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Solubilization of Chitosan in Strong Acid Medium*

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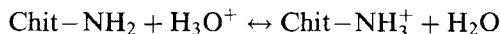
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This paper describes the equilibrium reaction of chitosan in the presence of a strong acid, HCl; the degree of protonation is determined as a function of the initial concentration of acid in which chitosan is dispersed. The solubilization of chitosan is obtained for a minimum degree of protonation $\alpha = 0.5$ at a stoichiometric ratio $[\text{HCl}]/[\text{Chit-NH}_2] \approx 0.5-0.6$. The degree of protonation is determined by potentiometry and conductimetry. The conductivity of an acidic solution decreases in the presence of chitosan; it passes through a minimum at a degree of protonation equal to approximately 1. Under the same conditions, the relative viscosity passes through a maximum corresponding to a degree of protonation of around 0.75; it then decreases because of an excess of highly dissociated acid. The salting out of chitosan is estimated at an acid concentration of larger than 1 M.

Keywords: Chitosan protonation; Solubility; Potentiometry; Viscometry; Conductimetry

INTRODUCTION

Chitosan in acidic medium becomes a polyelectrolyte due to the protonation of NH_2 groups. The following equilibrium describes the state of ionization:



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Different characteristics were investigated in this work, such as the pK_a , an apparent value of the pK which gives an intrinsic value pK_0 when the net charge goes to zero with

$$K_a = [\text{Chit-NH}_2][\text{H}_3\text{O}^+]/[\text{Chit-NH}_3^+]. \quad (1)$$

Different values for pK_0 are given in the literature; in absence of salt, pK_0 was found equal to 6.5^[1] and in 0.1 M NaCl, $pK_0 = 6.4$.^[2]

The role of the acid concentration used to protonate NH_2 groups was also examined in which the roles of a strong acid (HCl, assumed to be fully dissociated in dilute aqueous solutions) and of chitosan concentrations were studied. The degree of protonation as a function of the acid concentration and the critical degree of protonation causing the solubilization of chitosan were determined. These data were related to viscosity measurements as a function of the degree of solubility and of the expansion of the chain due to electrostatic interactions.

EXPERIMENTAL

Chitosan (Protan, Norway) was purified by dissolution in 0.5 M acetic acid and the solution was filtrated through 8-, 3-, 1.2-, 0.8- μm pore diameter cellulose nitrate membranes (Sartorius, Germany) successively and then neutralized with 10% sodium hydroxide to a pH of 8. The precipitate was washed with ethanol/water mixtures from 70/30 up to 100/0 v/v. The purified chitosan was characterized by ^1H NMR to obtain the degree of residual acetylation (DA) which was 0.12. NMR experiments were carried out on an AC300 Bruker spectrometer (Germany) at 353 K. The samples were dissolved at a concentration of 10 mg/mL in D_2O in the presence of hydrochloric acid (pH 4) and freeze-dried three times to exchange labile protons for deuterium atoms. The average viscosity molecular weight M_v was 295 000, estimated from the intrinsic viscosity determined in 0.3 M acetic acid/0.2 M sodium acetate using the Mark-Houwink parameters $a = 0.76$ and $K_\eta = 0.076$ at 25°C, where the viscosity is expressed in mL/g.^[3]

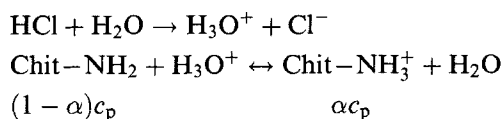
Solutions were prepared as follows: a given weight of chitosan (in the order of 30 mg) was added to 20 mL of the acidic solution with different concentrations from 2×10^{-5} up to 1 M. Equilibrium was

established after 24 h and controlled after different periods of 24 h by conductivity and pH measurements. The pH measurements were performed with a Minisis 6000 pH meter (Tacussel, France) with a glass electrode. The specific conductivity was determined with a conductimeter type CD 78 (Tacussel, France); the measurements were performed with a platinum platinated cell Ref. CM02/55/G at a frequency of 250 Hz. The viscosity was measured at low shear rate with a Low Shear 40 viscometer (Contraves, Switzerland). All measurements were performed at 25°C.

RESULTS AND DISCUSSION

Chitosan-HCl System

The reactions that take place are



in which α is the degree of protonation of chitosan amino groups and c_p is the total polymer concentration in the system, even if the unprotonated chitosan remains insoluble in the range of the lower acid concentration.

Potentiometric analysis pH measurements give the effective H_3O^+ activity assumed to be equal to the concentration. The total polymer concentration c_p is defined as $[\text{Chit-NH}_2] + [\text{Chit-NH}_3^+]$. The concentration of acid added (c_H) is known and $c_H = [\text{ChitNH}_3^+] + [\text{H}_3\text{O}^+]$, then from pH measurements, one deduces directly the fraction of charge

$$\alpha = [\text{ChitNH}_3^+]/c_p \quad (2)$$

thus

$$\text{p}K_a = \text{pH} + \log(\alpha/(1 - \alpha)). \quad (3)$$

The results giving the degree of protonation as a function of the acid concentration are shown in Figures 1 and 2. In Figure 1, the degree of

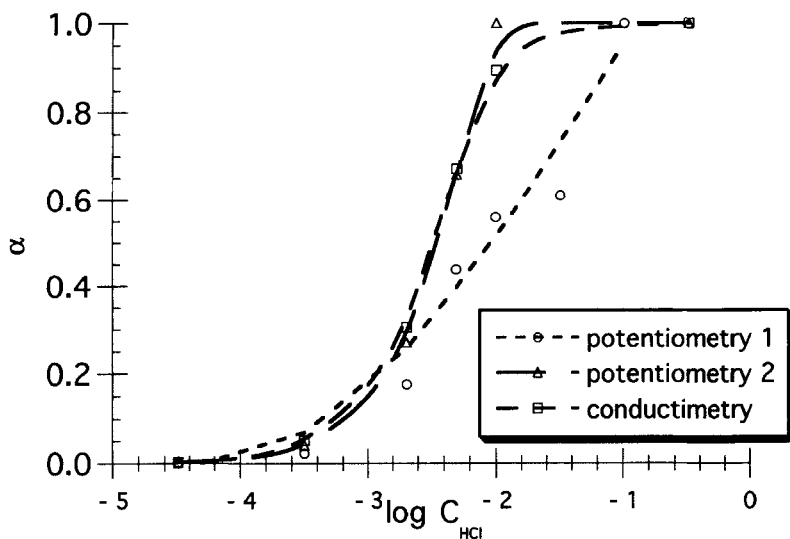


FIGURE 1 Determination of the degree of protonation of chitosan in HCl solutions: influence of the evaluation method; polymer concentration 7.52×10^{-3} monomol/L.

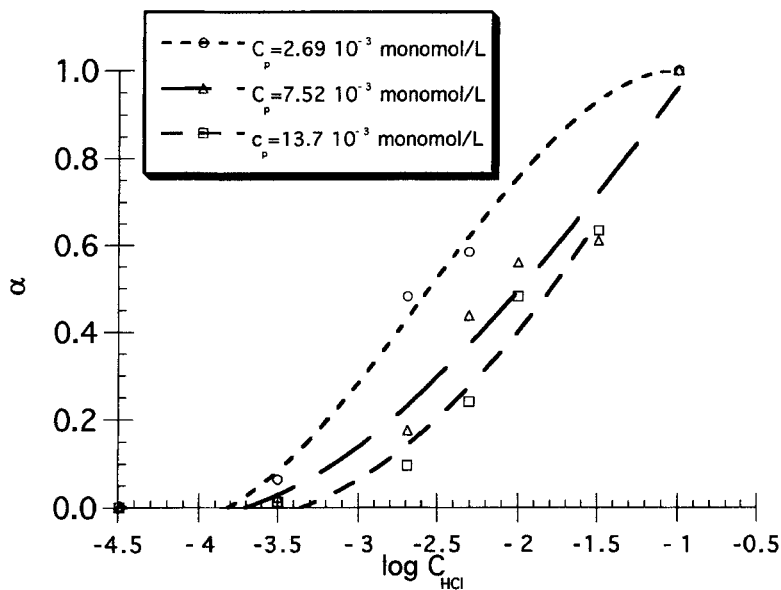


FIGURE 2 Determination of the degree of protonation of chitosan in HCl solutions: influence of the polymer concentration using potentiometric method 2.

protonation was determined using two methods. In the first method, the difference of pH at the same initial acid concentration without and with chitosan was considered (potentiometry 1). In the second method, the initial acid concentration was chosen and used to calculate the quantity of protons involved for the protonation of the amino groups from the pH value at equilibrium (potentiometry 2). The agreement is fair and the values obtained from method 1 are lower than from method 2; this is attributed to the imprecision of the determination of pH in the very dilute range or in a large excess of strong acid. In Figure 2, the degree of protonation of chitosan calculated from the initial acid concentration (method 2) was determined for different polymer concentrations. It must be pointed out that when the external acid concentration is much larger than the concentration of chitosan, a lack of precision is clear.

From the HCl content for which solubility is observed and considering the results given in Figures 1 and 2, we can demonstrate that solubility is obtained when $\alpha > 0.45$. Then, from the experimental conditions used, $\alpha \approx 0.5$ corresponds to a stoichiometric ratio $[\text{HCl}]/[\text{chitosan}] \approx 0.5-0.6$ which is the limiting value needed to solubilize chitosan. Under these conditions, $\text{pH} = 4.5-5$ for the three polymer concentrations tested. A series of experimental values is given in Table I.

For higher HCl concentration, a decrease in pH was observed and the degree of protonation was difficult to obtain with precision. For HCl concentrations larger than 1 M, salting out was observed.

Conductivity measurements From conductivity measurements the degree of protonation can also be determined. For each addition of

TABLE I Experimental data obtained for a series of acidic solutions

$c_{\text{HCl}} (\text{M} \times 10^{-3})$	pH_{init}	pH_{equil}	$\Delta_x (\mu\text{s} \times \text{cm}^{-1})$	η_{red}	α^*	α^{**}	α^{***}
3.22×10^{-2}	4.88	5.96	-5	1.17	0.0015	0.004	0.002
31.6×10^{-2}	3.62	5.31	-121	1.49	0.0235	0.041	0.052
204×10^{-2}	2.82	5.42	-713	7.27	0.177	0.271	0.306
496×10^{-2}	2.47	4.83	-1565	14.03	0.438	0.658	0.671
994×10^{-2}	2.15	2.62	-2084	10.09	0.560	1	0.894
32	1.62	1.70	-1210	5.26	0.611	—	—
100	1.10	1.14	-560	3.95	1.0	—	—

$c_p = 7.52$ monomol/L; $c_p = 1.42 \times 10^{-3}$ g/mL; *from pH determination (method 1); **from the initial acid concentration (method 2); ***from conductivity.

HCl in water

$$\chi_1 = 10^{-3}c_H(\lambda_{H^+} + \lambda_{Cl^-}) \quad (4)$$

in chitosan solution

$$\chi_2 = 10^{-3}[c_H\lambda_{Cl^-} + c_p\alpha\lambda_{p^+} + (c_H - c_p)\lambda_{H^+}] \quad (5)$$

where χ is the conductivity of the solution and λ_i is the mobility of ion i . Then, for the same acid concentration

$$\Delta\chi_{2-1} = \chi_2 - \chi_1 = 10^{-3}c_p\alpha(\lambda_{p^+} - \lambda_{H^+}) \quad (6)$$

assuming that the coefficient of transport equals 1 in the range of low degree of dissociation. From the decrease of conductivity for each concentration of HCl, α can be determined if λ_{p^+} is determined separately; in this work, $\lambda_{p^+} = 40 \Omega^{-1} \cdot \text{cm}^2 \cdot \text{equiv}^{-1}$ as often found for polyelectrolytes or large ions.^[4,5]

The conductivity of the acidic solution in the presence of chitosan is lower than in its absence due to the protonation and the mobility of the macroion formed (λ_{p^+}) which is smaller than that of the proton λ_{H^+} . The values of α are given in Figure 1 and are compared with the values obtained from potentiometry. The conductivity gives values in good agreement with those obtained by potentiometry based on the initial acid concentration method. The experimental values of $\Delta\chi$ obtained for different polymer concentrations are given in Figure 3. The $\Delta\chi$ curves pass through a minimum for a given acid concentration which depends on the chitosan concentration; the position of the minimum corresponds nearly to the complete protonation of chitosan (see Figure 1). Thus, the decrease of the absolute value of $\Delta\chi$ is attributed again to the large excess of strong acid and the lack of precision.

Viscometric behavior The viscosity was determined at equilibrium for all the acid concentrations tested. The viscosity first increased and then decreased in the range of high acid concentrations. The data are represented on Figure 4. The increase corresponds to the increase of solubilized materials and to the expansion of the chains due to the increase of the degree of protonation. In Figure 5, the reduced viscosity is plotted as a function of the degree of protonation (determined from the difference between initial and final pH; see Figure 1,

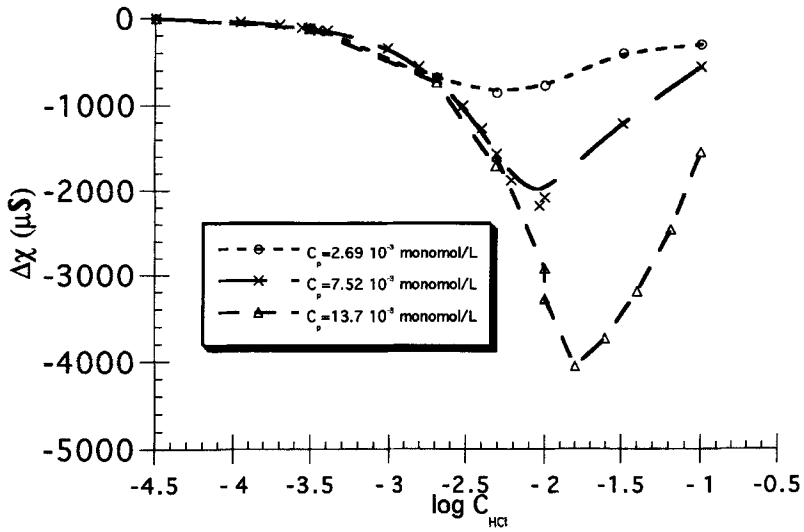


FIGURE 3 Variation of the conductivity (Equation (6)) as a function of HCl concentration for different polymer concentrations.

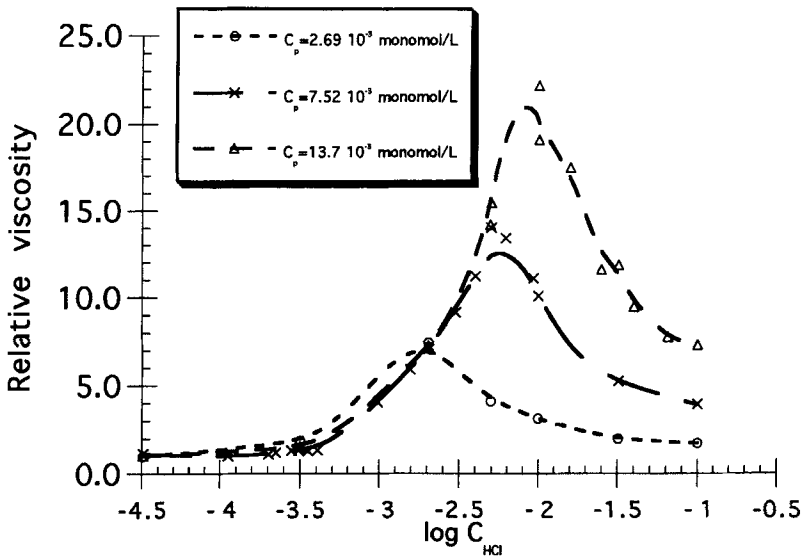


FIGURE 4 Variation of the relative viscosity as a function of HCl concentration for different polymer concentrations.

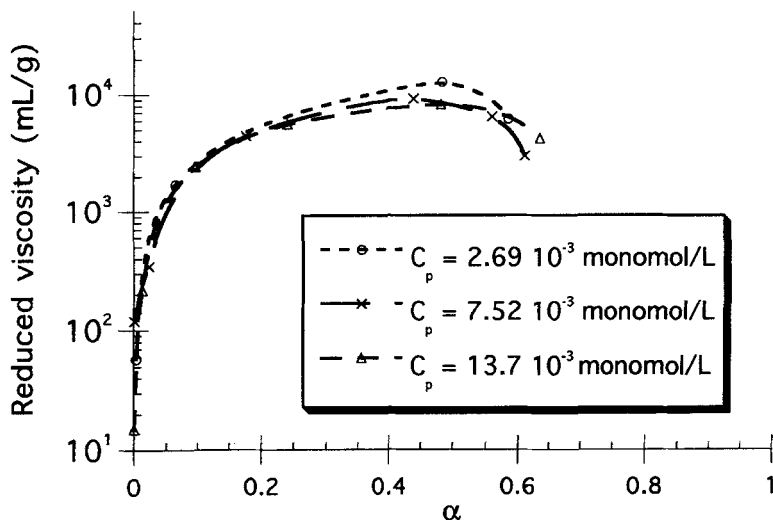


FIGURE 5 Variation of the reduced viscosity as a function of the degree of protonation (α) for different polymer concentrations.

method 1); all the experimental data are in agreement and demonstrate that the viscosity depends on the polymer concentration and the degree of protonation. Over the stoichiometry $[\text{HCl}]/[\text{Chit-NH}_2] = 1$ (i.e., approximately $\alpha \approx 0.75$ as obtained by potentiometry, method 2), the decrease in viscosity was caused by excess of acid which increases the ionic concentration and screens long-range electrostatic interactions. This effect was confirmed after isolation of the chitosan at different acid concentrations ($[\text{HCl}] = 0.01 \text{ M}$; $[\text{HCl}] = 0.1 \text{ M}$). The intrinsic viscosities of these two samples determined in the acetic acid/sodium acetate solvent were found to be identical, which demonstrated that polymer hydrolysis has not occurred.

Chitosan Chlorhydrate

The chlorhydrated form of chitosan was isolated in an excess of HCl and freeze-dried. It was then titrated by progressive addition of sodium hydroxide (0.1 M) causing the repression of protonation (α). From the neutralization curve, which gives the degree of neutralization (α' such as $\alpha' = 1 - \alpha$), the concentration of the chlorhydrate and the

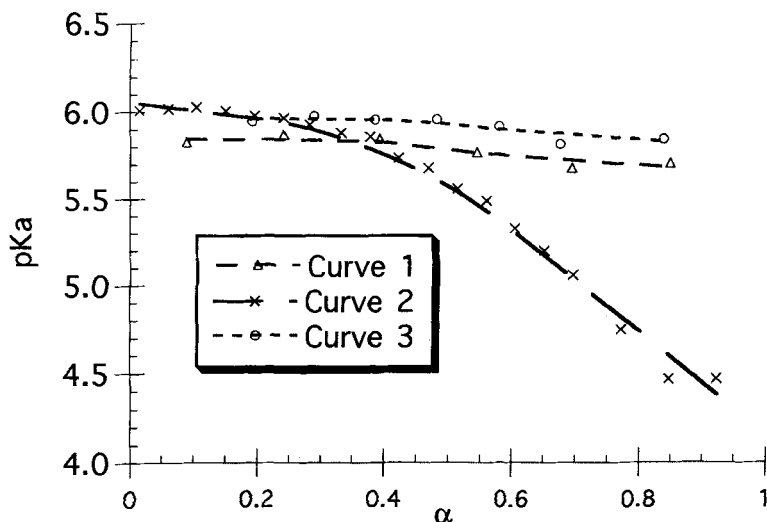


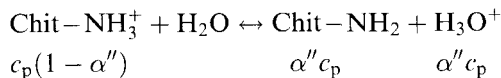
FIGURE 6 Determination of the intrinsic pK from the variation of pK_a with the degree of protonation of chitosan chlorhydrate: curve 1: the chlorhydrate form isolated from 6×10^{-3} M HCl; curve 2: the chlorhydrate form isolated from 0.1 M HCl; curve 3: previous solution dialyzed against distilled water.

pH for each degree of neutralization, the pK_a can be determined as a function of the net charge of the polymer (Equation (3)). The pK_a results are given in Figure 6 from which $pK_0 \approx 6 \pm 0.1$ is obtained by extrapolation to $\alpha = 0$.

First, from this measurement we find that depending on the acid concentration of the solution used to prepare the chitosan chlorhydrate, a fraction of the polymer is not in the salt state: 35% remains in the NH_2 form when isolated from a 6×10^{-3} M HCl solution (see Figure 6, curve 1) but it is completely in the chlorhydrate form when isolated from 0.1 M HCl. Under this condition, an excess of HCl remained in the sample after freeze drying (Figure 6, curve 2). This sample was dissolved and dialyzed against distilled water to extract excess acid (Figure 6, curve 3). The three series of data gave a similar pK_0 value.

From the initial pH of an aqueous solution of chitosan chlorhydrate, we can determine the degree of hydrolysis and follow the pK_a . The degree of hydrolysis remains small in the usual conditions: for $c_p = 1.77 \times 10^{-3}$ monomol $\cdot L^{-1}$, pH is 4.56 and the degree of

hydrolysis is $\alpha'' = 0.015$ following:



CONCLUSION

In this paper, we investigated conditions for the solubilization of chitosan in the presence of a strong acid, HCl, in aqueous solution. It was shown that solubility is obtained when the degree of protonation is approximately 0.5 and/or when the HCl concentration is nearly equal to that of the NH_2 groups. The determinations of the degree of protonation of chitosan were performed by potentiometry and conductimetry, which showed relatively good agreement with one another.

The pK_0 was determined and found to be equal to 6 ± 0.1 , which is close to the data given in the literature.^[1,2] The reduced viscosity determined under the same conditions passes through a maximum in the range of the degree of protonation of approximately 0.75.

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References

- [1] A. Domard (1987). *Int. J. Biol. Macromol.*, **9**, 98.
- [2] J.W. Park and H.K. Choi (1983). *Bull. Korean Chem. Soc.*, **4**, 68.
- [3] M. Rinaudo, M. Milas and P. Le Dung (1993). *Int. J. Biol. Macromol.*, **15**, 281.
- [4] R.A. Robinson and R.H. Stokes (1959). In: *Electrolyte Solutions*, 2nd Edn. (Butterworths: Scientific Publications, London), p. 463.
- [5] M. Rinaudo and M. Milas (1969). *J. Chim. Phys.* (France), **66**, 1489.