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Influence of acetic acid concentration on the solubilization of chitosan

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Abstract

This article concerns the protonation of chitosan in acetic acid aqueous solution with variable concentration. Comparison with HCl is also drawn. Potentiometry allows the determination of the degree of protonation and the pK_0. Conductimetry and viscometry are combined to follow the role of protonation on solubilization of chitosan. Solubilization occurs for a degree of protonation around 0.5 as previously found for HCl. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Chitosan is a partially deacetylated derivative obtained by alkaline treatment of chitin. Chitin is one of the most abundant natural polymer extracted from crustaceous shells or from some fungi [1].

Chitosan in acidic media becomes a polyelectrolyte because of the protonation of the $-NH_2$ groups. The following equilibrium reaction described the state of ionization:

$$\text{Chit-NH}_2 + \text{H}_3\text{O}^+ \leftrightarrow \text{Chit-NH}_3^+ + \text{H}_2\text{O}. \quad (1)$$

Different characteristics were investigated in this work such as the pK_a, the apparent value of $-NH_2$ pK which gives the intrinsic value pK_0 when the net charge goes to zero with

$$K_a = \frac{[\text{Chit-NH}_2][\text{H}_3\text{O}^+]}{[\text{Chit-NH}_3^+]}.$$

Different values for pK_0 have been given in the literature. In the absence of salt, pK_0 was found to be equal to 6.5 [2] and in NaCl 0.1 M p$K_0 = 6.4$ [3].

We have previously investigated the solubilization of chitosan in the presence of a strong acid, HCl [4]. It was demonstrated that solubility occurs in the range of a protonation degree $\alpha \approx 0.5$ and that p$K_0 = 6$. In the present work, our purpose is to investigate the solubilization in acetic acid, in order to determine the degree of protonation of chitosan in relation to the pK of the weak acid (acetic acid).

Acetic acid is commonly used to solubilize chitosan at a concentration of 0.1 M or 1%. The role of the respective concentration of chitosan and acetic acid will be discussed in the present work and the comparison with the behaviour in HCl acid will be established.

2. Experimental

The chitosan was purified by dissolution in 0.5 M CH_3COOH and the solution was filtered through 8, 3, 1.2, 0.8 μm cellulose nitrate membranes from Sartorius successively and then neutralized with 10% NaOH up to pH 8. The precipitate was washed with ethanol/water mixtures (from 70/30 up to 100/0 (v/v)). The purified chitosan was characterized by ^{1}H NMR to determine the degree of residual acetylation (DA; one finds DA = 0.12). The average viscometric molecular weight $M_v = 295\,000$ was estimated from the intrinsic viscosity determined in the solvent 0.3 M CH_3COOH/0.2 M CH_3COONa using the Mark–Houwink parameters $a = 0.76$, $K_\eta = 0.076$ at 25°C when the intrinsic viscosity is expressed in ml g^{-1} [5].

The solutions are prepared as follows: a given weight of chitosan (≈30 mg) was added to 20 ml of acidic solution, with different acid concentrations (from 2×10^{-4} M up to 16 M).

Equilibrium is established after 24 h and controlled after different periods of 24 h using conductivity and pH-measurements.

The pH-measurements were performed with a Tacussel Minisis 6000 pH-meter with a glass electrode. The specific conductivity is determined with a Tacussel conductimeter

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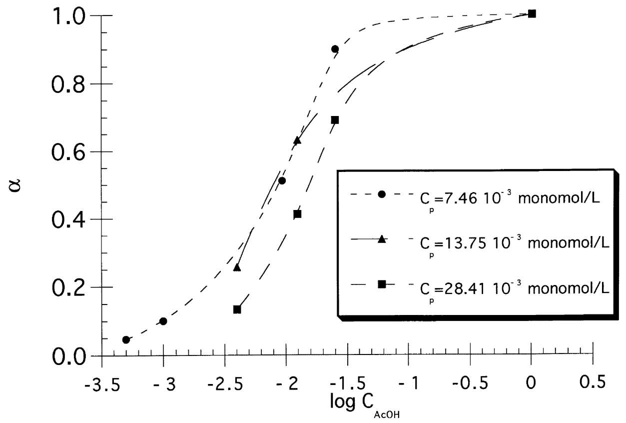

Fig. 1. Degree of protonation of chitosan as a function of the initial acetic acid concentration for different polymer concentrations.

type CD 78; the measurements were performed with a platinum platinated cell CM02/55/G at a frequency of 250 Hz. The viscosity is measured at low shear rate with a Low Shear 40 viscometer from Contraves. All the measurements were performed at 25 ± 0.1°C.

3. Results and discussion

When chitosan is dispersed in acetic acid solution at different concentrations the following equilibria have to be considered:

$$\underset{C_A(1-\alpha')}{CH_3COOH} + H_2O \leftrightarrow \underset{C_A\alpha'}{CH_3COO^-} + H_3O^+. \quad (2)$$

$$\underset{(1-\alpha)C_p}{\text{Chit-NH}_2} + H_3O^+ \leftrightarrow \underset{\alpha C_p}{\text{ChitNH}_3^+} + H_2O \quad (3)$$

where α' is the dissociation degree of acetic acid, α the protonation degree of chitosan, C_A and C_p, respectively, the total acid and polymer concentrations.

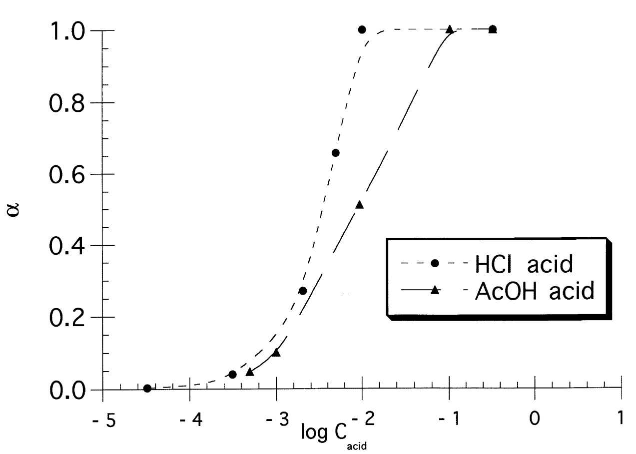

Fig. 2. Comparison of the degree of protonation of chitosan as a function of the acid concentration for HCl and acetic acid for a polymer concentration $C_p = 7.46 \times 10^{-3}$ monomol l^{-1}.

3.1. Potentiometry

The weak acid is characterized by its dissociation constant:

$$K = \frac{[H_3O^+][CH_3COO^-]}{[CH_3COOH]} = [H_3O^+]\left(\frac{\alpha'}{1-\alpha'}\right) \quad (4)$$

or $K = (\alpha'^2 C_A/(1-\alpha'))$ in pure water. K is a well-known constant at 25°C, i.e. p$K = 4.75$.

However, the degree of dissociation α' is directly controlled by the H_3O^+ concentration in the presence of chitosan and it becomes α''.

If the total concentration of acid (C_A) is known:

$$C_A = [CH_3COO^-] + [CH_3COOH]. \quad (5)$$

The degree of dissociation α'' is controlled by the protonation of chitosan which controls H_3O^+ based on its constant of protonation.

From pH measurements, the effective concentration in H_3O^+ is determined allowing to calculate α'' from relation (4) (α'' is the value of α' in presence of chitosan):

$$\alpha'' = K/(K + [H_3O^+]). \quad (6)$$

The degree of protonation of chitosan is α; as the electroneutrality imposes

$$C_A\alpha'' = C_p\alpha + [H_3O^+]$$

(neglecting $[OH^-]$ compared to $[H_3O^+]$ in an acidic medium), it becomes:

$$\alpha = (\alpha'' C_A - [H_3O^+])/C_p. \quad (7)$$

and the apparent constant of dissociation of Chit-NH_3^+ is:

$$K_a = K[(1-\alpha'')/\alpha''] \times [1-\alpha/\alpha] = [H_3O^+](1-\alpha)/\alpha. \quad (8)$$

From pH measurements, the degree of protonation α was determined and plotted as a function of the initial concentration of acid considered. The values of the degree of protonation for different polymer concentrations are given in Fig. 1.

From our data, we observed that complete solubilization is obtained when the degree of protonation is around 0.5 as previously found with HCl; the stoichiometry [AcOH]/[Chit–NH_2] = 0.6. For larger acid concentrations around the stoichiometry 1, a drop in pH values is observed; the pH decreases from 5 to values between 3 and 4.

In Fig. 2, the influence of acid concentration on the degree of protonation is shown for AcOH and HCl. It is clear that the protonation is larger in the strong than in the weak acid at a given molar concentration.

The value of α and pH allows to determine the pK_a (α); but, one has also isolated chitosan acetate from the freeze-drying of chitosan in acetic acid solution. The chitosan acetate is a water soluble neutral salt. It was neutralized by NaOH to get a direct determination of pK_a as a function of the degree of neutralization. The values of pK_a are plotted

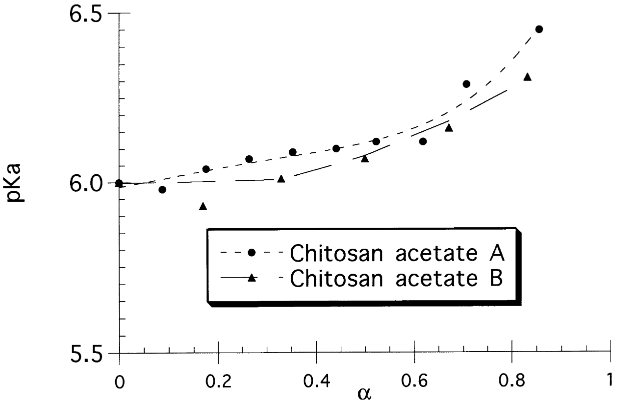

Fig. 3. Variation of the pK_a as a function of the degree of chitosan protonation obtained by neutralization of chitosan acetate neutral salt. Chitosan acetate A: freeze-dried from a 0.047 M acetic acid solution; chitosan B: freeze-dried from a 0.2 M acetic acid solution.

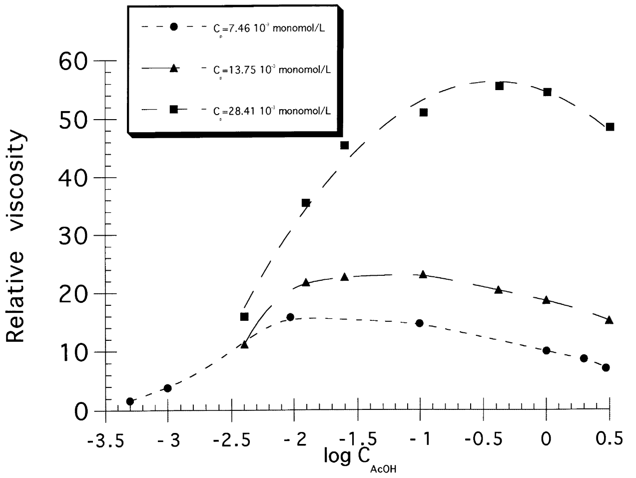

Fig. 5. Variation of the relative viscosity as a function of the initial acetic acid concentration for different polymer concentrations.

in Fig. 3; the value of pK_0 equals 6 ± 0.1 and is in good agreement with the value we recently determined in the presence of HCl [4].

3.2. Conductimetry

From conductivity it is also convenient to follow the protonation of chitosan. It is possible to write for each addition of CH_3COOH:

In water $\chi_1 = 10^{-3} C_A \alpha' (\lambda_{H^+} + \lambda_{A^-})$
In aqueous chitosan solution $\chi_2 = 10^{-3}[C_A \alpha'' \lambda_{A^-} + C_p \alpha \lambda_{p^+} + (\alpha'' C_A - \alpha C_p)\lambda_{H^+}]$

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

$$\Delta\chi_{2-1} = 10^{-3}[C_A(\alpha'' - \alpha'(\lambda_{A^-} + \lambda_{H^+}) + C_p \alpha(\lambda_{p^+} - \lambda_{H^+})] \quad (9)$$

assuming that the coefficient of transport equals 1 in the range of low degree of dissociation.

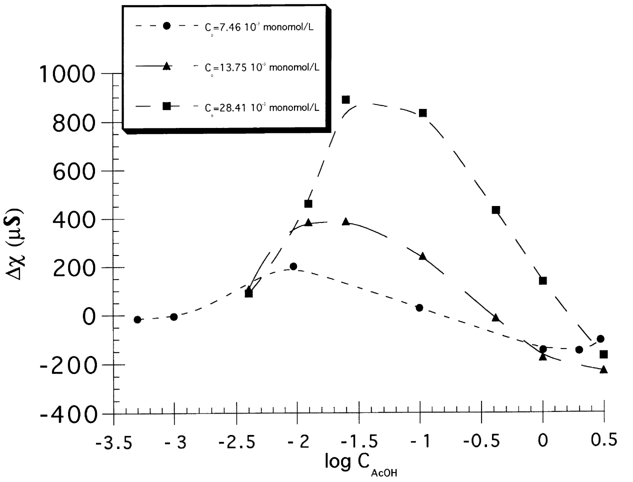

Fig. 4. Variation of the conductivity as a function of the initial acetic acid concentration for different polymer concentrations.

The first part of this relation is related to the change in dissociation of the weak acid because of the presence of chitosan.

The value of α' is determined from the dissociation constant (p$K = 4.75$) or from pH measurements of acetic acid in water. Then $\Delta\chi_{2-1}$ also allows us to determine α when α'' is known and λ_i determined [4]. It is clear that the conductivity in the presence of chitosan increases due to the displacement of the equilibrium of dissociation of the weak acid and progressive dissolution of protonated chitosan. The variations of $\Delta\chi_{2-1}$ are given in Fig. 4 for different concentrations of chitosan.

The curves $\Delta\chi_{2-1}$ (C_A) pass through a maximum located for a stoichiometry [AcOH]/[Chit-NH_2] ≈ 1 and a degree of protonation in the range of 0.5–0.7; then for higher acid concentration this difference seems to become negative. In fact, this domain corresponds to the range of acid

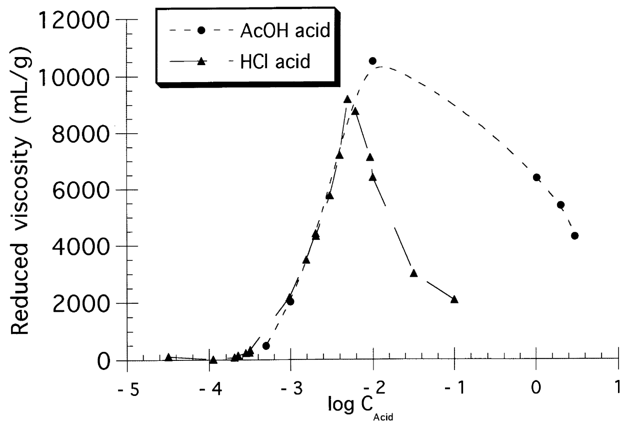

Fig. 6. Variation of the reduced viscosity as a function of initial acid concentration. Comparison between HCl and acetic acid for a polymer concentration $C_p = 7.46 \times 10^{-3}$ monomol l^{-1}.

concentrations where the viscosity also decreases because of an excess of ionic strength.

3.3. Viscometry

In the same conditions as in the previous experiments, viscosity, η, in different solutions was determined. The results are given in Fig. 5.

The relative viscosity (η/η_0 with η_0 the viscosity of the solvent) increases as shown previously in the presence of HCl and passes through a maximum for an acid concentration which depends on the polymer concentration. With acetic acid, the viscosity is less influenced by acid concentration than with HCl as shown in Fig. 6 where the reduced viscosities ($(\eta - \eta_0)/\eta_0 C$) are compared for the same polymer concentration (C). The remarkable situation is that the maximum of the reduced viscosity is nearly the same in HCl and in AcOH solutions.

For different polymer concentrations, the maximum obtained is in the range of degrees of protonation slightly larger than for conductivity. Nevertheless, the maximum in viscosity is very broad and remains nearly constant in a large range of initial acid concentration.

The situation is completely different from HCl for which the salting out occurs for HCl concentration larger than 1 M. In fact, only the dissociated fraction of the acid plays a role on the ionic strength of the solution and in excess of acetic acid, the degree of dissociation remains quite low. So, an excess of acetic acid or other weak acid is more favourable to chitosan solubility. No salting out was observed on the addition of concentrated acetic acid. Then chitosan was dispersed in pure acetic acid and increasing amount of water was added to obtain solubility. Solubility was observed in 16.5 M acetic acid.

4. Conclusion

In this article, the protonation of chitosan was investigated in acetic acid aqueous solution with different acid or/and polymer concentrations. The degree of protonation was determined and its variation with chitosan concentration established. For a given acid concentration, the degree of protonation depends on the pK of the acid used to solubilize the chitosan.

A value for the intrinsic pK, $\mathrm{p}K_0 = 6 \pm 0.1$ was determined which is in agreement with the value obtained previously in HCl solutions.

From conductivity measurements, the evolution of the protonation is clearly shown as well as that from the viscosity measurements. In fact, two factors are involved in the evolution of these parameters: the degree of protonation increases progressively involving a progressive solubilization of chitosan. Complete solubilization is obtained at $\alpha \geq 0.5$ and a stoichiometric ratio $[\mathrm{AcOH}]/[\mathrm{Chit\text{-}NH_2}] = 0.6$. The interesting behaviour of chitosan in acetic acid is that at large acid concentrations, the viscosity of chitosan solution remains nearly constant not only because of the complete protonation but also due to low ionic concentration in relation with the pK of acetic acid.

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