Viscosimetric studies of chitosan nitrate and chitosan chlorhydrate in acid free NaCl aqueous solution

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Abstract: Two salts of the biopolymer chitosan were prepared in aqueous medium by employing an excess of HCl or HNO_3 in order to ensure neutralization of all NH_2-chitosan groups. Chitosan salts were extensively dialyzed in dionised water and dried at 40 ºC until film formation. The films were characterized by thermogravimetry, FTIR and conductimetric tritration. QH^+Cl^- and $QH^+NO_3^-$ salts were viscosimetrically evaluated in free acid aqueous solutions in the presence of NaCl to control ionic strength of the medium. Unexpected high intrinsic viscosity values were obtained at low ionic strength when $QH^+NO_3^-$ salt were evaluated. Smidsrod´s approach was employed to estimate the stiffness parameter of both salts and B = 0.084 and 0.120 for QH^+Cl^- and $QH^+NO_3^-$, respectively, were obtained.

Introduction

In recent years considerable attention has been paid to chitosan due to the wide range of new and exciting applications that have been found for this material. It has been employed as flocculant agent for water treatment [1], as bactericide and fungicide in agriculture [2], as preservative in alimentary formulations [3], etc. Additionally, chitosan has demonstrated to be a promising material for biomedical applications such as controlled drug release [4], enzyme immobilization for biosensor preparation [5], gene therapy [6], etc. Most of these applications are related to their polyelectrolyte properties, which arise from the positive charges that can develop in the macromolecules due to protonation of amine groups in acid medium, as it is shown in the following scheme:
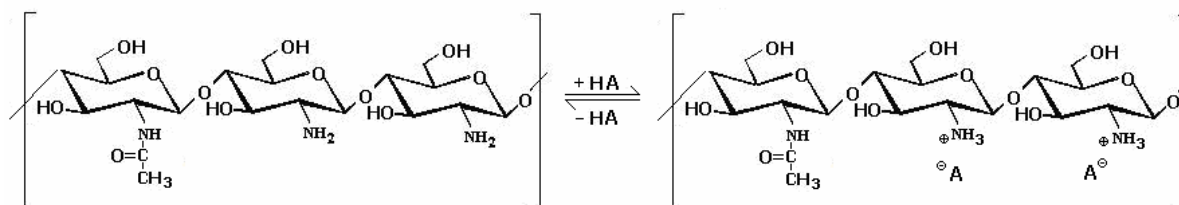
Charged moieties on the polymer chains originate from diverse conformational arrangements which contribute to the modeling of chitosan polyelectrolyte behavior. Conformational arrangements of polyelectrolyte chains in aqueous solution are controlled by temperature [7], pH [8], ionic strength [9], molecular weight [10], fraction of charged moiety [11], polyelectrolyte concentration [12], counterion nature [13], etc.

When polyelectrolyte chitosan is considered, it is well known that intrinsic viscosity [η] values decrease when solution temperature is increased, which could be attributed to a decrease of the radius of gyration/average molecular weight ratio, resulting in a higher chain flexibility and compactness of the molecule which, in turn, causes a decrease in the intrinsic viscosity [14]. However, this behavior can also be explained by a reduction of the hydrogen-bonded hydration water carried by polymer, which leads to the decrease of the specific volume [15].

On the other hand, pH effects on chitosan chain conformational arrangements in aqueous medium are easily understood due to the existence of the acid/base equilibrium $\sim NH_2 + H^+ \leftrightarrow \sim NH_3^+$, which is responsible for the charge extension on the polyelectrolyte chains [8]. Similarly, ionic strength (μ) can modulate conformational arrangements of the polyelectrolyte chains [9] depending on the screening that could induce small external electrolytes on charged moiety carried by polyelectrolyte, i.e., swelled hydrogels immersed in a high ionic strength medium usually collapse due to the additional ions causing a non perfect charge–charge electrostatic repulsion, leading to a decreased osmotic pressure difference between the hydrogel network and the external solution.

Polymer chain expansion is usually insignificant when materials with around two orders of magnitude difference in molecular weight are studied. However, as the molecular weight difference range expands polymer conformation could change, leading to a molecular weight-induced conformational transition. Chitosan conformational transition has been demonstrated to occur at 223 kDa [10].

Counterion nature is a determining factor on the conformational accommodations of the polyelectrolyte chains. Depending on the anionic counterions charge number, chitosan systems can show a completely different behavior, i.e., ionotropic gelation can occur by using multivalent anions but it usually does not occur with monovalent ions. This phenomenon has been extensively documented and some multivalent anions are well known to be good ionotropic-gelling agent for chitosan [16]. Similarly, chitosan-univalent counterion salt aqueous solution properties strongly depend on counterions size [17, 18].

In this work, the influence of ionic strength (μ) on the intrinsic viscosity [η] of two chitosan salts, chitosan hydrochloride (QH^+Cl^-) and chitosan nitrate ($QH^+NO_3^-$), is discussed. Additionally, Smisrdod´s aproach [19] was used to estimate the stiffness parameter (B), persistence length (a) and salt tolerance (S) of chitosan salts in acid-free NaCl aqueous medium.

Results and discussion

Materials characterization

Figure 1 shows FTIR spectra of both starting chitosan (KBr pellet) and chitosan salt (films). Chitosan and QH^+Cl^- spectra are very similar, even so QH^+Cl^- spectrum was better resolved due to higher transparency of film compared to KBr-chitosan pellet. As expected, no additional signals are observed coming from the chloride ion. On the other hand, two signals corresponding to nitrate anion at 1327 cm^{-1} (wide and strong) and 823 cm^{-1} (sharp and medium) can be clearly observed in the spectrum of $QH^+NO_3^-$ salt. FTIR spectra and conductimetric tritrations coming from both salts ensure purity of substances studied. TGA analysis provided humidity content for all samples ($\sim$ 5,5 % w/w) in order to correct the concentration of solutions.
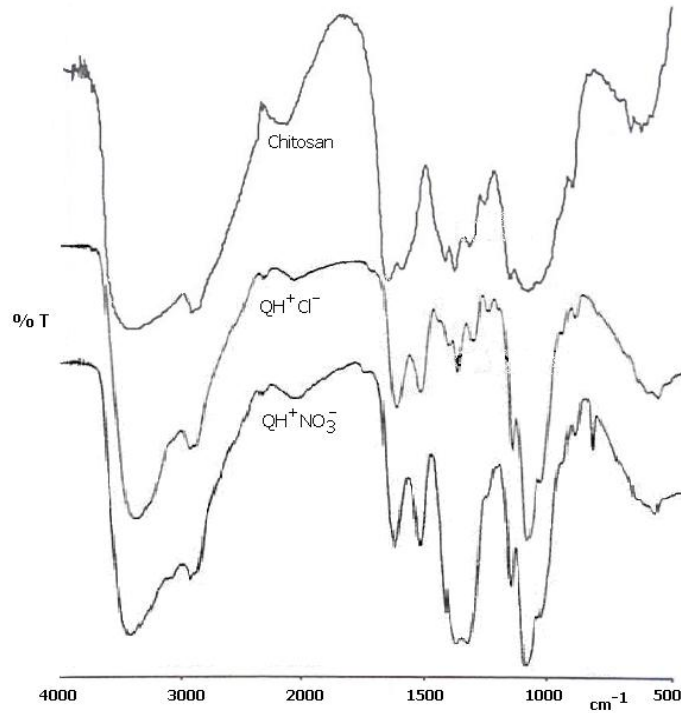
Fig. 1. FTIR spectra of chitosan (KBr) and chitosan salt films.

Viscosimetric studies

Figure 2 shows plots of η_{red} vs. c_p for QH^+Cl^- and $QH^+NO_3^-$ at different ionic strength solutions. Both systems show lower $[\eta]$ values as the ionic strength of solution increase, in accordance to the higher screening effect exercised by the external salt. Acceptable intrinsic viscosity values with good lineal correlation coefficients were obtained for the studied solutions.

This figure also shows that η_{red} values for $QH^+NO_3^-$ are higher than those obtained for QH^+Cl^- at lower ionic strength ($\mu = 0.05$), then they become very similar at $\mu = 0.20$ and, finally, lower when ionic strength is further increased ($\mu = 0.30$ and 0.60). This behavior clearly indicates that $QH^+NO_3^-$ is markedly more affected by the increasing ionic strength of the medium.

Figure 3 shows comparative plots for $[\eta]$ vs. $\mu^{-1/2}$ for both chitosan-salts, from which the intrinsic viscosity at infinite ionic strength ($[\eta]^\infty$) and salt tolerance (S) were estimated (from the intercept and the slope, respectively), according to Fixman theory. $[\eta]$ is usually expressed as [19]:

$$[\eta] = [\eta]^\infty + S.\mu^{1/2} \tag{1}$$

Interpolating at $\mu = 0.1$ M it is possible to obtain the intrinsic viscosity at this ionic strength ($[\eta]_{0.1}$), which can be used to calculate the stiffness parameter defined by Smidsrod, according to:

$$B = S/([\eta]_{0.1})^\nu \tag{2}$$

where the empirical exponent ν usually assumes a numeric average value of 1.3, which has been experimentally determined from a large series of polyelectrolyte [19]. However, it is important to remark that this average value of ν was obtained using

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experimental results that oscillate between 1.2 and 1.4, depending on the polyelectrolyte studied.
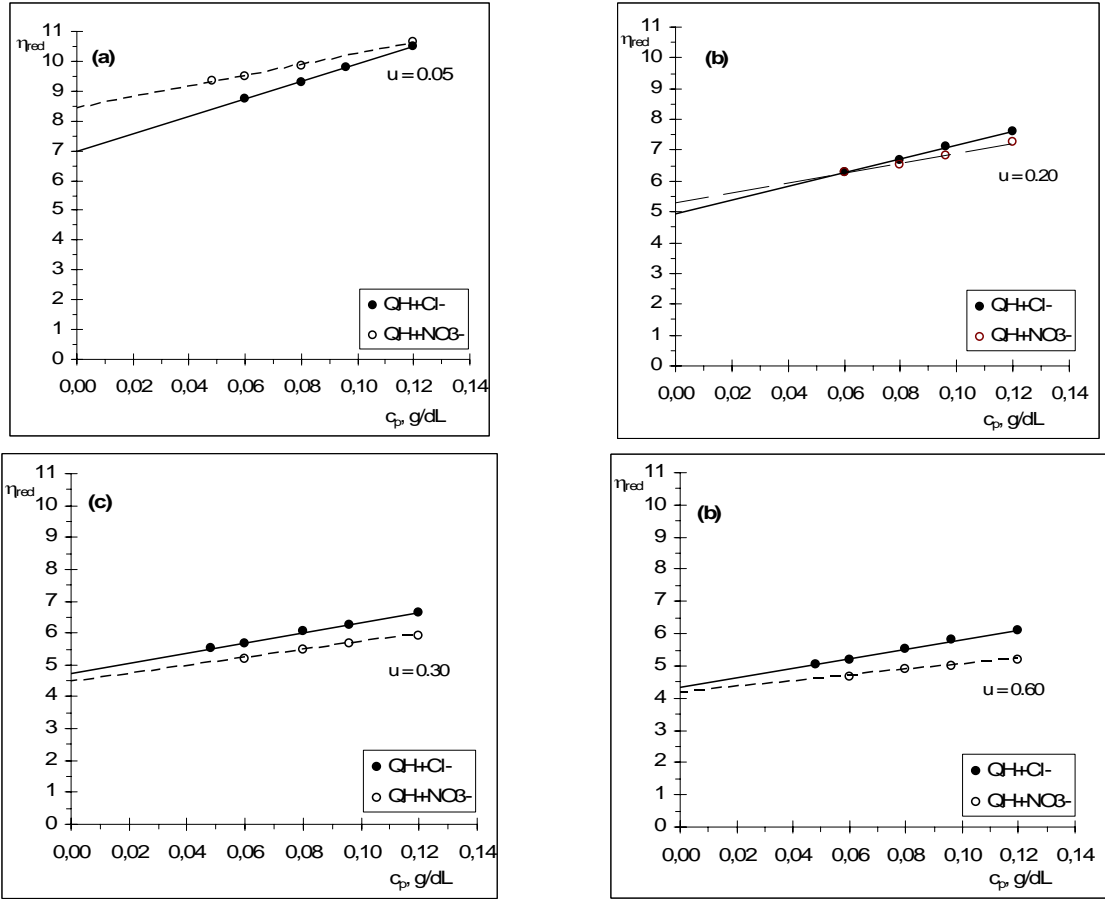

Fig. 2. η_{red} responses for QH^+ Cl^- and QH^+ NO_3^- vs. chitosan concentration at diverse ionic strength (NaCl). T = 25.0 ± 0.1 °C.
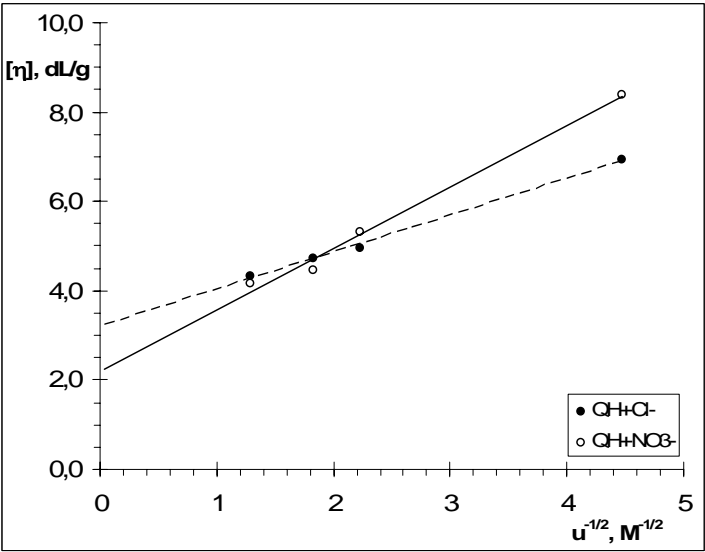

Fig. 3. Observed behavior for intrinsic viscosity of chitosan salts as function of $\mu^{1/2}$ (NaCl).

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Tab. 1. Comparative values of stiffness parameter, persistence length and salt tolerance estimate through Smidsrod´s approach for studied chitosan salts alongwith some reported values.

Chitosan salt	Medium	% GA	B	a (Å)	$[\eta]^\infty$ (dl.g^{-1})	S (dl.M$^{1/2}$.g^{-1})	Ref.
QH^+Cl^-	NaCl	18.2	0.112	93*		1.17	[20]
QH^+Cl^-	NaCl	22.6	0.06	173*			[21]
QH^+Cl^-	Sodium acetate/ Acetic acid	21	0.208*	50			[9]
QH^+Cl^-	NaCl	17	0.10	104*		0.38	[18]
$QH^+CH_3COO^-$	NaCl	17	0.064	163*		0.4	[18]
QH^+Cl^-	NaCl	15	0.084	116	3.21	0.83	This work
$QH^+NO_3^-$	NaCl	15	0.120	83	2.18	1.38	This work

* Values estimated using equation: a = 10.4/B and the corresponding values from reported data.

Table 1 shows the estimate values for B and S through Smidsrod´s approach for both chitosan salts which agrees with previous reports [18, 20, 21]. QH^+Cl^- salt has smaller B value than $QH^+NO_3^-$ (i.e., higher rigidity of the polyelectrolyte chain imply higher persistence length) as expected from size considerations for both counterions. As NO_3^- ion has a bigger size than Cl^- ion, it should produce a higher screening effect on positive charges carried by the polyelectrolyte. Thus, the polyelectrolyte should show a minor chain expansion, as it is reflected by the persistence length values shown in Table 1. $[\eta]^\infty$ values (Table 1) are indicative that at infinite ionic strength the polyelectrolyte chain will be more flexible at $QH^+NO_3^-$ than QH^+Cl^-. It could be explained considering that, under these experimental conditions, NO_3^- counterion is not completely exchanged by Cl^- counterion coming from NaCl employed as external salt, and higher screening caused by NO_3^- remainder counterions (neighbors to the polyelectrolyte) creates a less expanded and compact chitosan chain. Analogously, higher $[\eta]^\infty$ value founded for QH^+Cl^- can be explained by smaller screening produced by the minor sized Cl^- counterion which, in turn, causes a bigger chitosan chain expansion.

On the other hand, $[\eta]$ values obtained at lower ionic strength solutions are more difficult to explain because it should not be expected that NO_3^- counterions produce smaller screening of charged polyelectrolyte chains than those caused by Cl^- counterions. The differences between our B values and those from literature may be attributed to the diverse properties of the employed samples (molecular weight, deacetylation degrees, etc.), while the differences in B values from the studied salts in this work (which come from the same chitosan sample) would be attributed to the ion size differences as previously explained.

However, these different B values may also arise because an unique ν value (ν = 1,3) was used to calculate them. It is possible to obtain a unique B value for the

polyelectrolyte chain when a distinct ν value is employed for each salt, considering that Flory-Huggins parameter of interaction (χ) may be different for each system due to non similar thermodynamic compatibility between the two distinct polyelectrolyte and same external solution. Thus, if this consideration is made in the B calculations, values reported in Table 2 are obtained. As it can be seen, similar B values for both chitosan salts (~0.100) are obtained using ν values of 1,2 and 1,4 for QH^+Cl^- and $QH^+NO_3^-$, respectively.

Tab. 2. Calculation of B values of chitosan salts considering different exponential factor ν on Smidsrod´s approach.

Chitosan salt	ν	B
$QH^+ Cl^-$	1.2	→ 0.100
$QH^+ NO_3^{--}$	1.2	0.145
$QH^+ Cl^-$	1.3	0.084
$QH^+ NO_3^-$	1.3	0.120
$QH^+ Cl^-$	1.4	0.070
$QH^+ NO_3^-$	1.4	→ 0.099

However, it is convenient to note that equilibrium constants (K) in aqueous medium (without any salt added) have been recently reported to be 5.9×10^{-5} and 9.7×10^{-5} (25 ºC) for QH^+Cl^- and $QH^+NO_3^-$, respectively [22]. These values may indicate that at low ionic strength solutions there are a higher dissociated fraction of NO_3^- ions than Cl^- ions, in spite of having a bigger size than Cl^- ions, exercising a smaller screening effect on the charges carried by the polyelectrolyte.

Conclusions

Behavior of two chitosan salts, $QH^+NO_3^-$ and QH^+Cl^-, was studied in acid free NaCl aqueous solution in order to correlate conformational arrangements of the polyelectrolyte chain to the counterion size. According to B values obtained using Smidsrod´s approach, NO_3^- counterion causes a smaller stiffness than Cl^- counterion, which has been attributed to higher screening caused by the bigger sized NO_3^- ion. In order to explain the relative behavior of $QH^+NO_3^-$ salt, which produces unexpectedly high [η] values at low ionic strength, two proposals have been suggested: (a) at low ionic strength the higher equilibrium constant for $QH^+NO_3^-$ creates a higher chain expansion for $QH^+NO_3^-$ than QH^+Cl^- because of increased fraction of NO_3^- counterions and (b) ν can assume different values on Smidsrod´s equation, depending on the counterion considered. In order to clarify this situation, new studies using different external salts and counterions are conducted at present in our laboratory.

Experimental part

Chitosan was purchased from Fluka BioChemika (MW ~ 400.000). Acetylation degree (DA = 0.162) was determined by ^{1}H-NMR spectroscopy (Bruker, DRX 400 MHz) using D_2O as solvent in the presence of a small quantity of trifluoroacetic acid.

Chitosan salts were prepared as follows: (a) 3.0 g of chitosan was placed in 125 ml deionized water (Millipore Milli-Q^{tm}) and a small stoichiometric excess of HCl (Riedel of Haen, 37%) or HNO_3 (Riedel of Haen, 65%) was added. The resulting suspension was magnetically stirred until chitosan was completely dissolved. (b) The resultant solutions were dialyzed against deionized water until conductivity values of the external solution remained in similar levels to those of the used water, (c) dialyzed solutions were placed to dry off at 40 °C to obtain films, and kept in a dessicator until use. Visking tubes (The Scientific Instrument Centre) used during polyelectrolyte dialyzing were previously treated by boiling in deionized water (3 times), 1% EDTA aqueous solution (2 times), 1% $NaHCO_3$ aqueous solution (2 times), deionized water (1 time), 1% ethanol aqueous solution (1 time) and deionized water (3 times).

Thermogravimetric analysis (Perkin Elmer TGA-7) was performed in order to establish humidity content in the samples. Acid-base and conductimetric tritration of polyelectrolyte samples with normalized NaOH confirmed that there are no free acid moieties. FTIR spectra were recorded using a FTIR Perkin Elmer 2000 spectrometer. Viscosimetric studies were carried out at 25.0 ± 0.1 °C in a Gallenkamp thermostated bath using an Ubbelohde viscosimeter CANNON 50 E979. Measures were carried out at determined ionic strength starting with the more concentrated solution and then adding the required volume of solvent to obtain desired concentrations. All average η_{red} values were obtained by repeating each measurement five times.

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