Polymer Bulletin 55, 201–208 (2005)
DOI 10.1007/s00289-005-0418-x

Polymer Bulletin

Conductimetric studies of chitosan in aqueous medium

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Received: 1 March 2005 / Revised version: 9 June 2005 / Accepted: 20 June 2005

Summary

Conductimetric studies of two aqueous chitosan salts systems are presented. These salts were prepared dissolving the polyelectrolyte with desionised water and using an excess of the corresponding stoichiometric amount of acid (HCl or HNO_3) and then purified by dialysis. Limit equivalent conductance (Λ^o) was obtained by fitting the experimental conductimetric results to a fifth order polynomial function. This result was employed to determine classical conductimetric parameters in dilution limiting conditions, i.e., charge parameter (ξ), polyelectrolyte equivalent ionic conductivity (λ_p^o), fractional hydrodynamic coefficient (f), etc., employing Kohlrausch law for independent ions migration. We have found a remarkable agreement between experimental and theoretical results when Rinaudo equation was employed to calculate ξ. Additionally, our conductimetric studies show that equilibrium constants are similar for both systems when K values are obtained from plots of $\Lambda^2/[\Lambda^o(\Lambda^o - \Lambda)]$ *vs.* $1/c_p$.

Introduction

Chitosan has become quickly a very topical subject due to its bionatural origin and polyelectrolyte behavior which open a wide range of uses and applications **[1-3]**. However, despite the interest in this material, studies on chitosan polyelectrolyte properties are scarce. Most of the research effort has been focused towards obtaining either complexes with surfactants **[4]** or other types of polyelectrolytes (like interpolymeric complexes) **[5]**, especially to produce materials designed for encapsulation and/or release of substances. Even so, not much information has been published related to either polyelectrolyte behavior in solution or about their conductimetric properties **[6]**.

In order to investigate chitosan polyelectrolyte behavior it is necessary to take into account the acetylation degree (DA) of the samples, since this is directly related to the charges on the polyelectrolyte. Additionally, in samples with similar DA, the distribution of the acetylated groups along the polymer chain should produce an important effect over its polyelectrolyte behavior, as occurs with lysozyme degradation **[7]**, although at the present time there are not reports on this topic.

Line charge density parameter (ξ) is important to define the polyelectrolyte behavior of a material since it represents the density of lineal charge on the polymer. This is an adimensional parameter that can be obtained according to the following relationship:

$$\xi = l_B/b \qquad \mathbf{(1)}$$

where l_B is the Bjerrum distance and represents the distance at which two charges interact with energy k_BT (k_B = Boltzmann constant; T = absolute system temperature) and b is the mean distance between two consecutive charges on the polyelectrolyte.

In the case of polyelectrolytes with monovalent groups ξ has traditionally been obtained through following procedure: (a) Λ^o values are obtained from conductimetric measurements for two polyelectrolyte salts with different counterions (b) determination of the frictional hydrodynamical coefficient at limiting conditions (f^o) by means of the following equation:

$$f^o = (\Lambda_2^o - \Lambda_1^o)/(\lambda_{a2}^o - \lambda_{a1}^o) \qquad \mathbf{(2)}$$

where λ_{a2}^o and λ_{a1}^o are the ionic limit conductances of the counterions, which are reported in the literature, (c) ξ is calculated from the equations proposed by Manning for a long charged cylinder **[8]**:

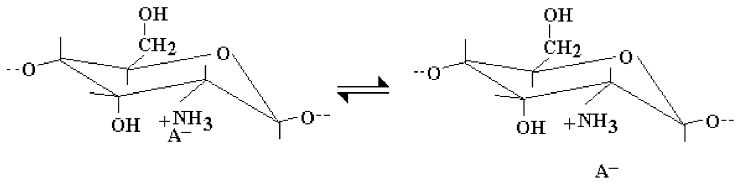
$$f = 1 - 0.55\xi^2/(\pi + \xi) \qquad (\xi < 1) \qquad \mathbf{(3a)}$$

$$f = 0.866/\xi \qquad (\xi \geq 1) \qquad \mathbf{(3b)}$$

On the other hand, in accordance with Rinaudo *et al* **[9]**, chitosan line charge density parameter can be calculated of the following equation:

$$\xi = 1.38(1 - \mathrm{DA})\alpha \qquad \mathbf{(4)}$$

where α is the polyelectrolyte dissociation degree in solution and represents the charged groups fraction on the polymer chain that are dissociated according to the following reaction:



This definition requires supposing that: (a) all the amino groups in the chain have been neutralized (i.e. there are not free $-NH_2$ groups) and (b) hydrolysis reaction of NH_3^+ groups does not occur to significant extension due to strong acids used in this study **[10]**.

In this work we present a conductimetric study for two aqueous systems based on chitosan, which has been neutralized with HCl and HNO_3 to produce the polyelectrolyte salts, denoted QH^+Cl^- and $QH^+NO_3^-$, respectively. All parameters obtained from experimental data, using the previously described procedure, are compared with those calculated through Manning´s equations using a ξ value obtained from equation 4 proposed by Rinaudo (denoted ξ_R).

Experimental

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

Samples for the conductimetric analysis were treated in the following way: (a) a weighed quantity of polymer is placed in desionised water (Millipore Milli-Q^{tm}) and a small excess of HCl (Riedel of Haen, 37%) or HNO_3 (Riedel of Haen, 65%) is added (b) the resultant solutions are dialyzed in desionised water until conductivity values of the external solution stay in similar levels to those of the used water, (c) dialyzed solutions are placed to dry off at 50 ºC to obtain films, and kept in a dissicator until use. Visking tubes (The Scientific Instrument Centre) used during polyelectrolyte dialyzing were previously treated by boiling in: desionised water (3 times), 1% EDTA aqueous solution (2 times), 1% $NaHCO_3$ aqueous solution (2 times), desionised water (1 time), 1% ethanol aqueous solution (1 time) and desionised water (3 times).

The conductimetric measurements were carried out with a Hanna Instruments conductimeter (model 8033). In a typical procedure 20 ml of an aqueous solution of the polyelectrolyte salt is placed in a graduate cylinder of 100 ml immersed in a water bath at 25 ºC, then the conductivities of the diluted solutions, generated by addition of known volumes of desionised water, are measured. Concentration of polyelectrolyte solutions is obtained considering samples moisture contents. Thermogravimetric analysis (Perkin Elmer TGA-7) showed that the samples usually contain ~5,5 % of humidity. Acid-base tritration of polyelectrolyte samples with normalized NaOH confirms that there are not free acid moieties.

Viscosimetric measurements were hcarried out at 25 ºC in a Gallenkamp thermostated bath using an Ubbelohde viscosimeter CANNON 50 E979.

Results and discussion

Λ vs. $c^{1/2}$ experimental curves for QH^+Cl^- and $QH^+NO_3^-$ systems are shown in figure 1. The Λ values were obtained subtracting water conductivity (0,9 µS/cm) to solution conductivities. Fifth order polynomial curves (continuous and dashed lines, respectively) show an excellent fit with experimental points. Λ^o values ($\Lambda_{QHCl}{}^o$ = 78.95 S.cm^2/eq, R^2 = 0.991; $\Lambda_{QHNO_3}{}^o$ = 75.27 S.cm^2/eq, R^2 = 0.997) are obtained from intercepts with y axe. Table 1 summarizes results obtained using ξ_R value (subscript R) and those calculated employing ξ_{exp}, which was obtained through the classical procedure previously described (subscript exp). As it can be seen from table 1, ξ_R and ξ_{exp} values are very similar (2 % difference), indicating the consistence of the experimental data. It is important to highlight that $\lambda_p{}^o{}_{exp}$ value is in accordance with that reported for a chitosan with DA = 0.20 **[6]**. This parameter has the same order of magnitude that small electrolytes, which could also imply that the mobility of the polyelectrolyte should be defined by the mobility of the charged segments in the chain i.e., monomer theory, which could be applied to a coiled polyion under conditions where cylindrical symmetry is operational **[11]**.

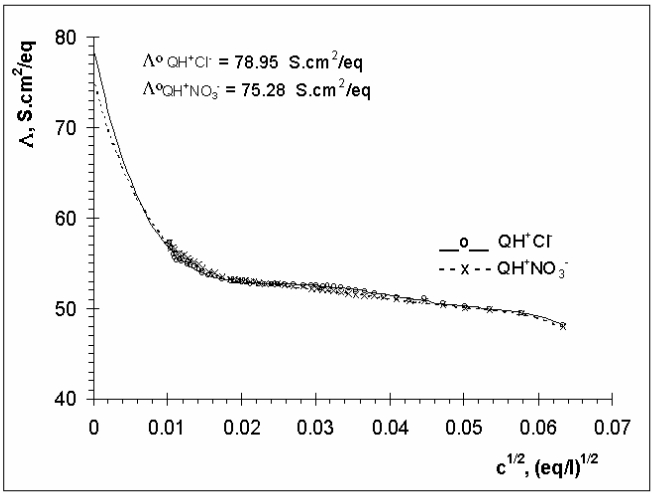


Figure 1.- Plots of Λ vs. $c^{1/2}$ for QH^+Cl^- and $QH^+NO_3^-$ systems. T = 25 °C.

Table 1.- Results obtained in the conductimetric studies for aqueous QH^+Cl^- and $QH^+NO_3^-$ systems. T = 25 °C.

System	$\Lambda^o{}_{exp}$ (S.cm^2/eq)	$\lambda_a{}^o$ (S.cm^2/eq)	ξ_R	f_R	$\lambda_p{}^o{}_R$ (S.cm^2/eq)	f_{exp}	ξ_{exp}	$\lambda_p{}^o{}_{exp}$ (S.cm^2/eq)
QH^+Cl^-	78.95	76.3	1.156	0.749	29.11	0.768	1.128	26.50
$QH^+NO_3^-$	75.27	71.5	1.156	0.749	28.99	0.768	1.128	26.50

On the other hand, theoretical polyelectrolyte ionic equivalent conductivity values ($\lambda_{p\ th}$) have also been calculated as function of concentration for the $QH^+NO_3^-$ system according to Manning equation:

$$\lambda_p = 0{,}866H\lambda_c{}^o|\ln(\kappa_D' a)|/(|z_c|\lambda_c{}^o + 0{,}134H|\ln(\kappa_D' a)|) \qquad \textbf{(5)}$$

where H is a standard electrophoretic factor defined as $H = 4\varepsilon\varepsilon_o RT/3\eta$; η is the dynamic viscosity of solvent, R the gas constant, ε the dielectric constant of medium, ε_o is the permissivity in vacuum, κ_D' the Debye screening constant (defined as $\kappa_D'^2 = F^2c_p/(\varepsilon\varepsilon_o RT\xi)$), *a* the polyelectrolyte radius and c_p is the polyelectrolyte concentration.

Figure 2 shows plots of Λ_{exp}, $\lambda_{p\ th}$ and λ_{ac} *vs* $c^{1/2}$ curves (subscripts refer to experimental (exp), theoretical (th) and calculated for $QH^+NO_3^-$ anion (ac)). $\lambda_{p\ th}$ values were calculated employing η = 0,000891 kg.m^{-1}s^{-1}, *a* = 6 Å (approximate distance between charged groups on polyelectrolyte chain) and T = 25°C data; ξ_R values were determined for each concentration. The λ_{ac} values have been obtained subtracting Λ_{exp}/f - $\lambda_{p\ th}$; f values were obtained using the appropriate Manning´s equation (3a or 3b equation) and ξ_R.

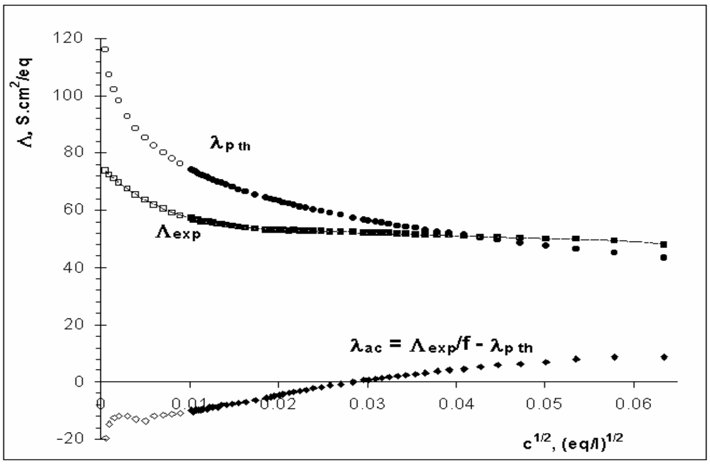


Figure 2.- Plots of Λ_{exp}, $\lambda_{p\ th}$ and λ_{ac} *vs* $c^{1/2}$ for $QH^{+}NO_3^{-}$ system. Bold points correspond to experimental data; open points correspond to extrapolated values using the fifth order polynomial curve that fit experimental data (continuous line).

As it can be seen from figure 2, $\lambda_{p\ th}$ and λ_{ac} results are very far from reasonable values, considering that: (a) at low concentration $\lambda_{p\ th}$ values are higher than Λ_{exp} and (b) λ_{ac} values are much smaller than those known for the NO_3^{-} ion at limiting conditions and even become negative for some concentrations.

On the other hand, $\lambda_{p\ th}$ values could be too high due to the drastic changes occurring in the viscosity, and probably in the dielectric constant, of the medium when concentration is increased, which have not been considered. In order to obtain viscosity corrected $\lambda_{p\ th}$ values, viscosimetric studies of the polyelectrolyte solutions were carried out (figure 3). Experimental η values have been used to correct the electrophoretic factor H in equation 5 and obtain a new set of $\lambda_{p\ th}$ and λ_{ac} values, which are denoted as $\lambda_{p\ thc}$ and λ_{acc}, respectively. Results are shown in the figure 4.

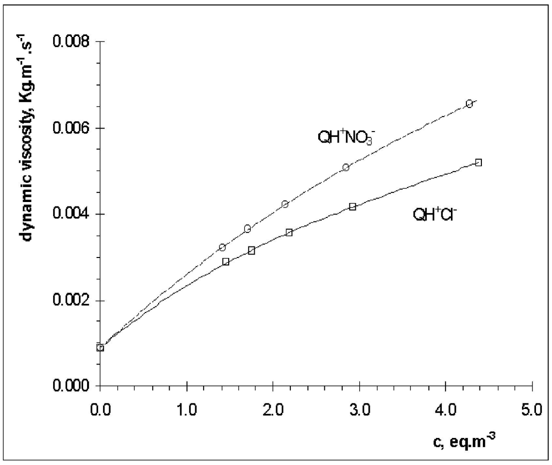


Figure 3.- Plots of the dynamic viscosity *vs.* c for $QH^{+}Cl^{-}$ and $QH^{+}NO_3^{-}$ system. T = 25°C.

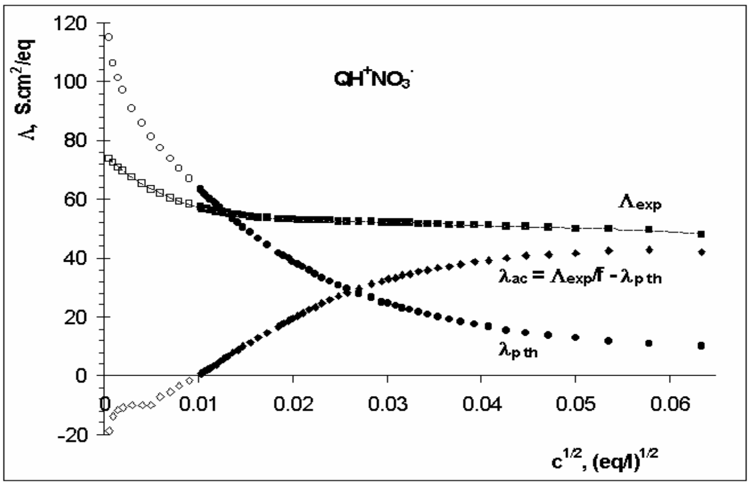


Figure 4.- Plots of Λ_{exp}, $\lambda_{p\,thc}$ and λ_{acc} *vs* $c^{1/2}$ for $QH^+NO_3^-$ system. Bold points correspond to experimental data; open points correspond to extrapolated values using the fifth order polynomial curve that fit experimental data (continuous line).

An improvement in $\lambda_{p\ thc}$ values was achieved when corrections were applied. However, at low concentrations, $\lambda_{p\ thc}$ is still higher than the reasonable values, causing that λ_{acc} values remain low. We believe that further experimental evaluation of the dielectric constant of the medium, when the polyelectrolyte concentration is varied, should be carried out to achieve a better adjustment of $\lambda_{p\,thc}$ values.

On the other hand, we have noted that $\lambda_{p\ thc}$ and λ_{acc} values could be improved when the electrophoretic factor H is adjusted using constant values for η. Figure 5 shows the results (renamed as $\lambda_{p\,thc1}$ and λ_{acc1}) when a value of η = 0,005 Kg.m^{-1}s^{-1} was used.

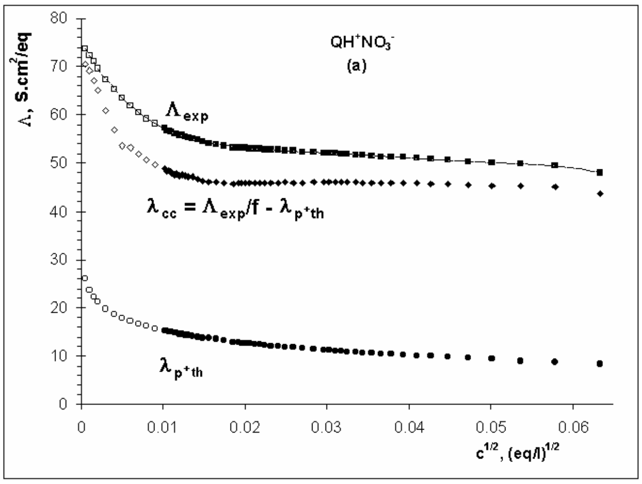


Figure 5.- Plots of Λ_{exp}, $\lambda_{p\ thc1}$ and λ_{acc1} *vs.* $c^{1/2}$ for $QH^+NO_3^-$ system. Bold points correspond to experimental data; open points correspond to extrapolated values using the fifth order polynomial curve that fit experimental data (continuous line).

Additionally, similar results from figure 5 appears when simulation was carried out employing $\eta = 0{,}000891$ kg.$m^{-1}s^{-1}$ and dielectric constant = 15. It is clear that to obtain a closer result to real behavior of the system, eq. 5 should consider simultaneous medium changes in viscosity and dielectric constant.

We are also interested in evaluating the equilibrium constant in both systems. A simple equilibrium has been considered:

$$QH^+A^- \leftrightarrow QH^+ + A^- \qquad \textbf{(6)}$$

If c_p is the initial polyelectrolyte concentration and x the quantity dissociated, the equilibrium constant will be defined as:

$$K = [QH^+]_{eq}[A^-]_{eq}/[QH^+A^-]_{eq} = x^2/(c_p - x) \qquad \textbf{(7)}$$

By definition the dissociation degree (α) is:

$$\alpha = x/c_p \qquad \textbf{(8)}$$

This has traditionally been determined from conductimetric measures according to:

$$\alpha = \Lambda/\Lambda^o \qquad \textbf{(9)}$$

Combining equations 8 and 9, x is obtained:

$$x = c_o\Lambda/\Lambda^o \qquad \textbf{(10)}$$

A new expression is obtained by substituting this result in equation 7:

$$\Lambda^2/[\Lambda^o(\Lambda^o - \Lambda)] = K/c_p \qquad \textbf{(11)}$$

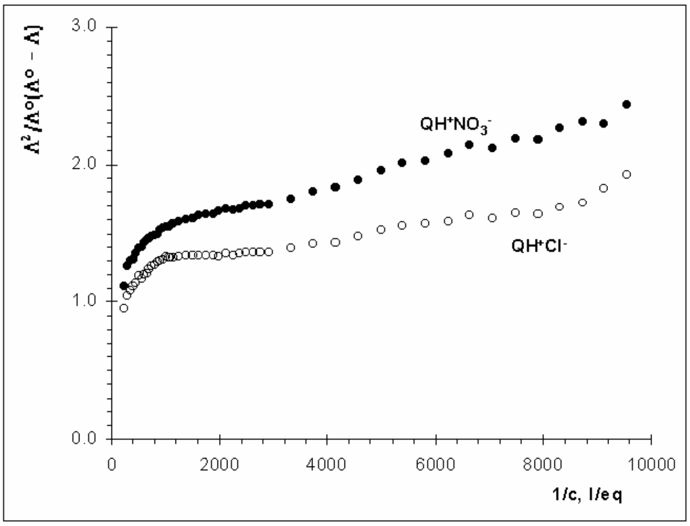


Figure 6.- Plots of $\Lambda^2/[\Lambda^o(\Lambda^o - \Lambda)]$ *vs.* $1/c_p$ for QH^+Cl^- and $QH^+NO_3^-$ system. T = 25ºC.

Thus, the equilibrium constant should be obtained from the slope of a plot of $\Lambda^2/[\Lambda^o(\Lambda^o - \Lambda)]$ *vs.* $1/c_p$, as it is predicted by equation 11. Figure 6 shows the curves obtained with the experimental data for the two studied systems. As it can be appreciated, in both cases, a remarkable linear region was obtained, from which the equilibrium constant for each system (K_{QHCl} ~ 5,9x10^{-5}; K_{QHNO_3} = 9,7x10^{-5}) was estimated.

Conclusions

Conductimetric results obtained for aqueous chitosan/HCl and chitosan/HNO_3 systems confirm that the polyelectrolyte limit ionic equivalent conductance is practically independent of the type of counterions here studied, i.e. these polyelectrolyte systems also obey the law of independent ion migration. The experimental value obtained for this parameter (λ_p^o ~ 26,5 S.cm^2/eq) is in accordance with that reported for chitosan with similar acetylation degree and posses the same order of magnitude that small electrolytes, which suggest a highly cooperative motion of the charged segments during polymer chain migration. We have also confirmed from our experimental results that the relationship proposed by Rinaudo for chitosan charge parameter as function of the acetylating degree is obeyed when the dissociation degree is one (infinite dilution limit).

The equilibrium constants obtained for the two studied systems were very close, which could be indicative that only the electrostatic polyelectrolyte/counterions interaction is important.

Acknowledgment. JSA thanks the received support from the CDCHT-ULA through the thesis project C-1234-04-08-F.

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