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# Viscosity Behavior of Chitosan and N,N,N-Trimethylchitosan Chloride Salts in Acid-Free Aqueous Solution

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*The viscosity behavior of the chloride salts of chitosan ($\overline{DA} = 18.2\%$) an N,N,N-trimethylchitosan ($\overline{DQ} = 50.4\%$; $\overline{DA} = 14.5\%$) was studied at 25°C. The dependence of the intrinsic viscosity ($[\eta]$) on the ionic strength ($\mu$) was investigated by using acid-free aqueous solutions of NaCl (0.06 M ≤ [NaCl] ≤ 0.3 M). The linear dependence of the intrinsic viscosity on the reciprocal of the square-root of the ionic strength ($\mu^{-1/2}$) and the decrease of the salt tolerance parameter (S) with decreasing molecular weight were observed in both cases. The method proposed by Smidsrød was employed to estimate the chain stiffness of the polyelectrolytes resulting in $B \cong 0.11$ for both polymers. Taking into consideration that the average degree of acetylation of N,N,N-trimethylchitosan is lower than that of chitosan and that most of the hydroxyl groups of the former polymer is also methylated, it is suggested that the content of acetamido groups is not the most important factor for the stiffness of chitosan.*

**Keywords** chitosan, N,N,N-trimethylchitosan chloride, viscosity behavior, chain stiffness, stiffness B-parameter

## Introduction

Chitosan, a $\beta(1 \rightarrow 4)$ aminoglycan prepared by the heterogeneous deacetylation of chitin, is a cationic polyelectrolyte when dissolved in aqueous media due to the protonation of its amino groups (1, 2). Since these are weakly basic groups, the solubility of chitosan is restricted to sufficiently acid aqueous media, usually the dilute solutions of acetic and hydrochloric acid of pH ≤ 5.5. Nevertheless, a polymer, which is soluble in a wide range of pH can be prepared via the homogeneous acetylation of chitosan to an average degree of acetylation close to 50% (3). The limited solubility of chitosan in aqueous media can also be overcome by introducing new functionalities through its derivatization such as, for instance, by sulphation and carboxymethylation reactions, however, such reactions also add an anionic nature to the cationic polylectrolyte character of the parent chitosan. An alternative route to improve the water solubility of chitosan without

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changing its cationic character is the introduction of an enough number of permanent positive charges in its chains. This can be done by preparing quaternary chitosan salts, via the covalent addition of a substituent containing a quaternary ammonium group (4) or by the quaternization of the amino groups already present in chitosan. The latter route has been studied by a number of authors (5–8) mainly by carrying out the methylation of the amino sites of chitosan. Indeed, this strategy was successfully used to improve the functionality of chitosan as a permeation enhancer for orally administrated drugs (9–11). The methylation of chitosan with iodomethane results in N,N,N-trimethylchitosan, a water soluble cationic polyelectrolyte permanently charged due to the presence of quaternized nitrogen atoms. Although many studies have been dedicated to the preparation and physical chemical characterization of this chitosan derivative (5–8,12, 13), as well as to evaluate its anti-bacterial and therapeutical properties and applications (9–11, 14–16), its viscosity behavior in acid-free aqueous solutions has not been reported thus far. Therefore, it seems very interesting to compare the viscosity behavior of chitosan and N,N,N-trimethylchitosan since both polymers have the same main chain, but the latter contains quaternized nitrogen sites whose presence can impart a different hydrodynamic response to this polymer. The viscosity behavior of chitosan is usually studied in aqueous media containing an excess of acid to assure its dissolution, but the occurrence of aggregation in these media has been reported (17). Since the N,N,N-trimethylchitosan is soluble in a wide range of pH and knowing that chitosan hydrochloride is readily soluble in pure water (18), the comparison of the viscosity behavior of these polymers in acid-free aqueous solutions may be predicted. The viscosity behavior of chitosan hydrochloride ($\overline{DA} = 22.6\%$), a water soluble form of chitosan, in aqueous NaCl solutions was studied and its stiffness parameter was reported as B = 0.06 (19). This value is in good agreement with the result reported in a study carried out with a chitosan ($\overline{DA} = 21\%$) dissolved in acetic acid/sodium acetate buffer (20). Other studies reported higher values for B when the viscosity behavior of less acetylated chitosans was investigated (21, 22). These results support the hypothesis that the stiffness of the chitosan chain depends on its average degree of acetylation, the acetamido groups being responsible for the establishment of hydrogen bonds with the H-O(C6) of adjacent repeating units and thus imparting a steric constraint to the chain rotation. Indeed, a recent work reported a comparison on the stiffness B-parameter of chitosan and poly(diallyldilethylammonium chloride) (23), concluding that the polysccharide chain was stiffer mainly due to the larger distance between consecutive repeating units and to the stronger steric restrictions to rotation found in chitosan.

In this work, the influence of the ionic strength on the intrinsic viscosity of the chloride salts of chitosan ($\overline{DA} = 18.2\%$) and N,N,N-trimethylchitosan ($\overline{DQ} = 50.4\%$ and $\overline{DA} = 14.5\%$) is studied by employing acid-free aqueous NaCl solutions and the approach of Smidsrød (24) is used to estimate and compare the chain stiffness of these polymers.

## Experimental

All reagents and solvents were of technical grade or P.A. and they were used as received unless when specified.

A commercial sample of chitosan (medium molecular weight chitosan from Fluka BioChimica; Lot 364955/144996) was purified and then used as the raw material for the preparation of N,N,N-trimethylchitosan. Therefore, the chitosan sample was dissolved in 1M lactic acid by stirring 1.0 g of the polysaccharide dispersed in 300 $cm^3$

of the acid aqueous solution overnight. The resulting solution was filtered through 5.0 μm mixed cellulose ester membrane (Millipore) and the chitosan was precipitated upon the addition of a concentrated ammonium hydroxide aqueous solution. Following its extensive washing with water and then with methanol, the purified chitosan was dried at ambient conditions, milled in a domestic blender and the fraction composed by particles having an average diameter lower than 125 μm was then used in the N-methylation reaction.

### *N,N,N-Trimethylchitosan Chloride*

The N-methylation reaction of chitosan aiming the preparation of N,N,N-trimethylchitosan chloride was carried out as described elsewhere (8) as follows: 1.0 g of purified chitosan was suspended in 40 $cm^3$ of N-methyl-2-pyrrolidone and the suspension was kept at room temperature and constant stirring for 15 h. Then, 2.4 g of sodium iodide, 5.5 $cm^3$ of aqueous sodium hydroxide 15% and 5.7 $cm^3$ of iodomethane were added. Further additions of sodium hydroxide and iodomethane (0.3 g NaOH and 1 $cm^3$ $H_3CI$) were carried out after 3 h, 6 h, 9 h and 12 h of reaction. The reaction proceeded for more 12 h after the last addition of reagents and the pH of the reaction medium was then adjusted to 7.5. The reaction medium was filtered to separate the fraction, which became insoluble upon neutralization and the filtered solution was then transferred to the appropriate dialysis membrane (10,000–12,000 g/mol molecular weight exclusion limit) where it was dialyzed against deionized water followed by dialysis against a 0.1M NaCl aqueous solution and against deionized water again. The final product was obtained after freeze-drying as a water-soluble chloride salt which will be referred to as sample TMCh.



### *Chitosan Chloride*

This water-soluble form of chitosan was prepared as follows: 1.0 g of commercial chitosan was dispersed in 150 $cm^3$ of 0.2 M aqueous acetic acid and the suspension was continuously stirred during 18 h. The resulting solution was sequentially filtered through 5.0 μm and 0.8 μm mixed cellulose esters membranes (Millipore). It was then transferred to the dialysis membrane (12,000–16,000 g/mol molecular weight exclusion limit) where it was dialyzed against a 0.2M NaCl aqueous solution and against deionized water. The chitosan chloride salt was obtained after freeze-drying and it will be referred to as sample Ch.

### *Depolymerization by Ultrasound Treatment*

To obtain samples of a lower molecular weight of chitosan and N,N,N-trimethylchitosan, these polymers were submitted to ultrasound treatment at a nominal potency of 30 W by using a Branson Sonifier 450 equipment.

In these experiments, the polymer solution was contained in a glass cell maintained at $28^\circ \pm 0.1^\circ C$, 2/3 of the ultrasound probe was immersed in it and the treatment proceeded for the desired time.

### *Depolymerization of Chitosan Chloride*

A 10 g/L solution of commercial chitosan in 0.2 M aqueous acetic acid was submitted to exhaustive dialysis against 0.2 M aqueous NaCl and against deionized water, it was

then sequentially filtered through 5.0 μm and 0.8 μm mixed cellulose ester membrane (Millipore). The solution was divided in 4 aliquots of 50 $cm^3$, which were then submitted to the ultrasound treatment for 1, 3, 8 and 15 min to result in the depolymerized samples $S_1$Ch, $S_2$Ch, $S_3$Ch, $S_4$Ch, respectively. These samples were freeze-dried and then stored in a dissicator containing $P_2O_5$.

### *Depolymerization of N,N,N-Trimethylchitosan Choride*

A 10 g/L solution of the sample TMCh in deionized water was divided in 3 aliquots of 50 $cm^3$, which were then submitted to the ultrasound treatment for 1, 5 and 12 min to result in the depolymerized samples $S_1$TMCh, $S_2$TMCh and $S_3$TMCh, respectively. After freeze-drying, these samples were stored in a dissicator containing $P_2O_5$.

### *Determination of Average Degrees of Acetylation ($\overline{DA}$) and Quaternization ($\overline{DQ}$)*

The average degrees of acetylation and quaternization were determined by $^1$H-NMR spectroscopy. Thus, the $^1$H-NMR spectra of the chloride salts of chitosan (Ch) and N,N,N-trimethylchitosan (TMCh), as well as of their depolymerized samples were acquired at 80°C by using a 200 MHz spectrometer (Bruker AC200). For these analyses, the samples of N,N,N-trimethylchitosan were dissolved in $D_2O$ at a concentration of 10 g/L while chitosan was dissolved in $D_2O$/HCl (100/1 v/v) at the same concentration. The average degrees of acetylation ($\overline{DA}$) of chitosan and sonicated samples were determined as previously described (25) through the ratio between the intensities of the signals due to the hydrogen of the methyl groups of the acetamido moieties ($I_{Nac}$) and those corresponding to the hydrogen bonded to C2 ($I_{H2}$) by using the expression:

$$\overline{DA} = \left[\frac{I_{NAc}}{3} \times \frac{1}{I_{H_2}}\right] \times 100 \quad [1]$$

The average degrees of acetylation ($\overline{DA}$) and quaternization ($\overline{DQ}$) of N,N,N-trimethylchitosan and sonicated samples were determined from the attributions of signals reported in the literature (11) by using the expressions:

$$\overline{DA} = \left[\frac{I_{NAc}}{3} \times \frac{1}{S}\right] \times 100 \quad [2]$$

$$\overline{DQ} = \left[\frac{I_{NQ}}{9} \times \frac{1}{S}\right] \times 100 \quad [3]$$

$$S = \frac{I_{NQ}}{9} + \frac{I_{ND}}{6} + \frac{I_{NAc}}{3} \quad [4]$$

where $I_{NQ}$ and $I_{ND}$ correspond, respectively, to the intensities of the signals due to quaternized and dimethylated nitrogen sites present in the chains of TMCh. The average degree of quaternization ($\overline{DQ}$) of the sample TMCh was also determined from its titration with aqueous 0.1 M $AgNO_3$. Thus, an aliquot of the aqueous solution of TMCh ($C_P = 1.2$ g/L) was transferred to a glass cell maintained at 25.0° ± 0.1°C and the solution conductivity was measured by the Handylab LF1 conductivimeter upon the addition of aqueous $AgNO_3$ by the Titronix Universal automatic burette, both from

Schott-Geräte. The average degree of quaternization was then determined by using the expression:

$$\overline{GQ} = \left[\frac{M_{TMCh} \times V \times [AgNO_3]}{m}\right] \times 100 \qquad [5]$$

where $M_{TMCh}$ is the molecular weight (g/mol) of the repeating unit of TMCh containing the quaternized site, $V$ ($dm^3$) and [$AgNO_3$] (mol/$dm^3$) are the equivalent volume and concentration of $AgNO_3$ aqueous solution, respectively, and m (g) is the mass of TMCh.

### *Viscosity Measurements in Acid-Free NaCl Aqueous Solution*

These experiments were carried out with aqueous solutions of the chloride salts of chitosan (samples Ch, $S_1$Ch, $S_2$Ch, $S_3$Ch, $S_4$Ch) and N,N,N-trimethylchitosan (samples TMCh, $S_1$TMCh, $S_2$TMCh and $S_3$TMCh) containing different concentrations of NaCl ($0.06M \leq$ [NaCl] $\leq 0.3$ M). The polymer solutions were previously filtered through 0.80 μm and 0.45 μm mixed cellulose ester membrane (Millipore) and the polymer concentrations were such that $2.0 > \eta_{rel} > 1.2$, from the initial solution toward the more diluted solution whose viscosities were measured in this study. The AVS-350 viscometer coupled to the AVS-20 automatic burette, both from Schott-Geräte, was used for the viscosity measurements. The glass capillary ($\phi = 0.53$ mm) containing 15 $cm^3$ of the polymer solution was immersed in a water bath maintained at $25^\circ \pm 0.01^\circ$C and previously programmed volumes of the solvent, i.e., the aqueous solutions of NaCl ($0.06\,M \leq$ [NaCl] $\leq 0.3$ M), were sequentially added to proceed the desired dilution. The intrinsic viscosities were determined from the curves of the reduced viscosity ($\eta_{sp}/C$) vs. polymer concentration (C) and the values of S, salt tolerance, resulting from the curves of the intrinsic viscosity at a given ionic strength ($[\eta]_\mu$) vs. the reciprocal root-square of the ionic strength ($\mu^{-1/2}$).

## Results and Discussions

### *Average Degrees of Acetylation ($\overline{DA}$) and Quaternization ($\overline{DQ}$)*

Since the hydrodynamic behavior of polyelectrolytes depends on the content of charged groups in its chains, it is important to determine the average number of amino groups and quaternized amino groups present in the chains of chitosan and N,N,N-trimethylchitosan, respectively. Indeed, the protonated amino groups of chitosan hydrochloride and the quaternized sites of N,N,N-trimethylchitosan hydrochloride are positively charged sites responsible for the cationic polyelectrolyte character of these polymers. Moreover, as some authors claim that the average degree of acetylation has an important role on the intrinsic stiffness of chitosan-like chains, the content of acetylated repeating units present in the chains of N,N,N-trimethylchitosan must also be known. Hence, the $^1$H-NMR spectroscopy was used for the determination of the average degrees of acetylation and of quaternization of these polymers.

The $^1$H-NMR spectra of the samples Ch (chitosan) and TMCh (N,N,N-trimethylchitosan) are shown in Figures 1 and 2, respectively. The intensities of the signals at $\delta \cong 2.0$ and $\delta \cong 3.2$ in the spectrum of chitosan (Figure 1), which correspond, respectively, to the methyl hydrogens of the acetamido groups and to the hydrogen bonded to C2 of the glypiranose ring, were taken for the determination of the average degree of acetylation of the sample Ch, resulting in $\overline{DA} = 18\%$.

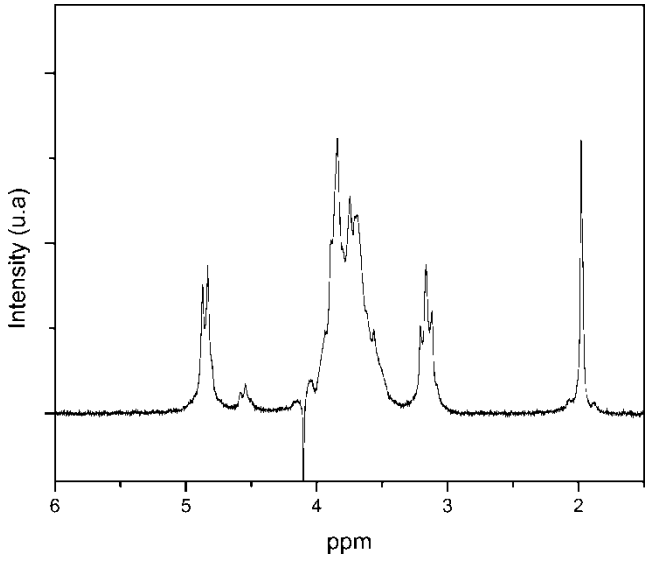


**Figure 1.** $^{1}$H-NMR spectrum of 1% chitosan chloride (Ch) in $D_2O$/HCl (100:1 v/v) acquired at 80°C.



However, the same ratio cannot be used to determine the average degree of acetylation of the sample TMCh as a consequence of the occurrence of superimposition of signals. Indeed, the signal due to the hydrogen bonded to C2 of the glypiranose ring becomes broader and it is shifted to the region $2.42 \leq \delta \leq 3.05$ in this case (Figure 2), being superimposed to the signal attributed to the dimethylated nitrogen sites (11).

The average degree of quaternization of N,N,N-trimethylchitosan could be determined from its $^{1}$H-NMR spectrum from the ratio between the intensities of the signals due to the quaternized amino sites and of the signal attributed to the hydrogen bonded

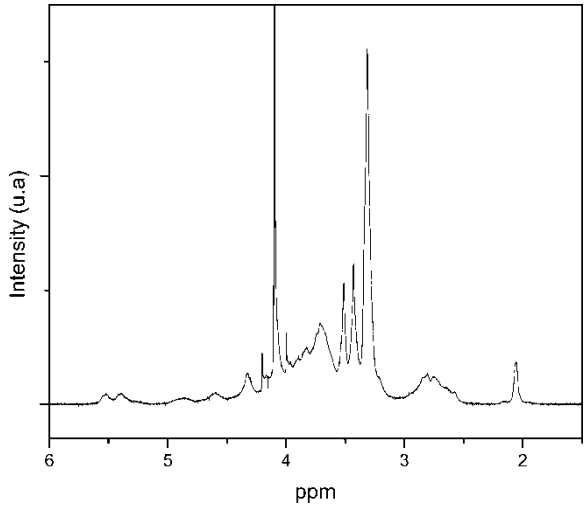


**Figure 2.** $^{1}$H-NMR spectrum of 1% N,N,N-trimethylchitosan chloride (TMCh) in $D_2O$ acquired at 80°C.

to C2, but as pointed out above, the signal due to dimethylated amino group is superimposed to the latter signal precluding its use as a reference. In fact, the use of intensity of the signal of the hydrogen bonded to C2, as a reference, will result in the underestimation of the average content of acetylated and quaternized amino sites present in the TMCh chains. Indeed, an important underestimation occurs in this case since the intensity due to six hydrogen atoms of two methyl groups of the N,N-dimethylated sites will be inadvertently added to that of the hydrogen bonded to C2, increasing the denominator of the ratio used for the calculation of $\overline{DA}$ and $\overline{DQ}$.

Usually, the average degree of quaternization of N,N,N-trimethylchitosan is determined from the ratio between the intensity of the signal due to the quaternized amino sites and the set of signals attributed to the anomeric hydrogen (11). However, these signals are very weak, the resolution of spectrum is not good enough (Figure 2) and the use of this ratio to determine the average degree of quaternization of the sample TMCh resulted in $\overline{DQ} = 21.3\%$, an underestimation of the content of quaternized amino groups considering the reaction conditions employed in the methylation of chitosan (8). Alternatively, the use of the expressions [2] and [3] for the determination of the average degrees of acetylation and quaternization of the TMCh sample will result in more realistic estimations of $\overline{DA}$ and $\overline{DQ}$ since in this case the denominator of the ratio used to calculate them will also be increased as a consequence of the superimposition of signals but much less than in the former case. In fact, the intensity due to the hydrogen bonded to C2 will be added to those of the nine hydrogens of three methyl groups of the quaternized sites, six hydrogen atoms of two methyl groups of the dimethylated sites and three hydrogen of the methyl group of the acetamido moiety. Thus, the impact of the superimposition of signals on the determination of $\overline{DA}$ and $\overline{DQ}$ is relatively much less important in this case than that pointed out above. Also, for this alternative method to determine $\overline{DA}$ and $\overline{DQ}$ be valid it is assumed that all amino groups of N,N,N-trimethylchitosan are methylated (di- and trimethyl amino groups) or acetylated, a reasonable assumption since the methylation of chitosan was carried out in the presence of a very large excess of the alkylating agent and for a long reaction time. Thus, by taking into account the signals due to trimethylated and dimethylated sites centered at 3.30 ppm and 2.60 ppm, and by using the expression [3], resulted in $\overline{DQ} = 49.6\%$ for the sample TMCh, a value which is approximately 2.3 times higher than that determined by using the signals due to the anomeric hydrogens as a reference.

The average degree of quaternization of the sample TMCh was also determined from the titrimetric curve resulting from the dosage of chloride, the only counter-ion of the quaternized group of the TMCh hydrochloride, with $AgNO_3$ (Figure 3) resulting in $\overline{DQ} = 55.0\%$, a value which is less than 10% higher than that determined by $^1$H-NMR spectroscopy by using the expressions [3] and [4].

The values of the average degrees of acetylation and of quaternization of the chitosan and N,N,N-trimethylchitosan as well as those corresponding to the sonicated samples are shown in the Table 1.

Recent works have shown that the ultrasound treatment of chitin and chitosan in aqueous solutions provokes no changes in the average degree of acetylation of these polymers, but results in random chain depolymerization (19, 26, 27). However, no data were thus far available to confirm that the acetylated and methylated sites on N,N,N-trimethylchitosan were not affected by the ultrasound treatment. Indeed, the data on Table 1 shows that the ultrasound treatment did not provoke either deacetylation nor demethylation on the samples Ch and TMCh, since the average degrees of acetylation and quaternization of the ultrasound treated samples are quite similar to those of the

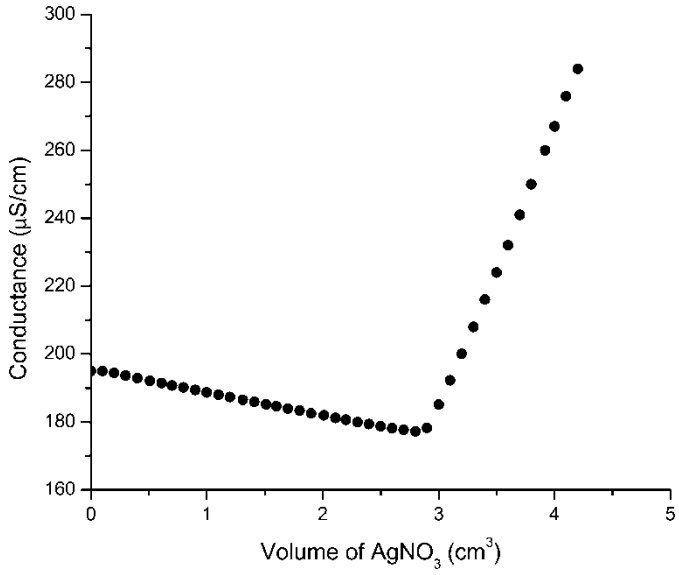


**Figure 3.** Curve of conductance vs. volume of aqueous 0.1 M $AgNO_3$ solution used to titrate the aqueous solution of N,N,N-trimethylchitosan chloride.



parent polymers. Thus, 18.2% and 14.5% can be taken as the mean values of $\overline{DA}$ for the entire set of samples of chitosan and N,N,N-trimethylchitosan, respectively, while 50.4% is the representative value for the $\overline{DQ}$ of the TMCh samples. From these data, it is also observed that the N-methylation of chitosan resulted in its simultaneous deacetylation as the mean value of the average degree of acetylation of TMCh is approximately 20% lower than that of the parent chitosan.

**Table 1**
Average degrees of acetylation ($\overline{DA}$) and quaternization ($\overline{DQ}$) of the chitosan chloride (Ch), N,N,N-trimethylchitosan chloride (TMCh) and their sonicated samples ($S_n$Ch and $S_n$TMCh)

| Sample | $\overline{DA}$ (%) | $\overline{DQ}$ (%) |
|---|---|---|
| Ch | 18.0[a] | — |
| $S_1$Ch | 18.7[a] | — |
| $S_2$Ch | 18.3[a] | — |
| $S_3$Ch | 17.8[a] | — |
| $S_4$Ch | 18.1[a] | — |
| TMCh | 14.8[b] | 49.6[c] (55.0)[d] |
| $S_1$TMCh | 14.2[b] | 50.1[c] |
| $S_2$TMCh | 14.4[b] | 48.7[c] |
| $S_3$TMCh | 14.7[b] | 48.6[c] |

[a]Determined by $^1$H-NMR spectroscopy using expression [1].
[b]Determined by $^1$H-NMR spectroscopy using expression [2].
[c]Determined by $^1$-NMR spectroscopy using expression [3].
[d]Determined by titration with $AgNO_3$.

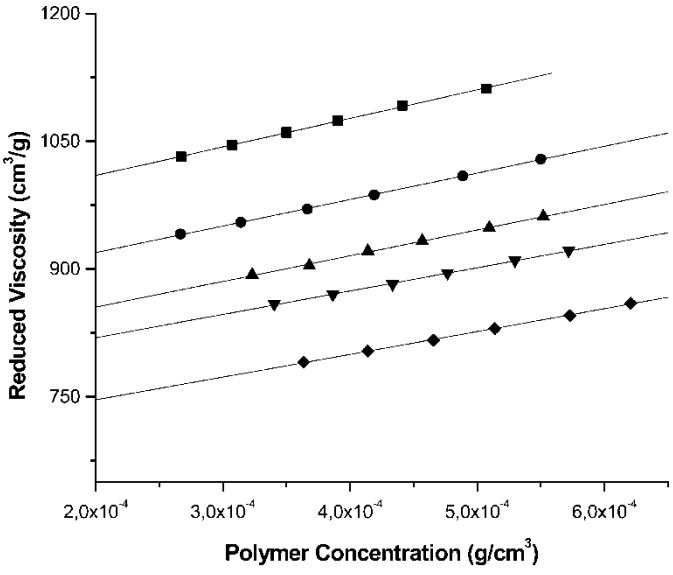


**Figure 4.** Reduced viscosity of the aqueous solutions of the chitosan chloride (Ch) as a function of [NaCl]: (■) 0.06M; (●) 0.10M; (▲) 0.14M; (▼) 0.2M; (◆) 0.3M.



### *Effect of the Ionic Strength on the Viscosity Behavior of Chitosan and N,N,N-Trimethylchitosan Chloride Salts*

Typical curves $\eta_{sp}/C$ vs. C for the samples Ch and TMCh are shown in Figures 4 and 5, respectively, while those corresponding to the samples $S_2$Ch and $S_2$TMCh are seen in Figures 6 and 7, respectively. These curves show that in all cases the reduced viscosity values are progressively lower the higher the ionic strength, a behavior which is typical of polyelectrolytes and which is attributed to the progressive screening of the like charges along the polymer chains as the concentration of the low molecular weight salt is increased. Also, the curves in Figures 4 and 5 reveal that regardless of the ionic strength, the values of the reduced viscosity corresponding to the sample Ch are higher than those of the sample TMCh, indicating that the methylation of chitosan resulted in its simultaneous depolymerization, probably due to hydrolysis in the strongly alkaline medium.

The treatment of the viscosity data by employing the Huggins equation (28), which resulted in all cases, in straight lines whose experimental data show good linearity ($r \geq 0.99$; Tables 2 and 3). It is also observed that the values of intrinsic viscosity of the samples which were submitted to the ultrasound treatment are lower than those from which they were prepared, the decrease being higher the longer the treatment. Thus, as already reported in the literature (8), such behavior may be attributed to the lower molecular weight of these samples as a consequence of the ultrasound treatment to which they were submitted. The data in the Tables 2 and 3 also show that the values of the Huggins constant are inserted in the range $0.27 \leq k_H \leq 0.55$, except for those corresponding to the samples $S_3$Ch and $S_4$Ch in [NaCl] = 0.3M which attain 0.78 and 0.72, respectively. However, even these values are sufficiently small to support the assumption that the aqueous solutions of chitosan and N,N,N-trimethylchitosan hydrochlorides studied in this work do not contain large aggregates.

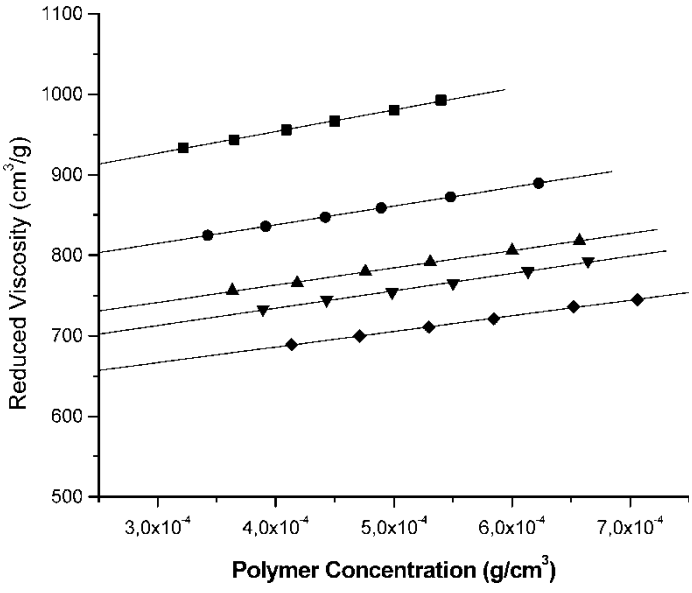


**Figure 5.** Reduced viscosity of the aqueous solutions of the N,N,N-trimethylchitosan chloride (TMCh) as a function of [NaCl]: (■) 0.06M; (●) 0.10M; (▲) 0.14M; (▼) 0.2M; (◆) 0.3M.



### *The Stiffness of Chitosan and N,N,N-Trimethylchitosan*

The intrinsic viscosities of both polymers, Ch and TMCh, displayed linear dependences on the reciprocal root-square of the ionic strength (Figures 8 and 9), a behavior already reported in other works concerning polyeletrolytes and including chitosan (19–24, 29–31)

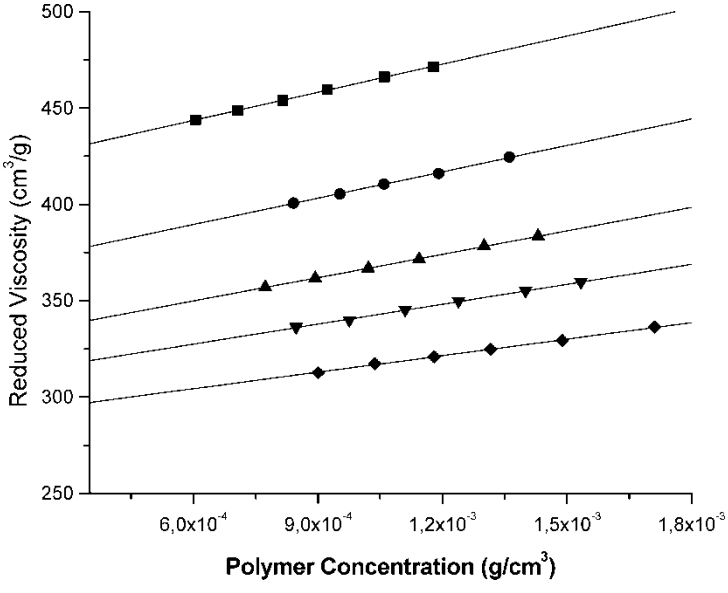


**Figure 6.** Reduced viscosity of the aqueous solutions of sonicated chitosan chloride ($S_2$Ch) as a function of [NaCl]: (■) 0.06M; (●) 0.10M; (▲) 0.14M; (▼) 0.2M; (◆) 0.3M.

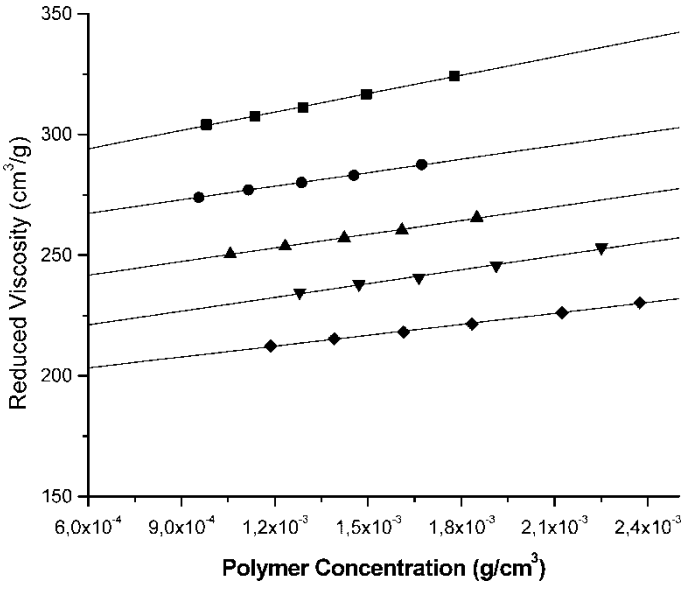


**Figure 7.** Reduced viscosity of the aqueous solutions of sonicated N,N,N-trimethylchitosan chloride ($S_2$TMCh) as a function of [NaCl]: (■) 0.06M; (●) 0.10M; (▲) 0.14M; (▼) 0.2M; (◆) 0.3M.



but which was not yet described for N,N,N-trimethylchitosan. The curves shown in Figures 8 and 9 obey the following expression:

$$[\eta]_\mu = [\eta]_\infty + S\mu^{-1/2} \qquad [6]$$

where $\mu$ is the ionic strength, $[\eta]_\mu$ and $[\eta]_\infty$ stand for the intrinsic viscosity at finite and infinite ionic strength, respectively, and S is the salt tolerance.

The values of the intrinsic viscosity extrapolated to infinite ionic strength and salt tolerance factors, S, issued from the curves of Figures 8 and 9 are shown in Table 4.

These data show the dependence of both parameters, $[\eta]_\infty$ and S, on the duration of the ultrasound treatment. Thus, the values of $[\eta]_\infty$ and S decrease with the increasing length of the ultrasound treatment, a tendency that reflects that the molecular weight of the treated samples is progressively lower the longer the time of sonication. In fact, it is assumed that at infinite ionic strength the polyelectrolyte is very close to the $\theta$ condition, a particular condition where the polymer chain attains its dimensions unperturbed by excluded volume effects and the polymer behavior approaches that of an uncharged macromolecule (32). Thus, the differences among the values of $[\eta]_\infty$ for untreated and sonicated samples may be attributed to differences of their molecular weights, the higher the value of $[\eta]_\infty$ the higher the molecular weight. The comparison of the values of S for the untreated and sonicated samples also supports this assumption since the dependence of S on the molecular weight of the polymer has already been reported (24). Moreover, the comparison among the values of $[\eta]_\infty$ and S of the samples Ch and TMCh reveals that the methylation of the chitosan resulted in its severe depolymerization since the methylated derivative exhibits much lower values of

**Table 2**
Intrinsic viscosity ([$\eta$]) of the chitosan chloride (Ch) and sonicated samples ($S_n$Ch) as a function of the [NaCl] and values of the Huggins constants ($k_H$) from the curves of reduced viscosity vs. polymer concentration for each ionic strength

| Sample | [NaCl] (M) | [$\eta$] ($cm^3/g$) | $k_H$ $(cm^3/g)^2$ |
|---|---|---|---|
| Ch | 0.06 | 943 | 0.38 |
| | 0.10 | 856 | 0.42 |
| | 0.14 | 795 | 0.48 |
| | 0.20 | 764 | 0.47 |
| | 0.30 | 692 | 0.56 |
| $S_1$Ch | 0.06 | 895 | 0.39 |
| | 0.10 | 807 | 0.42 |
| | 0.14 | 743 | 0.47 |
| | 0.20 | 697 | 0.48 |
| | 0.30 | 644 | 0.55 |
| $S_2$Ch | 0.06 | 846 | 0.37 |
| | 0.10 | 745 | 0.42 |
| | 0.14 | 677 | 0.46 |
| | 0.20 | 648 | 0.51 |
| | 0.30 | 608 | 0.52 |
| $S_3$Ch | 0.06 | 720 | 0.36 |
| | 0.10 | 635 | 0.42 |
| | 0.14 | 587 | 0.49 |
| | 0.20 | 561 | 0.51 |
| | 0.30 | 507 | 0.78 |
| $S_4$Ch | 0.06 | 599 | 0.40 |
| | 0.10 | 524 | 0.41 |
| | 0.14 | 500 | 0.46 |
| | 0.20 | 477 | 0.49 |
| | 0.30 | 441 | 0.72 |



$[\eta]_\infty$ and S than chitosan. Knowing the values of the tolerance factor, S, and intrinsic viscosity at ionic strength 0.1M, $[\eta]_{0.1}$, for chitosan, N,N,N-trimethylchitosan and the corresponding sonicated samples, the B-parameter standing for the chain stiffness (24) of these polymers can be determined by using the expression:

$$S = B\left([\eta]_{0.1}\right)^{\nu} \qquad [7]$$

It is usually done by plotting the logarithm form of the equation [7] and extrapolating the resulting straight line whose slop is $\nu$ to $[\eta]_{0.1} = 1.0 dl/g$. Such plots for chitosan and N,N,N-trimethylchitosan are shown in the Figures 10 and 11 and the resulting values of B are compared in the Table 5.

**Table 3**
Intrinsic viscosity ([$\eta$]) of the N,N,N-trimethylchitosan chloride (TMCh) and sonicated samples ($S_n$TMCh) as a function of the [NaCl] and values of the Huggins constants ($k_H$) from the curves of reduced viscosity vs. polymer concentration for each ionic strength

| Sample | [NaCl] (M) | [$\eta$] ($cm^3/g$) | $k_H$ $(cm^3/g)^2$ |
|---|---|---|---|
| TMCh | 0.06 | 414 | 0.28 |
| | 0.10 | 362 | 0.34 |
| | 0.14 | 326 | 0.38 |
| | 0.20 | 307 | 0.37 |
| | 0.30 | 287 | 0.35 |
| $S_1$TMCh | 0.06 | 382 | 0.27 |
| | 0.10 | 325 | 0.35 |
| | 0.14 | 306 | 0.34 |
| | 0.20 | 279 | 0.42 |
| | 0.30 | 254 | 0.41 |
| $S_2$TMCh | 0.06 | 327 | 0.32 |
| | 0.10 | 290 | 0.33 |
| | 0.14 | 265 | 0.37 |
| | 0.20 | 245 | 0.37 |
| | 0.30 | 227 | 0.39 |
| $S_3$TMCh | 0.06 | 279 | 0.33 |
| | 0.10 | 256 | 0.29 |
| | 0.14 | 230 | 0.36 |
| | 0.20 | 210 | 0.43 |
| | 0.30 | 194 | 0.40 |



According to Smidsrod, the stiffness parameter is relatively insensitive to the stoichiometric charge density and it can be correlated to the unperturbed dimensions of the polymer chains, the lower the B value, the stiffer is the polymeric chain (24). Thus, as the data in Table 5 shows that there is no appreciable difference between the values of B for chitosan and N,N,N-trimethylchitosan, one may conclude that both polymers are equally stiff/flexible. Yet, the values of B exhibited by these samples are close to those reported for chitosan ($9\% < \overline{DA} < 21\%$) in the literature (21–23), although some lower values ($B \cong 0.06$) are also reported for chitosan having slightly higher contents of acetamido groups (19, 20).

The data of this work shows that the samples Ch and TMCh have different average degrees of acetylation, which are however inserted in the range ($9\% < \overline{DA} < 21\%$). Moreover, the $^1$H-NMR spectrum of the sample TMCh (Figure 2) exhibits signals at 3.51 ppm and 3.43 ppm, which are attributed to O(C3)-$CH_3$ and O(C6)-$CH_3$ sites (32). Thus, since part of the H-O(C6) groups originally present in the chitosan chains become methylated after its reaction with iodomethane resulting in the TMCh sample, they are not able to establish hydrogen bonds with the acetamido groups of adjacent repeating units in the polymer chain. Indeed, a recent work reports that most

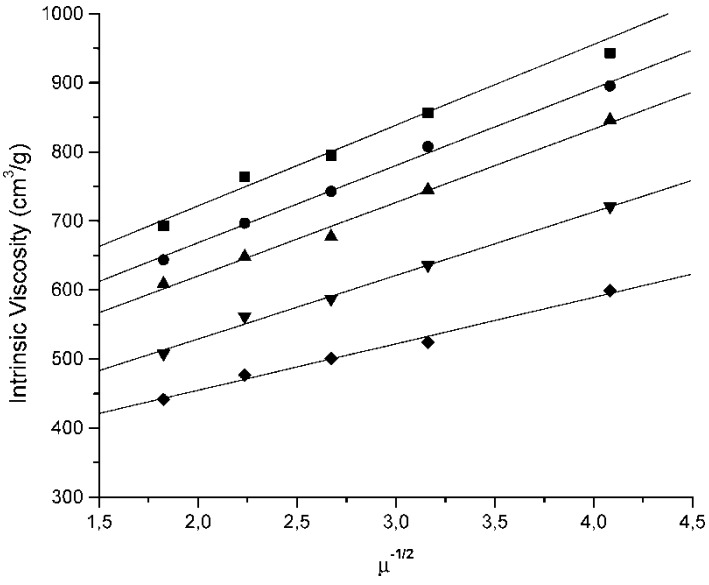


**Figure 8.** Dependence of the intrinsic viscosity of the chitosan chloride samples on the reciprocal square-root of the ionic strength ($\mu^{-1/2}$). (■) Ch; (●) $S_1$Ch; (▲) $S_2$Ch; (▼) $S_3$Ch; (◆) $S_4$Ch.



of the hydroxyl groups of a TMCh sample prepared in conditions similar to that employed in this work are methylated (8). Therefore, assuming that the establishment of hydrogen bonds between acetamido and H-O(C6) groups has an important influence on the stiffness of the chitosan chain and considering the facts mentioned

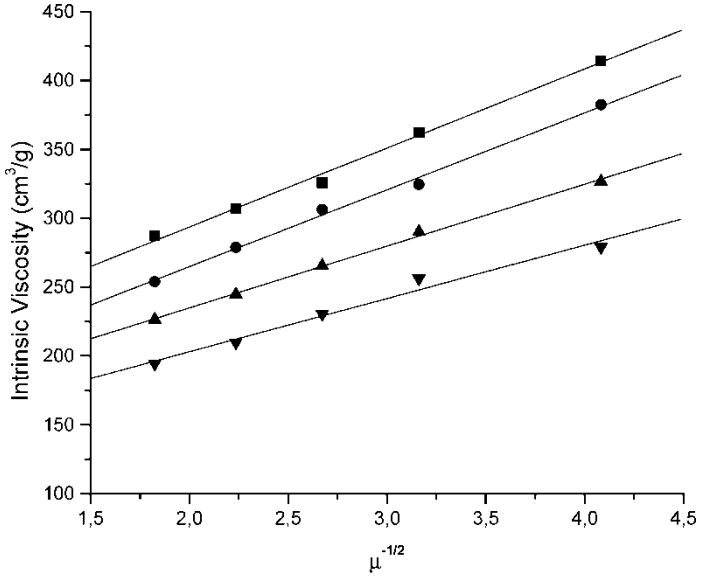


**Figure 9.** Dependence of the intrinsic viscosity of the N,N,N-trimethylchitosan chloride samples on the reciprocal square-root of the ionic strength ($\mu^{-1/2}$). (■) TMCh; (●) $S_1$TMCh; (▲) $S_2$TMCh; (▼) $S_3$TMCh.

**Table 4**
Values of intrinsic viscosity at infinite ionic strength, $[\eta]_\infty$, and salt tolerance factor, S, from the curves of intrinsic viscosity at a given ionic strength ($[\eta]_\mu$) vs. the reciprocal square root of the ionic strength ($\mu^{-1/2}$)

| Sample | $[\eta]_\infty$ (dl/g) | S (dl $M^{1/2}$/g) |
|---|---|---|
| Ch | 4.88 | 1.170 |
| $S_1$Ch | 4.45 | 1.117 |
| $S_2$Ch | 4.07 | 1.064 |
| $S_3$Ch | 3.45 | 0.919 |
| $S_4$Ch | 3.20 | 0.672 |
| TMCh | 1.79 | 0.573 |
| $S_1$TMCh | 1.53 | 0.558 |
| $S_2$TMCh | 1.45 | 0.448 |
| $S_3$TMCh | 1.26 | 0.387 |



above, it was expected that the TMCh chain should be less stiff than that of chitosan. On the other hand, considering that the introduction of methyl substituents on the hydroxyl groups may restrict the chain rotation for they having a greater volume than the hydrogen atoms of the H-O and $H_2N$ groups, one can postulate a compensation between the steric freedom resulting from the loss of capacity to hydrogen bonding mentioned above and the steric restriction due to the presence of voluminous methyl groups. However, no experimental data are thus far available to support this hypothesis.

Despite the apparent lack of sensitivity of the stiffness parameter to the different structural features of chitosan and N,N,N-trimethylchitosan, it should be remembered that B

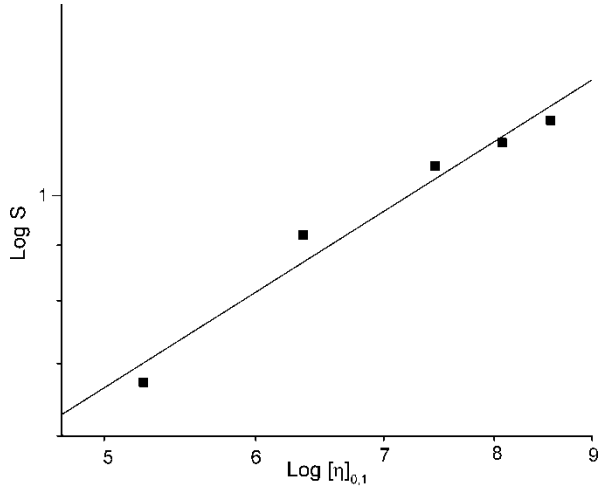


**Figure 10.** Plot of the logarithm of the salt tolerance factor, log S, vs. th logarithm of the intrinsic viscosity at 0.1M NaCl, log $[\eta]_{0.1}$, for the chitosan chloride.

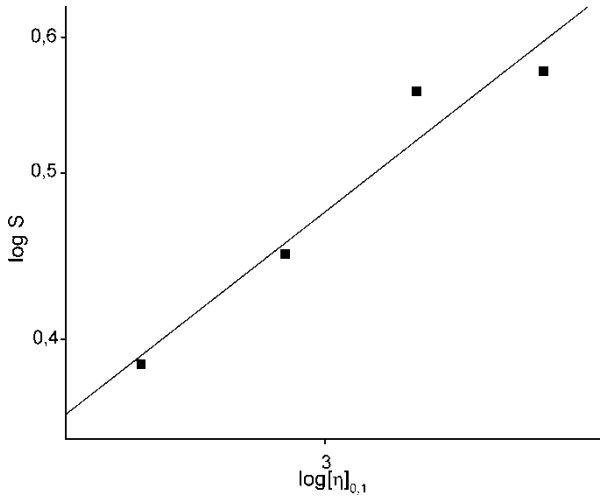


**Figure 11.** Plot of the logarithm of the salt tolerance factor, log S, vs. the logarithm of the intrinsic viscosity at 0.1M NaCl, log $[\eta]_{0.1}$, for the N,N,N-trimethylchitosan chloride.



**Table 5**
Average degrees of acetylation and quaternization of chitosan (Ch) and N,N,N-trimethylchitosan (TMCh) and corresponding values of the stiffness parameter (B)

| Sample | % $\overline{GQ}$ | % $\overline{GA}$ | B | $\nu$ |
|---|---|---|---|---|
| Ch | — | 18.2 | 0.112 | 1.100 |
| TMCh | 50.4 | 14.5 | 0.125 | 1.217 |

must be seen as a preliminary evaluation of the polymer stiffness. Hence, methods better founded from a theoretical point of view, such as those proposed by Yamakawa (32) and Odijk (34), should be employed to allow the determination of the stiffness of chitosan and its derivatives, including N,N,N-trimethylchitosan, and the influence of the average degree of acetylation and other structural features on it. An important contribution in better understanding the dependence of the chain stiffness of chitosan-like polymers on its structural features should be produced from light scattering measurements.

## Conclusions

The preparation of chitosan and N,N,N-trimethylchitosan as water-soluble chloride salts allowed the study and comparison of the viscosity behavior of these polymers in acid-free aqueous solutions of NaCl. The ultrasound treatment allowed the preparation of depolymerized samples, but neither the concomitant deacetylation nor demethylation of chitosan and N,N,N-trimethylchitosan were observed. This study showed that both polymers display typical polyelectrolyte behavior, exhibiting a linear decrease of the intrinsic viscosity as a function of the reciprocal square root of the ionic strength. Also,

these polymers have similar values of the stiffness parameter, leading to the conclusion that their chains are equally stiff, the occurrence of O- and N-methylation and some deacetylation upon the methylation of chitosan do not resulting in different chain stiffness as evaluated by the Smidsrod's approach.

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