a molecular weight induced conformational transition occurred and as a consequence they determined different values of B as a function of the molecular weight of the chitosan (Tsaih & Chen, 1997). Other differences in these studies concern: (i) the values of S, the salt tolerance of chitosan; (ii) the values of $[\eta]_{0.1}$, the intrinsic viscosities at 0.1 M ionic strength; (iii) the values of ν, the slopes of the curves $S = f([\eta]_{0.1})$.

In the study of Anthonsen et al. (1993) chitosan samples in the chloride salt form were dissolved in 0.02 M acetate buffer/NaCl (C_s), the concentration of this latter salt being mainly responsible for the maintenance of the ionic strength ($0.05\ \text{M} \leq \mu \leq 1.07\ \text{M}$). From viscosity measurements at 20°C they found that the values of S ranged from 0.036 to 1.272 ($S = 0.883$ and $S = 1.07$ for the chitosan having $M_n = 125,000$ and $M_n = 245,000$, respectively, both samples with $F_A = 0.15$); the values of $[\eta]_{0.1}$ ranged from 1.35 to 9.31 dl/g ($[\eta]_{0.1} = 5.67$ dl/g and $[\eta]_{0.1} = 7.80$ dl/g for chitosans having $M_n = 125,000$ and $M_n = 245,000$, respectively, both samples with $F_A = 0.15$). These authors also found that ν was greater ($\nu = 1.728$) for the more acetylated sample of chitosan ($F_A = 0.6$) than for the less acetylated ones ($\nu = 1.217$ and $\nu = 1.219$ for chitosans having $F_A = 0.15$ and $F_A = 0$, respectively). Considering the maximum relative viscosity of the chitosan solutions employed in that study ($\eta_{rel.} = 3$–4), the occurrence of aggregation may not be discarded. Indeed, a light scattering study carried out by the same authors showed the occurrence of a concentration-dependent aggregation of chitosan chains in this solvent (Anthonsen, Varum & Hermansson, 1994).

Tsaih and Chen applied the same value of ν as found by Anthonsen ($\nu = 1.217$) for chitosans of different molecular weights and $F_A = 0.17$ and their ranges of S and $[\eta]_{0.1}$ were as follows:

- $0.091 \leq S \leq 0.74$ (S = 0.39 and S = 0.174 for chitosans having $M_w = 280,000$ and $M_w = 120,000$, respectively).
- $3.785\ \text{dl/g} \leq [\eta]_{0.1} \leq 0.862\ \text{dl/g}$ ($[\eta]_{0.1} = 2.231$ dl/g and $[\eta]_{0.1} = 1.216$ dl/g, for chitosans having $M_w = 280,000$ and $M_w = 120,000$, respectively).

In this case, neutral chitosan samples were dissolved in 0.01 N HCl/NaCl (C_s) in the ionic strength range of $0.01\ \text{M} \leq \mu \leq 0.3\ \text{M}$, the viscosity measurements being performed at 30°C. However, in such an acid solution (pH ≅ 2.0) it is well known that chitosan has a limited solubility and in this case the occurrence of aggregation must also be suspected. Moreover, these authors have not applied the Smidsrod treatment to their data as it is usually done since they employed the equation relating the salt tolerance factor, S, to intrinsic viscosity at ionic strength 0.1 M, $[\eta]_{0.1}$, to each individual sample by using the same value of ν as determined by Anthonsen. However, by doing the usual treatment to their data a good straight line is obtained ($r = 0.9963$), a single value of B may be found equal to 0.121 and the slope of the curve $S = f([\eta]_{0.1})$ is $\nu = 1.40$.In the work done by Rinaudo et al. (1993b) the values of S ranged from 0.6 to 1.4 ($S \cong 0.6$ for chitosan having DA = 21% and $M_v = 127,000$), the values of $[\eta]_{0.1}$ ranged from 6.0 to 12 dl/g ($[\eta]_{0.1} \cong 4.9$ dl/g for chitosan having DA = 21% and $M_v \cong 127,000$ g/mol), $\nu \cong 1.3$, and the authors claimed that good chitosan solutions were obtained as evaluated by the values of the Huggins constant ($k' = 0.3$) in AcOH 0.3 M/AcONa 0.2 M.As mentioned before, aggregation processes may have occurred in the studies for the determination of stiffness of the chitosan chain done by Anthonsen et al. (1993) and Tsaih and Chen (1997). This could explain their higher values for the Smidsrod stiffness parameter compared with that obtained by Rinaudo and in this work. The presence of aggregates may also explain the lower values for intrinsic viscosities and its weak dependence on the ionic strength expressed by the salt tolerance factors. Indeed, as has been discussed elsewhere (Rinaudo et al., 1993b), aqueous solvents containing HCl/NaCl are not good for the study of solution properties of chitosan since the aggregation of macromolecules occurs and overestimates of molecular weights are obtained. Moreover the hydrodynamic behaviour may be drastically influenced by the occurrence of aggregation of the chitosan chains and the effect of increasing ionic strength on the stiffness of the macromolecule may also be affected.

4. Conclusions

The preparation of chitosan hydrochloride from a commercial chitosan resulted in a purified sample which does not require the presence of acid to promote its solubility in aqueous solvents. This may be seen as an advantage since the study of chitosan acid-free aqueous solutions makes comparison with other polyelectrolytes easier. The

depolymerization of the commercial sample by ultra-sound treatment of its aqueous acetic acid solutions followed by purification produced chitosan hydrochlorides of lower molecular weights but with identical degrees of acetylation. This latter finding supports the assumption that the sonication of chitosan in aqueous acid media has no effect on the degree of acetylation of the polymer. Good solutions of all chitosan hydrochlorides were obtained as evaluated by the values of the Huggins constants ($0.31 \leq k' \leq 0.63$) in acid-free aqueous solvents containing different concentrations of NaCl ($0.06\text{M} \leq \mu \leq 0.3$ M). Viscosity measurements on these solutions allowed the determination of the characteristic stiffness parameter of chitosan hydrochloride, $B = 0.06$, in close agreement with the value reported for chitosan in AcOH 0.3 M/AcONa 0.2 M. The disagreement with the higher values of B reported in the literature for chitosan may be attributed to the occurrence of aggregation in the acid-containing aqueous solutions employed in those determinations. Considering the results presented here, the acid-free aqueous solutions of purified chitosan hydrochlorides may allow the study of the solutions properties of chitosan with minimum influence due to aggregation, which may contribute to the characterization and to the determination of structure/properties of chitosan.

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