

Removal of Cr VI on chitosan gel beads. Kinetic modelling

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Abstract: The treatment of chromium in wastewaters has been widely investigated. Chitosan has demonstrated a great ability at removing metal ions. In this study it was conditioned as gel beads, in order to expand the diffusion network. The beads were crosslinked with glutaraldehyde to avoid partial dissolution in acidic or alkaline media. The study of the kinetics showed the importance of external and intraparticle diffusion. A model in two distinct steps was proposed.

Key words: chitosan, gel, beads, chromium, adsorption, diffusion

Introduction

In the field of environmental pollution there are few subjects that have developed as rapidly in recent years as the study of toxic metals. Biosorption has been revealed in the last thirty years as a promising way to reduce the contamination of surface waters from industrial effluents. Several works have demonstrated the high affinity of chitosan for metal ions such as cadmium, chromium, copper, lead, mercury, uranyl (1-11). However, its performances depend on diffusion mechanisms. There are several processes to enhance diffusion properties, such as conditioning it as gel beads. The protocole is derived from a standard alginate bead formation procedure (12-14). The crosslinking of chitosan in glutaraldehyde (14), epichlorhydrin (11), ethylene glycol glycidyl ether (15), enhances the resistance of sorbent beads against chemicals (14, 16-18). The aim of this work is to study the influence of several parameters (size of the beads, crosslinking, metal ion concentration) on metal ion diffusion. The study investigates sorption kinetics and tries to model them.

Materials and methods

Materials

Chitosan was supplied by ABER-Technologie (France) as a flaked material, with a deacetylation percentage around 80 %, defined by IR spectrometry (19-20). Glutaraldelyde was an Acros product

(25 wt % in aqueous media). Other reagents such as sodium hydroxide and acetic acid were Prolabo reagents.

Bead formation procedure

Milled chitosan flakes were dissolved in 3.5 wt % acetic acid. The final percentage of chitosan was approximately 3.5 %. The solution was then pumped and dripped into a casting solution of sodium hydroxide (2.5 M) using the apparatus presented in Figure 1. The diameter of the needle was 0.6 mm and that of the annular space of the apparatus is 3 mm. Depending on the diameter of the beads to be manufactured, air was blown through the apparatus at a variable flow rate. The formed beads were left to stand in the precipitation bath for 16 hours and then washed in several demineralized water baths. The collected rinsed beads were then crosslinked.

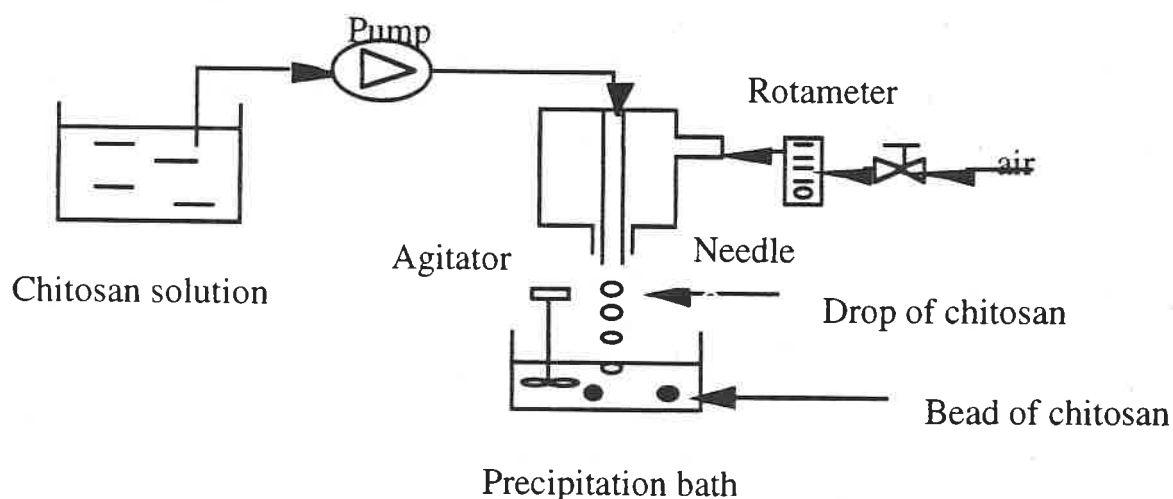


Figure 1 : Bead formation apparatus

The crosslinking bath was a 2.5 wt % glutaraldehyde solution. The ratio between glutaraldehyde and chitosan beads was 2.5 g.l⁻¹ (dry mass of beads). The crosslinking takes place over 24 hours. The chitosan beads were extensively rinsed with demineralized water. Physical properties of the products are given in Table I.

Product	Size (mm) (mean diameter)	ρ (kg.m ⁻³)	water content
Beads 3 NCL	2.70	1020	96.3 %
Beads 1 CL	1.03	1023	94.6 %
Beads 2 CL	1.46	1008	96.3 %
Beads 3 CL	2.70	1012	94.0 %

Table I : Physical properties of chitosan beads (NCL for non crosslinked and CL for crosslinked)

Experimental procedure : metal ion sorption

Chromium solutions at 25 mg.l^{-1} were prepared. Solution pH was maintained at the required level of 4 (previous work showed that the optimum pH of adsorption was 4), by regulation with NaOH or H_2SO_4 concentrated. Kinetic studies were performed by mixing 1 l of chromium solution with crosslinked chitosan wet beads (the dry equivalent mass is 200 mg), the agitation being provided by a mixer at 200 rpm. Samples were collected and filtered. The chromium (VI) content was estimated using the diphenyl carbazide colorimetric assay method (21).

Sorption kinetics modelling

Sorption kinetics

The adsorption of solutes on solid particles has been extensively studied. The overall mechanism can be described as the succession of various steps (22-23), the external mass transfer resistance and intraparticle mass transfer resistance being the major controlling stages.

Limiting rate

The slope of plots of amount adsorbed (q) against the square root of time has been defined as a rate parameter k, characteristic of the rate of adsorption in the region when the intraparticle diffusion is rate controlling (24). Extrapolation of the linear portions of the plots back to the time axis provides intercepts ($\sqrt{t_{\text{lim}}}$) which are proportional to the extent of the boundary layer thickness, ca. the larger the intercept, the greater the boundary layer effect. These coefficients have been calculated and were greater than 0.8, meaning the boundary layer was not restricted to a thin layer.

The pore diffusion coefficient (D) was estimated using the following equation (25) :
$$t_{\frac{1}{2}} = 0.03 \frac{a^2}{D} \quad (1)$$

where $t_{\frac{1}{2}}$ is the time for half adsorption and a the radius of the

adsorbent. A value of D in the order of $10^{-10} \text{ mm}^2.\text{min}^{-1}$ is indicative of pore diffusion (26). In our case however, the values of D were sufficiently higher than $10^{-10} \text{ mm}^2.\text{min}^{-1}$ to conclude that pore diffusion was not the main rate determining step.

So, these comments tend to indicate that both the external and intraparticle diffusion have to be taken into account. The problem is to treat them simultaneously. Indeed, such a model needs considerable computing resources. Therefore, in order to simplify, a model in two distinct steps was chosen.

Modelling

The external diffusion is given by:

$$-V \frac{dC}{dt} = k_f A (C(t) - C_s(t)) \quad (2)$$

with A the sorbent exchange surface (m^2), k_f the external mass transfer coefficient ($m \cdot min^{-1}$), and $C_s(t)$ the concentration at the interface ($mg \cdot l^{-1}$). At early times of contact $C_s(t) = C(a,t) \rightarrow 0$ Equation (2) can then be written:

$$-V \frac{dC}{dt} = k_f A C(t) \quad (3)$$

Plotting $\ln [C(t)/C_0]$ versus t provides k_f . $C(t)$ is modelled by:

$$C = C_0 e^{-\frac{k_f A}{V} t} \quad (4)$$

In a second step, the intraparticle diffusion is the preponderant rate controlling factor. Crank (39) applies Fick's laws for a spherical particle with a radial equation:

$$\frac{\partial q}{\partial t} = D \left(\frac{\partial^2 q}{\partial r^2} + \frac{2}{r} \frac{\partial q}{\partial r} \right) \quad (5)$$

which is solved by the Laplace transform :

$$q(r,t) = q_e \operatorname{erfc} \frac{r}{2(Dt)^{1/2}} \quad (6)$$

The mass balance between liquid and solid phases is written:

$$-V \frac{dC}{dt} = m \frac{d\bar{q}}{dt} \quad (7)$$

with the average concentration of the solute in the solid, \bar{q} :

$$\bar{q} = \frac{3}{a^3} \int_0^a q(r,t) r^2 dr \quad (8)$$

Then \bar{q} is calculated in order to give $C(t)$ by the equation :

$$\bar{q} = q_e \operatorname{erfc} \frac{a}{2(Dt)^{1/2}} - 2 \frac{q_e}{a} \left(\frac{Dt}{\pi} \right) \exp \left(-\frac{a^2}{4Dt} \right) + 8 \frac{q_e}{a^3 \sqrt{\pi}} (Dt)^{3/2} \left(1 - \exp \left(-\frac{a^2}{4Dt} \right) \right) \quad (9)$$

Then equation (9) gives $C(t)$:

$$C(t) = C_i - \frac{m}{V} \left(-\bar{q}_i + q_e \operatorname{erfc} \frac{a}{2(Dt)^{1/2}} - \frac{2q_e}{a} \left(\frac{Dt}{\pi} \right)^{1/2} \exp \left(\frac{a^2}{4Dt} \right) + \frac{8q_e}{a^3 \sqrt{\pi}} (Dt)^{3/2} \left(1 - \exp \left(\frac{a^2}{4Dt} \right) \right) \right) \quad (10)$$

where t_i , time when intraparticle diffusion begins to be rate controlling . At t_i , $C = C_i$ and $q(t) = q(t_i)$

Equation 10 is used through trial and error to determine the overall intraparticle diffusivity which best fits experimental data. An example is given in Figure 2.

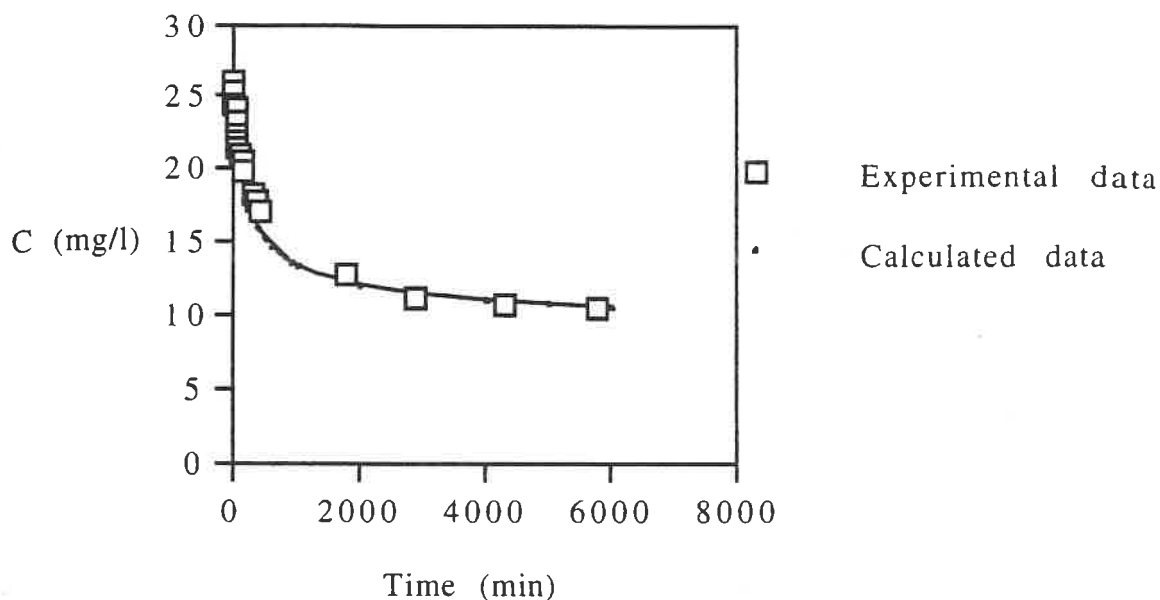


Figure 2 : Kinetic Modelling on beads 3 CL

As can be shown, there is a good fit between experimental and calculated data. This model was therefore chosen to calculate the different diffusion coefficients, in order to evaluate their impact on kinetics.

Results and discussion

Effect of bead size

Figure 3 shows the impact of bead size on kinetics.

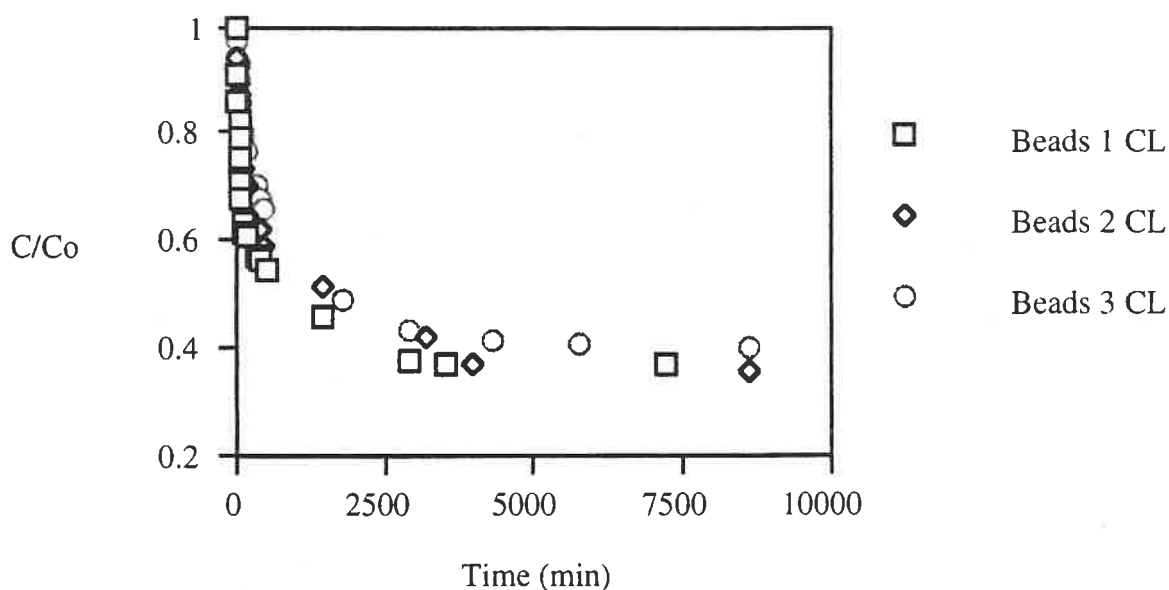


Figure 3 : Effect of bead size on kinetics

It appears that increasing bead size increases the time to reach equilibrium (48 h, 66 h and 96 h for beads 1, 2 and 3 respectively): the metal ion has a greater intraparticle distance to diffuse before the polymer becomes saturated. Table III gives the diffusion coefficients.

Beads	$k_f A/V$ (min^{-1})	k_f ($\text{mm} \cdot \text{min}^{-1}$)	D ($\text{mm}^2 \cdot \text{min}^{-1}$)
Beads 1 CL	$12 \cdot 10^{-3}$	$2.1 \cdot 10^{-3}$	$3 \cdot 10^{-3}$
Beads 2 CL	$9 \cdot 10^{-3}$	$2.2 \cdot 10^{-3}$	$3 \cdot 10^{-3}$
Beads 3 CL	$5 \cdot 10^{-3}$	$2.2 \cdot 10^{-3}$	$3 \cdot 10^{-3}$

Table II : Effect of beads size on diffusion coefficients

The intraparticle diffusion coefficient D is independent of bead size, as should be expected. Indeed, increasing bead size should have no impact on pore size.

Effect of crosslinking on beads

Figure 4 illustrates the impact of crosslinking on kinetics. Table III gives the diffusion coefficients.

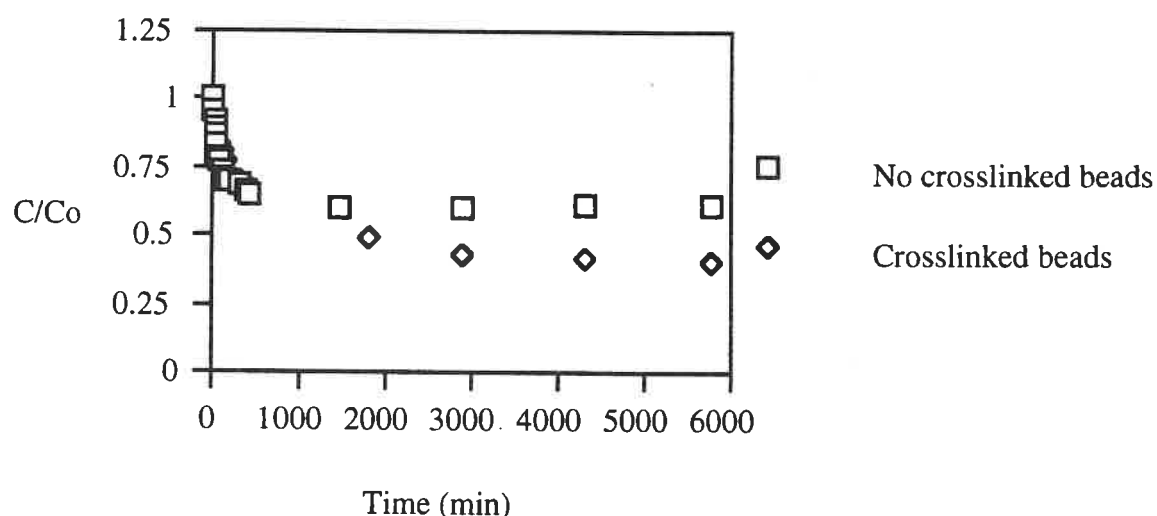


Figure 4 : Effect of crosslinking on kinetics (Beads 3)

Beads	$k_f A/V$ (min^{-1})	k_f ($\text{mm} \cdot \text{min}^{-1}$)	D ($\text{mm}^2 \cdot \text{min}^{-1}$)
Beads 3 NCL	$7 \cdot 10^{-3}$	$3.2 \cdot 10^{-3}$	$3 \cdot 10^{-2}$
Beads 3 CL	$5 \cdot 10^{-3}$	$2.2 \cdot 10^{-3}$	$3 \cdot 10^{-3}$

Table III : Effect of crosslinking on diffusion coefficients

It is clear that only intraparticle diffusion is affected by crosslinking. The time to reach equilibrium is greater in the case of crosslinked beads (32 h for crosslinked beads and 24 h for non crosslinked beads). The intraparticle diffusion coefficient is ten times lower in the case of crosslinked beads. This result can be

explain by the fact that the spacing between chitosan chains is reduced by the crosslinking, rendering access to adsorption sites more difficult.

Effect of initial metal ion concentration

Figure 5 illustrates the effect of the initial metal ion concentration on kinetics.

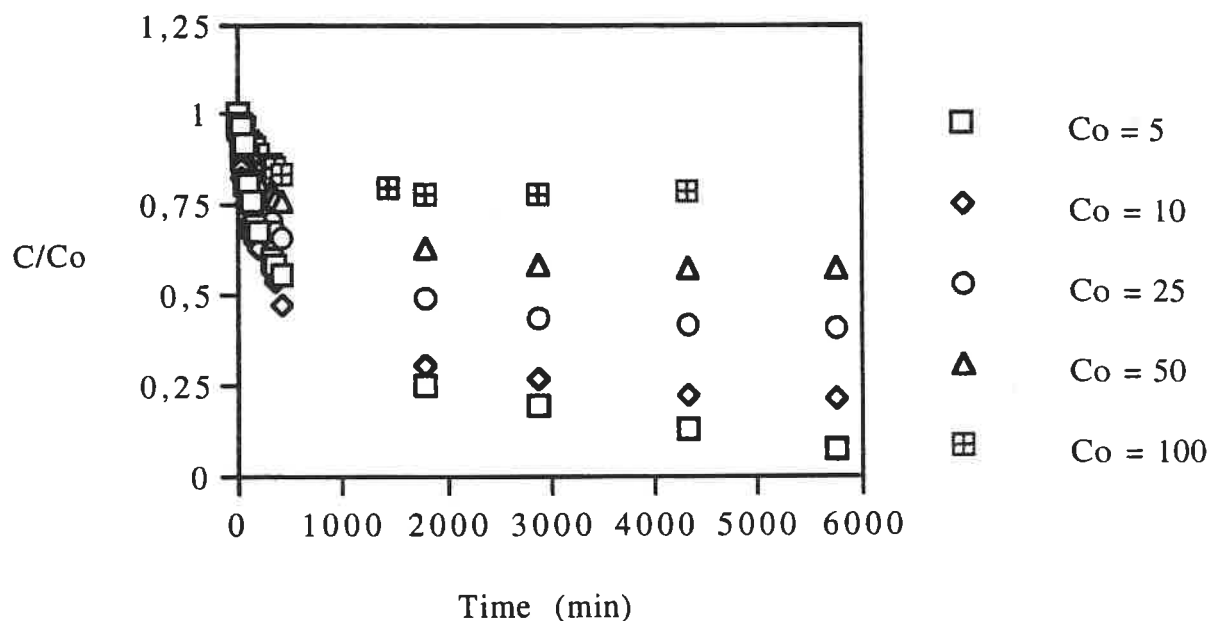


Figure 5 : Effect of C_0 on kinetics (Beads 3 CL)

It appears that the time to reach equilibrium increases when C_0 decreases. The explanation of such a phenomenon could be a pore blockage mechanism, as proposed by Rorrer *et al.* (14). At low initial metal ion concentrations, the initial flux of metal ions through the porous matrix is low and so the metal binds with exposed amine sites on internal surfaces near the outer surface of the bead. Eventually, the adsorbed metal clusters clog the pore near the outer surface so the metal ions can no longer diffuse to exposed amine sites deep within the interior surfaces of the bead. In this case, the metal is loaded in a shell near the outer surface of the bead. In contrast, at high metal ion concentrations, the initial flux of metal ions is high and so metal ions shoot deep into the interior porous matrix until the pores are finally clogged, and the adsorption capacity is thus higher.

C_0 (mg.l ⁻¹)	$k_f A/V$ (min ⁻¹)	k_f (mm.min ⁻¹)	D (mm ² .min ⁻¹)
5.05	4.10^{-3}	$1.8.10^{-3}$	3.10^{-3}
11.6	8.10^{-3}	$3.6.10^{-3}$	2.10^{-3}
26.02	5.10^{-3}	$2.2.10^{-3}$	3.10^{-3}
51.67	3.10^{-3}	$1.35.10^{-3}$	2.10^{-3}
101.68	1.10^{-3}	$0.45.10^{-3}$	4.10^{-3}

Table VI : Effect of C_0 on diffusion coefficients

Table IV indicates that k_f depends on C_0 . In the range of initial concentrations from 10 to 100 mg.l⁻¹, increasing C_0 leads to a decrease in k_f . This result is contradictory to that of McKay *et al.* (1993), for the adsorption of copper on chitosan.

Conclusion

The study of the kinetics of chromium on chitosan gel beads has shown that :

- both external and intraparticle diffusion have to be taken into account to explain the kinetics; the model proposed postulates that in the first step, the external diffusion is preponderant, and in the second step, it is intraparticle diffusion
- the time to reach equilibrium depends on the size of the beads: the metal ion has a greater intraparticle distance to diffuse with larger beads
- the time to reach equilibrium increases when C_0 decreases: this result can be explained by a pore blockage mechanism.

References

- (1) Coughlin, R.W., Deshaies, M.R. and Davis, E.M. *Environ. Prog.* 1990, 9, 35
- (2) Eiden, C.A., Jewell, C.A. and Wightman, J.P. *J. Appl. Polym. Sci.* 1980, 25, 1587
- (3) Muzzarelli, R.A.A., Tanfani, F. and Emanuelli, M. *Carbohydr. Polym.* 1984, 4, 137
- (4) Muzzarelli, R.A.A., Tanfani, F., Emanuelli, M. and Bolognini, L. *Biotechnol. Bioeng.* 1985, 27, 1115
- (5) Jha, I.N., Iyengar, L. and Prabhakara Rao, A.V.S. *J. Environ. Eng. ASCE*, 1988, 114, 962
- (6) Udaybhaskar, P., Iyengar, P. and Prabhakara Rao, A.V.S. *J. Appl. Polym. Sci.* 1990, 39, 739

- (7) Guibal, E., Saucedo, I., Roussy, J., Roulph, C. and Le Cloirec, P. *Water SA* 1993, **2**, 119
- (8) Guibal, E., Saucedo, I., Roussy, J., and Le Cloirec, P. *React. Polym.* 1994(a), **23**, 147
- (9) Guibal, E., Saucedo, I., Jansson-Charrier, M., Delanghe, B. and Le Cloirec, P. *Wat. Sci. Technol.* 1994(b), **30**, 183
- (10) Peniche-Covas, C., Alvarez, L.W. and Argüelles-Monal, W. *J. Appl. Polym. Sci.* 1992, **46**, 1147
- (11) Argüelles-Monal, W. and Peniche Covas, C. *Angew. Macromol. Chem.* 1993, **207**, 1
- (12) Tanaka, H., Matsumara, M. and Veliky, I.A. *Biotechnol Bioeng.* 1984, **26**, 53
- (13) Jang, L.K., Brand, W., Resong, G M., Mainieri, W. and Geesey, G.G. *Environ. Progress.* 1990, **9**, 269
- (14) Rorrer, G.L., Hsien, T.Y. and Way, J.D. *Ind. Eng. Chem. Res.* 1993, **32**, 2170
- (15) Kawamura, Y., Mitsuhashi, M., Tanibe, H. and Yoshida, H. *Ind. Eng. Res.* 1993, **32**, 386
- (16) Masri, M.S., Garcia Randall, V. and Piiman, A.G. *Polym. preprints. Am. Chem. Soc., Division of polymer Chemistry.* 1978, **19**, 483
- (17) Tong, P., Baba, Y., Adachi, Y. and Kawazu K. *Chem. Lett.* 1991, 1529
- (18) Hsien, T.Y. and Rorrer, G.L. *Sep. Sci. Technol.* 1995, **30**, 2455
- (19) Domszy, J.G. and Roberts, G.A.F. *Macromol. Chem.* 1985, **186**, 1671
- (20) Baxter, A., Dillon, M., Taylor, K.D.A. and Roberts, G.A.F. *Int. J. Biol. Macromol.* 1992, **14**, 122
- (21) Rodier, J. *L'analyse de l'eau, eaux naturelles, eaux résiduaires, eaux de mer.* 8^{ème} édition, 1996, Paris, Dunod.
- (22) McKay, G. Sorption of metal ions by chitosan. In: *Immobilisation of ions by biosorption.* H. Eccles and S. Hunt, Eds., Ellis Horwood Limited : Chichester, U.K., 59-69, 1986
- (23) Hand, D.W., Crittenden, J.C. and Thacker, W.E. *J. Environ. Eng. Div.* 1983, **109**, 82
- (24) Weber, W.J. and Morris, C.J. *Advances in water pollution research.* Proc. 1st Int. Conf. on Water Pollution Res., Pergamon Press, New York, 1962
- (25) Hellferich, F. *Ion Exchange*, McGraw Hill Book Company Inc., New York. 1962
- (26) Michelsen, L.D., Gideon, P.J., Pace, E.G. and Kutal, L.H. Removal of solute mercury from wastewater by complexing techniques. U.S.D.I Office of Water Research and Technology Bull. No. 74. 1975
- (27) McKay, G., Findon, A. and Blair, H.S. *J. Environ. Sci. Health.* 1993, **A28 (1)**, 173

INTERACTIONS BETWEEN CHITOSAN AND RADIOACTIVE ELEMENTS DIFFERENT FROM URANYL IONS

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Abstract

This paper deals with the study of the interactions of chitosan with three radioactive elements : ^{238}Pu , ^{241}Am and ^{85}Sr . We determined the optimal experimental conditions for each of them (pH, ionic strength, carbonate concentration, DA of the polymer, ...) and attempted to explain the mechanism of interaction. In this case, the study of the nature and the percentage of the metallic ionic species present in solution as a function of the experimental conditions was performed by means of the TOT software. We showed that the addition of carbonate ions made the interactions between chitosan and strontium ions possible and concluded to the formation of a ternary complex $-\text{NH}_2\text{-Sr}^{++}\text{-CO}_3^{2-}$. We also showed a good fixation of ^{238}Pu and ^{241}Am on chitosan for pH's between 11-12.

Keywords : Chitosan, Chitin, ^{238}Pu , ^{241}Am , ^{85}Sr , Carbonate ions, Ternary Complex.

Introduction

Chitosan is an amino polysaccharide derived from chitin, one of the most widespread polysaccharides in the biomass. For the last forty years, a lot of researches have been done about the interactions between chitosan and different metals, particularly heavy metals [1,2]. This polymer is now recognized as a very interesting biosorbant thanks to the free doublet of its nitrogen atoms. Among radioactive elements, uranyl ions fixation is now well explained [3], but none or few studies were carried out with α emitters like plutonium or americium and γ emitters like ^{85}Sr . The removal of such radioactive elements from industrial effluents is very important with regard to the pollution of these long-life radioactive elements. Moreover, some studies were performed about the interactions between chitosan and alkaline-earth elements, but this polymer seemed to be ineffective towards alkaline or alkaline-earth metal ions. Nevertheless, some interactions of these elements with chitin or chitosan derivatives (phosphorylated [4] or carboxymethyl [5] derivatives) have been shown and the mechanism proposed was a chelation process.

In this paper, we are interested with the study of the interactions of chitosan with three radioactive elements, two from the actinide elements : ^{238}Pu , ^{241}Am and one from the alkaline-earth one : ^{85}Sr . No interaction was detected between chitosan and strontium ions alone, confirming the results of a lot of researches. In contrast, we showed that the addition of carbonate ions renders these interactions possible. We determined then the optimal experimental conditions (pH, carbonate concentration, ionic strength (FI), physical form and DA of the polymer). In the case of plutonium and americium, we also showed that a good fixation was obtained and studied the role of the same parameters. For

these three ions, we attempted to explain the mechanism of interaction and determined thus the nature and the percentage of the metallic ionic species present in solution by means of the TOT software [6].

Materials and methods

Material

Chitin and chitosan (batch n° A32E03 and A13G19) were samples from Aber Technologies (France). They were in the form of flakes of average length 2,5mm. Their acetyl contents (DA) were determined by I.R. spectrometry on films obtained from solutions of chitosan [7,8] and on KBr pellets of chitin powder. The DA was found to be 2% for chitosan-batch A32E03, 10% for chitosan-batch A13G19, and 70% for chitin. These chitin and chitosan samples were crude products used as received from the manufacturer. Chitosan nitrate in a lyophilised form was prepared as previously described [9]. A lyophilised form of chitin was also used and obtained in a similar manner as described in a previous paper [3].

^{85}Sr , ^{238}Pu and ^{241}Am were samplings of dilute solutions provided by the CEA (France) dispersed in deionized water containing NaNO_3 and Na_2CO_3 at different concentrations.

The different salts used : NaNO_3 , Na_2CO_3 and NaOH were from Aldrich (Normapur).

Methods

In order to define the most appropriated experimental conditions (pH, ionic strength, carbonate concentration, physical form of the polymer, ...) kinetics studies were performed in 100 to 200 ml of deionized water containing various salts at different concentrations for each element. The concentration of ^{85}Sr was near 7 to 10×10^{-12} mol/l, corresponding to an activity of the solution of 600 to 800 kBq/l. In the case of ^{238}Pu , ^{241}Am , the concentrations were respectively near 10^{-9} and 10^{-8} mol/l, corresponding to a solution activity of 350 to 450 kBq/l. Chitin or chitosan were added directly in the radioactive solution at the beginning of the kinetics, without previous hydration, except in one case (see below). The pH of the media was adjusted with NaOH or HNO_3 1M. Samplings of about 7ml were taken at chosen time and centrifuged (5000 tr/min) to eliminate the possible polymer particles in suspension. The initial and remaining activity of the different solutions was determined in 5 ml of the supernatants by γ spectrometry for ^{85}Sr , and by α scintillation for ^{238}Pu and ^{241}Am . These activities are directly proportional to the concentration.

A possible precipitation of ^{85}Sr has been investigated in all the conditions of pH and ionic strength studied, but none appeared considering the state of trace of ^{85}Sr in solution. In the case of the actinides elements, the formation of polymerized, precipitated or colloidal species has been found until a concentration in solution as low as 10^{-11} mol/l of these two elements [10]. In order to bypass this problem, we made all the experiments twice, one with polymer and the other without and considered the difference. The results given in this paper take thus into account a possible precipitation.

Techniques

All the experiments were performed at the CEA (Cadache) in conditions of high protection against possible radioactive contamination.

Characterization of the DA was made by means of a Perkin-Elmer 1760 FT-IR spectrophotometer (10 scans) on films or KBr pellets. The pH of the dispersion was verified with a Tacussel Minisis 8000 pH meter equipped with a double junction glass electrode. The results of fixation were deduced after determination of the activity of ^{85}Sr in the supernatants with a γ spectrophotometer EGSP 2000-20R from EURISYS Mesures. The counting were made on 5 ml liquid samplings, during 5 to 10 minutes and results were given in kBq/l. For ^{238}Pu and ^{241}Am the activity were determined with a α,β liquid scintillation counter WALLAC 1409-011 on samplings containing 0,1 ml of radioactive solution and 14.9 ml of scintillating liquid (Instagel).

Results and discussion

* Interactions with Strontium 85

We were first interested with ^{85}Sr . According to the litterature, chitosan seems to be ineffective towards alkaline or alkaline-earth metal ions [11]. In this work we verified that no fixation of ^{85}Sr appeared on chitosan, whatever the pH. In contrast, we showed that the addition of carbonate ions to the media made these interactions possible, but only for a pH over 11. These interactions are optimum for a pH close to 12.5. As the pKa of dissociation of hydrogenocarbonate ions is 10.35, we can suppose that it is the presence of carbonate ions which is necessary for the interactions between chitosan and ^{85}Sr . We have thus studied these interactions at pH 12.5, they are characterized by the decontamination factors (FD), such as :

$$\text{FD} = A_i / A_f \quad \text{with} \quad A_i, \text{the initial activity of the solution (kBq/l) and} \\ A_f, \text{the final activity of the solution (kBq/l).}$$

Influence of carbonate concentration

As we showed that without carbonate ions no interaction appeared, we first studied the influence of their concentration, at the optimum pH of 12.5. We used the lyophilised nitrate chitosan of DA 2% at a concentration of 3 g/l, and varied the Na_2CO_3 concentration from 10^{-3} to $5 \cdot 10^{-1}$ mol/l. The decontamination factor obtained after five hours of polymer-solution contact are represented on Figure 1. No interaction appeared for carbonate concentration below 10^{-2} mol/l, but they were maximum for 10^{-1} mol/l and then decreased progressively with a further increase of the carbonate concentration.

In order to understand this evolution, we determined the repartition curves of the strontium species present in solution for all the different salt concentrations with the use of the TOT software [6]. The percentage of the two carbonated species present at pH 12.5 versus the carbonate concentration of the solution are reported Figure 2. We observed that the evolution of SrCO_3^0 appeared very similar to the evolution of the decontamination factor (Figure 1). This could signify that SrCO_3^0 is the ionic species which interacted with chitosan. But we also noticed the occurrence of the species $\text{Sr}(\text{CO}_3)_2^{2-}$ in solution for a carbonate concentration of 0.1 mol/l. This species could also be involved in the interactions with chitosan. For important carbonate concentrations ($5 \cdot 10^{-1}$ mol/l), an important decrease of fixation is visible, probably due to the increase of the ionic strength

as this evolution does not correspond to a lower percentage of the carbonated strontium species present in solution.

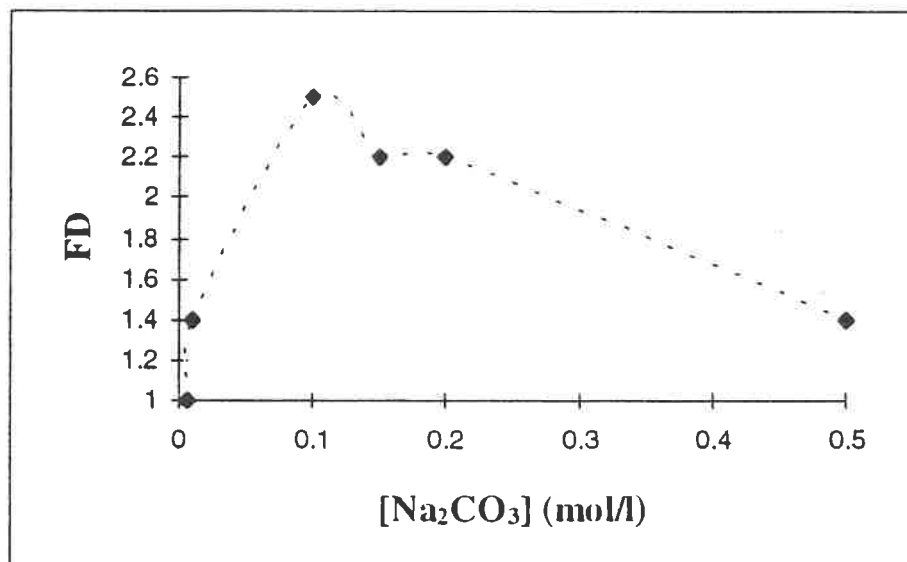


Figure 1. Variation of FD of the ^{85}Sr solution ($A_i = 600 \text{ kBq/l}$) versus Na_2CO_3 concentration after the addition of chitosan (3 g/l) and 5 hours of polymer-solution contact.

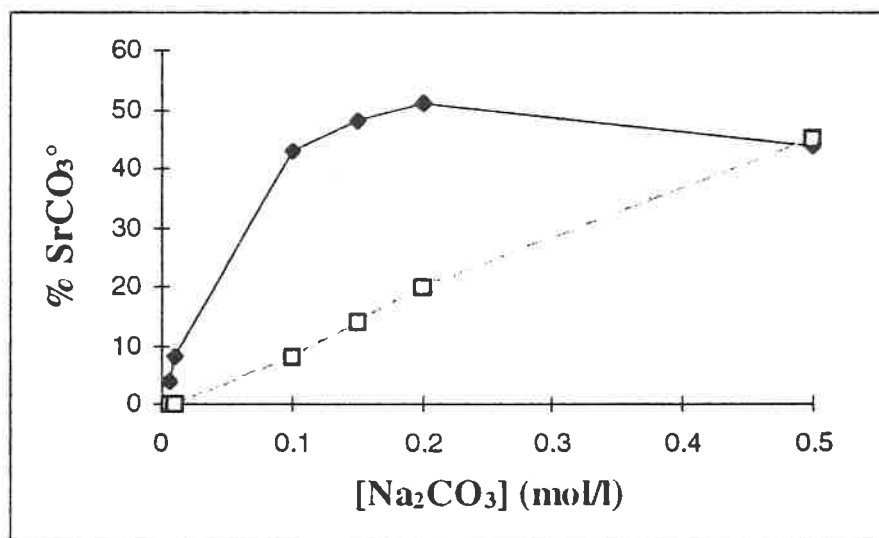


Figure 2. Variation of the carbonated strontium species in solution versus Na_2CO_3 concentration : -♦- SrCO_3° , - - $\text{Sr}(\text{CO}_3)_2^{2-}$

Influence of the ionic strength

The influence of the ionic strength was then studied at pH 12.5, with the optimal carbonate concentration (0.1 mol/l), by varying the NaNO_3 concentration from 0 to 50 g/l. Ionic strength corresponding to these different concentrations and the decontamination factors obtained in each case after three hours of polymer-solution contact are presented in Table 1. The maximum of fixation was obtained for the lower ionic strength and progressively decreased with increasing this ionic strength. We cannot explain this decrease by an possible evolution of the nature of the strontium ionic species in solution, as SrCO_3° and $\text{Sr}(\text{CO}_3)_2^{2-}$ remain largely present whatever the NaNO_3 concentration.

Moreover, at such an alkaline pH, interactions of electrostatic nature cannot appear and explain this evolution of fixation. Indeed, if there was electrostatic interactions between the alcoholate groups present on chitosan at this pH and the positively charged strontium ions, carbonate ions would not be necessary. If electrostatic interactions arised with $\text{Sr}(\text{CO}_3)_2^{2-}$ species, it would necessitate positively charged groups on chitosan, but none are present at pH 12.5. The influence of the ionic strength could be explained by the weak formation constant of SrCO_3 ($\log \beta = 1.3$ [12]) which allows us to assimilate this species to an ion pair. An increase of the ionic strength will thus :

- induce a decrease of the formation of SrCO_3 ,
- have a shielding effect.

Table 1. Influence of the NaNO_3 concentration on the $\text{FD}(\text{chitosan}/^{85}\text{Sr})$ for solutions containing Na_2CO_3 at 10^{-1} mol/l and ^{85}Sr at 10^{-12} mol/l ($A_i=600$ kBq/l), after addition of lyophilised nitrate chitosan (DA 2%) at 3 g/l and for 3 hours of polymer-solution contact.

NaNO_3 (g/l)	0	5	10	15	25	50
FI	0.33	0.39	0.45	0.51	0.63	0.92
FD (3h)	3.2	2.9	2.6	2.5	2.3	2.1

The negative influence of the ionic strength explains the decrease of fixation observed previously for important carbonate concentrations ($5 \cdot 10^{-1}$ mol/l). We can deduce from these results that the interactions involve the species SrCO_3 and are not of electrostatic nature.

Influence of the physical form and of the acetylation degree of chitosan

We then studied the influence of the DA in order to understand if the chitosan amino groups were involved or not in these interactions. Different chitosan were then tested at a concentration of 10 g/l :

- a lyophilised nitrate chitosan of DA 2%
- a lyophilised nitrate chitosan of DA 10%
- a lyophilised chitin of DA 70%
- a crude chitosan of DA 2%

The FD obtained after 5 hours of polymer solution contact are given in Table 2.

Table 2. Influence of the DA and the physical form of the polymer (10 g/l) on the $\text{FD}(\text{chitosan}/^{85}\text{Sr})$ in a solution containing Na_2CO_3 at 10^{-1} mol/l and ^{85}Sr at 10^{-12} mol/l ($A_i=600$ kBq/l) after 5 hours of polymer-solution contact.

	Chitosan nitrate (DA 2%)	Chitosan nitrate (DA 10%)	Lyophilised chitin (DA 70%)	Crude chitosan (DA 2%)
FD (5h)	5.9	2	1	1.3

We observed a decrease of fixation with an increase of the acetylation degree, for a DA of 70%, no more interaction appeared. The same result was obtained with an increase of the bulk density and of the crystallinity of the polymer. Indeed, the crude chitosan has a DA of 2%, but its bulk density was 0.12 g/cm^3 and its percentage of crystallinity 40% (as previously determined [9]). In the case of the lyophilised nitrate chitosan of DA 2%, the bulk density was only 0.04 g/cm^3 and the percentage of crystallinity 14% [9]. As

previously shown for the uranyl ions [9], we can conclude that the amino groups are involved in the interactions, and that the higher the accessibility to these groups, the more important are the interactions.

Proposition of mechanism

From these results we can deduce that moderate interactions exist between chitosan, carbonate ions and strontium ions. These interactions are not of electrostatic nature, but involve the chitosan amino group and the ion pair $\text{Sr}^{++}\text{CO}_3^{2-}$, they could be interpreted by the formation of a ternary complex between these ion pairs and the amino groups, as proposed on the Figure 3.

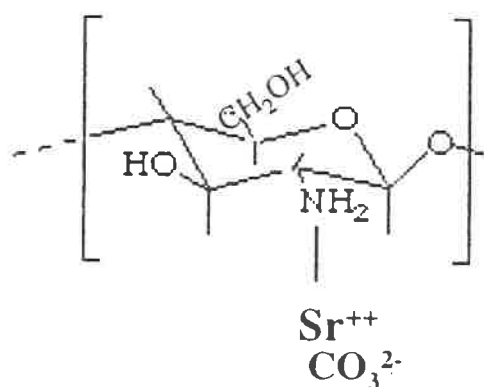


Figure 3. Proposed representation of the ternary complex between chitosan, strontium ion and carbonate ion.

The same behaviour has been observed with Baryum, another alkaline-earth element : to observe interactions with chitosan, the presence of carbonate ions at a pH over 11 was necessary. It seems thus that we can generalize the existence of interactions between alkaline earth elements and chitosan in the presence of carbonate ions.

* Interactions with ^{248}Pu and ^{241}Am

The fixation of α emitters has been carried out in deionized water containing NaNO_3 0.17 mol/l, with lyophilised nitrate chitosan of DA 2% at a concentration of 1 g/l. We took the precautions precised before (see *Materials and methods*) in order to have reliable results.

The influence of the pH has first been studied, and the FD obtained with ^{241}Am and ^{238}Pu are represented Figures 4 and 5 versus the pH after 2 hours of polymer-solution contact.

In the case of ^{241}Am , good fixation was obtained from pH 8 to 12.5 with FD over 20. For ^{238}Pu , fixation occurred only for pH's within 11-12.5 and was maximal at pH 12. The use of TOT software for americium allowed us to determine the nature of the species present in the solution versus the pH (for a carbonate concentration corresponding to the atmospheric conditions, a sodium nitrate concentration of 0.17 mol/l and an americium conditions of 10^{-8} mol/l) :

pH 7-8.5	: $\text{Am}(\text{OH})_2^+$
pH 8.5-12.5	: $\text{Am}(\text{OH})_3^0$
pH > 12.5	: $\text{Am}(\text{OH})_4^-$

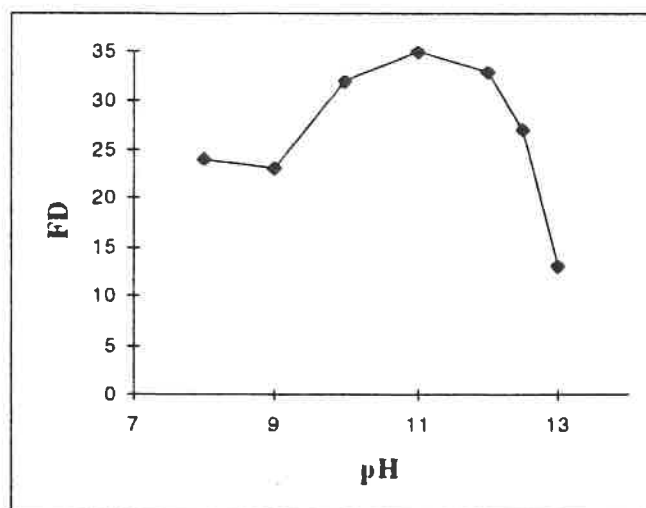


Figure 4. Variation of the $FD(\text{chitosan}/^{241}\text{Am})$ versus the pH after 2 hours of kinetics for a chitosan concentration of 1 g/l and a ^{241}Am concentration of 10^{-8} mol/l.

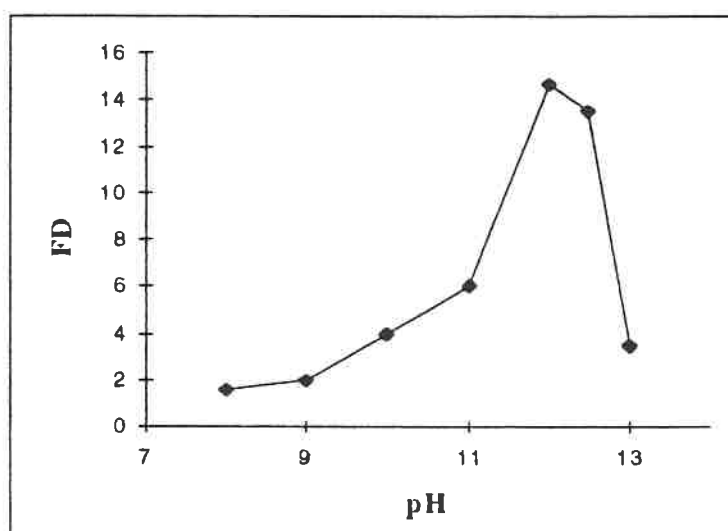


Figure 5. Variation of the $FD(\text{chitosan}/^{238}\text{Pu})$ versus the pH after 2 hours of kinetics for a chitosan concentration of 1 g/l and a ^{238}Pu concentration of 10^{-9} mol/l.

We can suppose that interactions with the species $\text{Am}(\text{OH})_3^0$ are majoritary. In contrast, at pH over 12.5, the appearance of the anionic species $\text{Am}(\text{OH})_4^-$ could explain the decrease of fixation, due to the electrostatic repulsion of this species with alcoholate groups present on chitosan. For plutonium, the exact simulation of the ionic species present in solution has not been possible, but we can suppose that the neutral species $\text{Pu}(\text{OH})_4^0$ is majoritary at pH's 11 -12 and interacts with chitosan. For higher pH's, the presence of the anionic species $\text{Pu}(\text{OH})_5^-$ could explain the decrease of the interactions, as for americium. We suppose that for these two α emitters, the interactions involve the chitosan amino groups, as for the other heavy metal ions.

We have then studied the role of the ionic strength for the two elements, at pH 12.5, by varying the sodium nitrate concentration. An increase of the ionic strength induced a strong decrease of the interactions. This could be explained by :

- the shielding effect,
- a modification of the pKa of the alcoholate groups of chitosan to a lower value.

The addition of carbonate ions has also be tested, as carbonate are strong complexant of these two elements [13]. At pH 12.5, the addition of carbonate induces the formation of strong hydrogenocarbonate and carbonate complexes of americium or plutonium, complexes which no longer interact with chitosan.

Conclusion

This work allow us to conclude that interactions of chitosan with alkaline-earth elements is possible, but only with the presence of carbonate ions and at a pH over 11. These interactions are very weak in comparison with those observed with heavy metals. The proposed mechanism of fixation is the formation of a ternary complex between an ion pair (alkaline-earth ion / carbonate ion) and the amino group of chitosan. We also showed interactions of chitosan with α emitters, the optimal pH being within 11 and 12. A high ionic strength or the addition of complexants hinder these interactions. We suppose that the interactions involve the chitosan amino group.

References

- [1] Tsezos, M. and Volesky, B. *Biotechnol. Bioeng.*, 1981; 23:583.
- [2] Domard, A. *Int. J. Biol. Macromol.*, 1987; 9:98.
- [3] Piron, E. and Domard, A. To be Published In *Int. J. Biol. Macromol.*, Parts 1 and 2.
- [4] Nishi, N., Maekita, Y., Nishimura, S.I., Hasegawa, O. and Tokura, S. *Int. J. Biol. Macromol.*, 1987; 9:109.
- [5] Tokura, S., Nishimiura, S.I. and Nishi, N. *Pol. Journal*, 1983; 5:597.
- [6] Rosset, R., Bauer, D. and Desbarres, J. In: *Chimie des solutions et informatique* (R. Rosset, D. Bauer, and J. Desbarres, eds), Masson, Paris, 2nd Ed, vol.1, 1991.
- [7] Varum, K.M., Anthonsen, M.W., Grasdalen, H. and Smisrod, O. *Carbohydrate Research*, 1991; 211: 17.
- [8] Miya, M., Iwamoto, P., Yoshikawa, S. and Mima, S. *Int. J. Biol. Macromol.*, 1980; 2:323.
- [9] Piron, E., Accominotti, M. and Domard, A. *Langmuir*, 1997; 13:1653.
- [10] Piron, E. *Ph.D. Thesis*, C. Bernard Lyon I University ; Lyon, France, 1997.
- [11] Hsien, T.Y. and Rorrer, G.L. *Separation Sc. and Techn.*, 1995; 30:2455.
- [12] Högfeld, E. In *IUPAC Chemical Data*, Series 21 (Pergamon Press, eds) Oxford- New York, 1982.
- [13] Weigel, F., Katz, J.J. and Seaborg, G.T. In *The chemistry of the actinides elements - Plutonium* (Chapman and Hall, eds), London-New York, 1986.

RHEOLOGICAL PROPERTIES OF CHITOSAN AND ITS BLENDS IN SOLUTION

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Abstract

The present work is concerned with rheological characteristic properties of semi dilute chitosan solutions. Effects of deacetylation degree and molecular weight of chitosan with changing solution concentration and pH, a shear rate and a temperature are investigated. In general, chitosan solutions behave as a typical non-Newtonian shear-thinning fluid. Because of a strong intermolecular interaction the chitosan molecules have a tendency to form a network. It was found that the solution structure and thus its rheological behavior (also the apparent activation energy for viscous flow) depend very strongly on the concentration of solution, deacetylation degree of chitosan and shear rate applied but weakly on pH.

The effect of weight fraction of chitosan in the blend with PEO and PVA in solution on the rheological characteristics was found and discussed. For example a negative deviation from the additivity of parameter A indicates on good compatibility of the components in acetic aqueous system due to the presence of polar interactions between macromolecules.

Keywords: Chitosan solutions, rheological parameters, blends, polyethylene oxide, polyvinyl alcohol, pH effect.

Introduction

Chitosan is a biopolymer with unique properties including bioactivity, biocompatibility and biodegradability favorable for a variety of industrial and biomedical applications.(1-5)

At present chitosan is produced mainly by the thermochemical deacetylation of chitin. Degree of deacetylation of chitosan has been found to influence physical and chemical properties and biological activities of chitosan.

Chitosan fibrils, sheets, films, membranes and fibers are prepared from solutions. Thus the data have been accumulated on the solubility and rheological characteristics and fiber forming ability of chitosan. They have formed the basis for further applied research and for commercial applications.

Chitosan forms ionic complexes with a number of water soluble polymers like poly (ethylene oxide) - PEO, poly (vinyl alcohol) - PVA. (6,7) Rheological behavior of chitosan solution and the compatibility in solution of chitosan (DD = 73.3 %) with PEO ($M_w = 1 \cdot 10^5$) and PVA (M_w - low) were investigated.

Materials and methods

The examined chitosan is to be made of α -chitin obtained from Antarctic Krill's shells. It was produced in the Sea Fishery's Institute (Gdynia, Poland) by thermochemical deacetylation of the chitin. Chitosan samples with various deacetylation degree (DD) from 62.8 to 86.7 % and molecular weight (M_w) from 1.05 to $5.5 \cdot 10^5$ were investigated.

Polyethylene oxide (PEO) of $M_w = 1 \cdot 10^5$ was obtained from Polysciences, Inc., polyvinyl alcohol (PVA) of low M_w was a commercial product.

The aqueous solution of acetic acid (1 wt %) was used as a solvent for the chitosan of various DD. Aqueous solutions of PEO and PVA were prepared reaching the maximum concentration 5g/100ml and 10g/100ml, respectively.

Blend solutions were prepared by mechanical mixing of chitosan (DD=73.3 %) and the respective polymer solutions in relevant proportions. Semi-dilute chitosan (DD=78.4 %) systems of 3 wt % and 1.5 wt % in varying aqueous solutions of 0.5, 1 and 5 % acetic acid were also prepared to study pH effect on their rheological properties.

Flow measurements were carried out using a rheometer with concentric cylinder and cone-plate units-Rheotron (version 1.8) over a range of temperatures 20-45°C and with various shear rates up to 10^3 1/s divided into three ranges of shear rate: up to 10^1 1/s, 10^2 1/s and 10^3 1/s or $0.5 \cdot 10^3$ 1/s. The studies were performed in the three different steps:

1. Rheological studies of semi-dilute chitosan solutions in 1 % aqueous solution of acetic acid (various DD and M_w of chitosan) with changing concentration of chitosan and temperature under study.
2. Rheological studies of chitosan (chosen sample, DD = 78.4 %) solutions with varying pH value of the system.
3. Rheological measurements on the chitosan blends with PEO and PVA in solutions of various concentration and weight fractions of the components.

All the systems investigated have been found to obey the power-law equation:

$$\tau = A \cdot \dot{\gamma}^n, \quad \eta = \tau / \dot{\gamma} = A \cdot \dot{\gamma}^{n-1} \quad (1)$$

where: τ -shear stress, η -shear viscosity, n and A -rheological parameters.

Results and discussion

It was noticed that a rheological behavior of chitosan macromolecules in solution depends on many factors such as deacetylation degree, molecular weight, polymer concentration, shear stress, pH of the solution. In order to gain a more detailed insight into these phenomena we have carried out rheological measurements on semi-dilute systems of chitosan solution and in the presence of various amounts of the water soluble polymer such as PEO and PVA.

Chitosan solutions-concentration and DD effect.

The results on rheological characteristics of semi-dilute solution of chitosan of various DD and M_w in 1% acetic acid has been previously reported in detail (8). Here we have presented some results of the studies. Figure 1 shows the shear viscosity drawn versus solution concentration found for various samples of chitosan with DD ranging from 62.8 % to 86.7 % (sample 1-5) measured at 25°C. The shift procedure is used to calculate master curves from the shear viscosity data of the chitosan solution dependent on temperature and concentration. Figure 2 presents one of the non well fitted curves

(DD=78.4 %) obtained using the procedure. At increasing concentrations of chitosan the flow curves present a more pronounced non-Newtonian behavior (parameter n decreases) (8, 9), due to increasing physical interactions between entangled chitosan macromolecules and internal friction of the system.

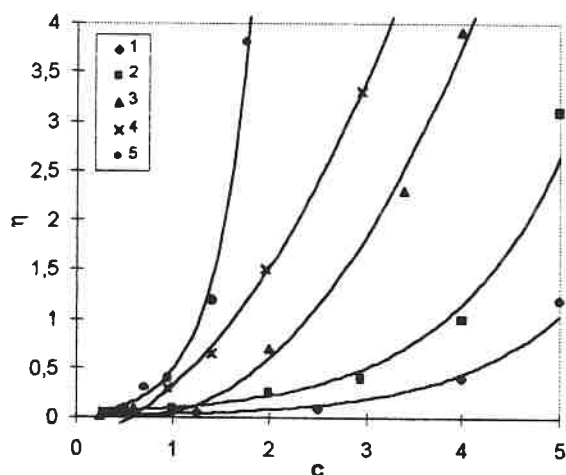


Fig. 1. Shear viscosity η (for shear rate $\dot{\gamma}=2 \cdot 10^2$ 1/s) at 20°C versus concentration c drawn for samples 1-5 (curves 1-5).

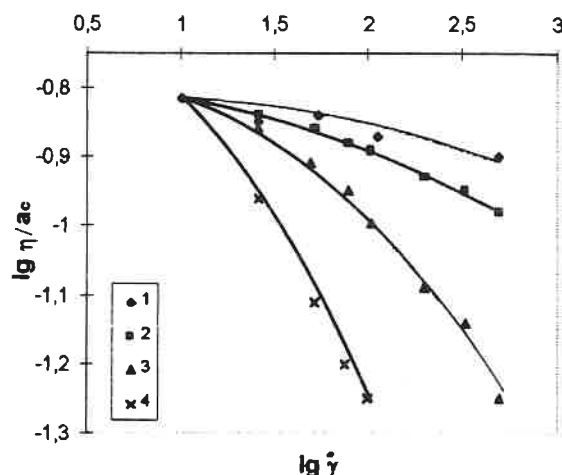


Fig. 2. Vertical shifting of shear viscosity-shear rate plots of various concentrations c of chitosan solutions: curves 1-4 for $c=0.5, 1.0, 2.0, 4.0$ g/100ml, respectively; $a_c=c/c_0$. Where $c_0=1$ g/100ml, $\dot{\gamma}=10$ 1/s.

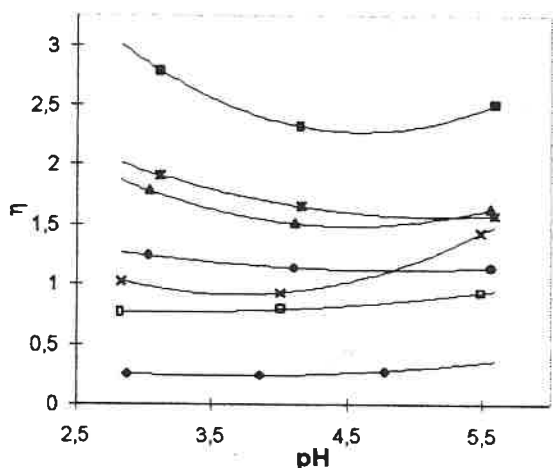


Fig. 3. Shear viscosity ($\dot{\gamma}=10$ 1/s upper curves and $\dot{\gamma}=100$ 1/s lower curves) measured at $25, 35$ and 45°C drawn versus pH value of solution, concentration of solution $c=1.5\%$ (—♦—) and 3% (others).

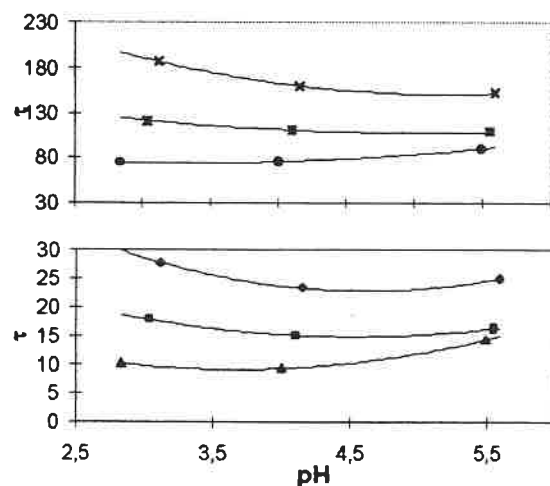


Fig. 4. Shear stress ($\dot{\gamma}=100$ 1/s, upper curves and $\dot{\gamma}=10$ 1/s, lower curves) measured at $25, 35$ and 45°C , drawn versus pH value of solution $c=3\%$.

The apparent activation energy of viscous flow E_a , also calculated from Arrhenius plots depends on solution concentration (which is shown in Figure 10 (DD = 78.4 %)) reflecting the influence of the solution structure (see also paper (8)). The value of the apparent activation energy for viscous flow of chitosan solutions depends on few factors: the

concentration of solution, shear rate applied (decreases with increasing shear rate) and deacetylation degree. All the facts are attributed to the molecular structure of the solution due to a polymer-solvent interaction (quality of the solvent) and the presence of entanglements changing with the shear stress applied or concentration.

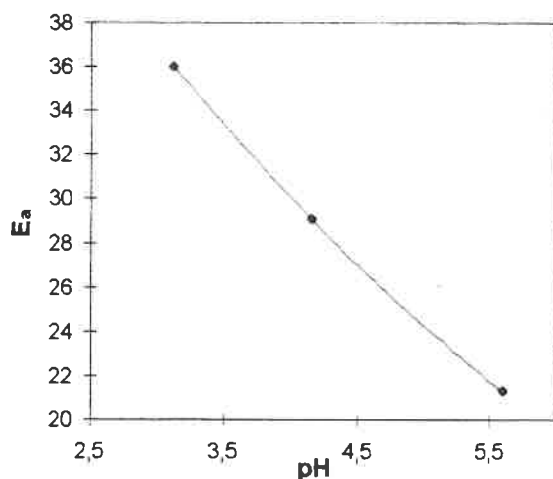


Fig. 5. Activation energy for viscous flow versus pH value, $c=3\%$.

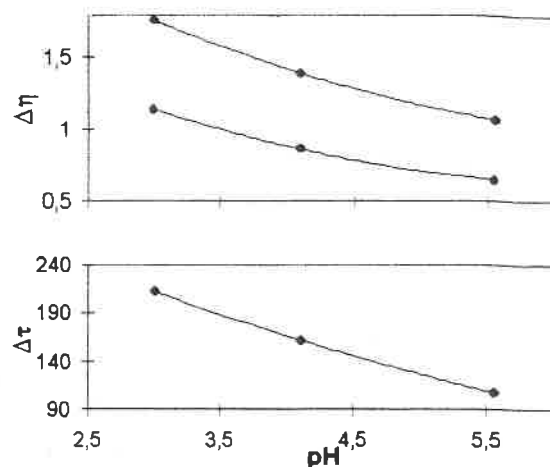


Fig. 6. Change of shear viscosity $\Delta\eta$ ($\dot{\gamma}=10$ 1/s upper curve and $\dot{\gamma}=100$ 1/s) and shear stress $\Delta\tau$ ($\dot{\gamma}=500$ 1/s) for $\Delta T=20^\circ\text{C}$ versus pH value of solution; $c=3\%$.

Chitosan solutions-pH effects.

In order to get more data on the rheological phenomena of chitosan solution the measurements at different pH values of solution were carried out. The results of a shear viscosity, a shear stress and an apparent activation energy for viscous flow are presented in the following Figures 3-6. The observed effect of pH on shear viscosity and shear stress (Fig. 3 and 4) is weak. At pH values around 4 most of the amino groups of chitosan are assumed to be protonated (10). Increasing protonation can cause increasing electrostatic repelling between changed groups of the same sign which leads to better dissolution of the polymer molecules from the initial network structure. Increasing pH values above 4 lead to observed small increasing viscosity due to poorer thermodynamic conditions for the solubility of chitosan molecules. The apparent activation energy shows a tendency to decrease with increasing pH value (in the pH range under study) due to worse dissolution and aggregates formation (Fig. 5). Figure 6 presents a decrease of shear viscosity $\Delta\eta$ and shear stress $\Delta\tau$ with increasing temperature by 20°C drawn versus pH value of chitosan solution ($c=3\%$). The higher pH value the larger effect of temperature is observed. In the investigated region of pH value the effect of pH on parameter n is not clear. For shear rate reaching 10 1/s Newtonian flow ($n\approx 1$) and for shear rate reading 500 1/s, non Newtonian flow ($n=0.6\rightarrow 0.75$) behaviors are observed for various pH values.

Chitosan blend solutions

Figure 7 presents the plot of \lg shear viscosity (measured at two temperatures 20°C and 40°C) on weight fraction w_1 of chitosan in the blend solution. Shear viscosity shows positive deviation from additivity in the case of 10 % of PEO and PVA in the blend solution. It can be explained by the formation of some complexes due to interaction between chitosan PEO or PVA molecules through active amino groups and PEO ether

groups or PVA hydroxy groups, which lead to increase of the system viscosity. Increasing amount of PEO and PVA in solution causes an the decrease of the system viscosity.

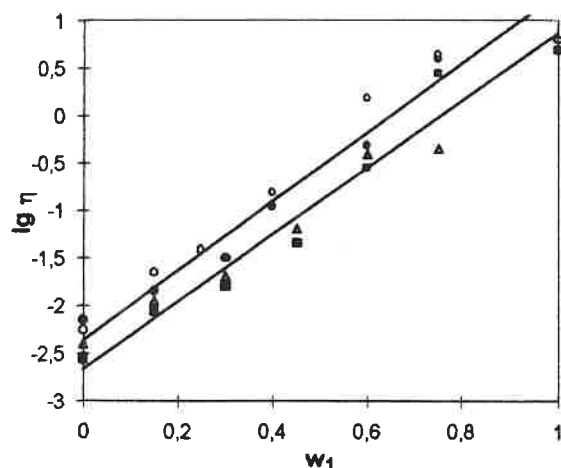


Fig. 7. \lg shear viscosity of blend solutions: chitosan-PEO and chitosan-PVA ($\gamma=100$ 1/s) versus weight fraction of chitosan w_1 at 40°C (lower values) and 20°C ; ○—△—PVA, —●—■—PEO

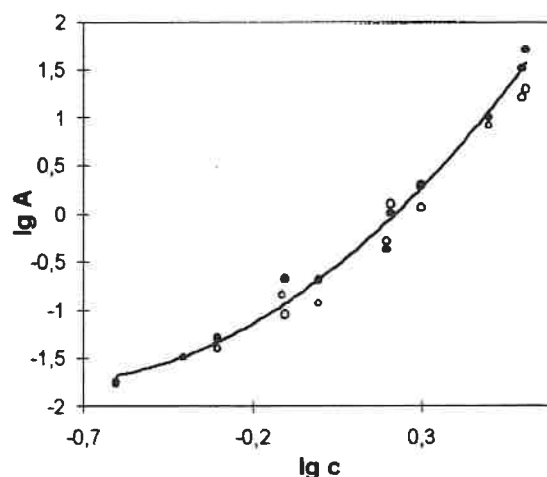


Fig. 8. $\lg A$ versus $\lg c$; c -concentration of chitosan in the blend solution with —●— PEO and —○— PVA.

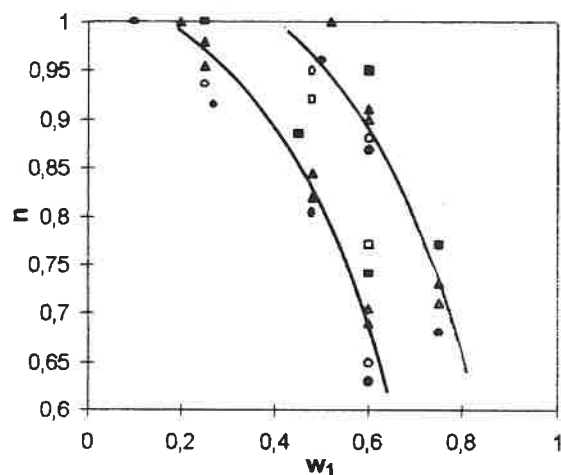


Fig. 9. n parameter found for two regions of shear rate $10 < \dot{\gamma} < 100$ 1/s (curve 1) and $200 < \dot{\gamma} < 500$ 1/s (curve 2) drawn versus weight fraction w_1 of chitosan in the blends $\gamma=100$ 1/s ($c=5$ g/100ml for pure PEO and with PEO and PVA; higher values n for higher temperatures (20° - 40°C).

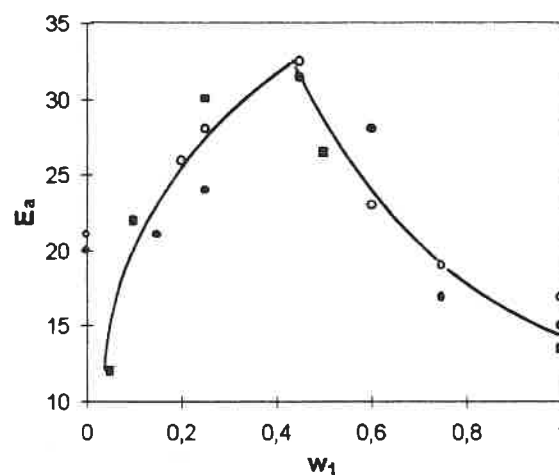


Fig. 10. Activation energy values versus weight fraction w_1 of chitosan in the blend solutions with —●— PEO and —○— PVA ($\gamma=100$ 1/s ($c=5$ g/100ml for pure PEO and with PVA solutions) —■— versus concentration of chitosan solution.

The rheological parameter A (equal $\lg \eta$ for $\dot{\gamma} \rightarrow 0$) drawn in the double \lg scale versus chitosan concentration in solution shows clearly a negative deviation from additivity line (Fig. 8), which indicates compatibility of both components in the solution. At the same time the value of a parameter n increases to 1 with decreasing weight fraction of chitosan, tending to more Newtonian flow character (Fig. 9). Figure 10 shows the apparent activation energy for viscous flow E_a drawn versus weight fraction of chitosan in the

solution and for a comparison E_a value found for pure chitosan solution drawn versus concentration of the solution. The concentrations of pure components were taken $c=5\text{g}/100\text{ml}$ for PEO and PVA in aqueous solution and $c=4\text{g}/100\text{ml}$ for chitosan in 1 % acetic acid solution. The curves show a similar character of dependence. Increasing viscosity of chitosan solution with the concentration of chitosan molecules results from the formation of spacial structures due to intermolecular interaction by polar groups, hydrogen bonds and molecular entanglements. Thus the activation energy for viscous flow reflecting energy of forming molecular cavity into which a molecule flows, when leaving its temporary equilibrium position also increases, when free volume in the liquid decreases with formation of the polymer network structure. Decreasing E_a value for higher concentration of chitosan can be explained by the formation of another structure of chitosan solution, containing larger molecular aggregates or micelles, in the solvent not good enough thermodynamically. A destruction of network structure of the chitosan solution can occur also by increasing a shear rate if the time of creation of the physical crosslinking is much longer than the opposite mechanism of its destruction. It leads also to a decrease in the observed value of the activation energy for viscous flow (8).

Conclusions

Chitosan solutions behaves in a broad range of concentrations as non-Newtonian shear thinning fluids. At high DD values and low concentration of solution Newtonian flow is observed (8,10). Rheological characteristics indicate that in chitosan systems where strong intermolecular interactions are present the network structure in solution shows a tendency to change with solution concentrations, DD and M_w of chitosan, shear rate applied and complexing with others polymers well miscible with chitosan. The decreasing apparent activation energy for viscous flow with increasing shear rate, pH of solution and concentration chitosan in solution above some value results from the disruption of the network structure of molecules or a formation of aggregates in a thermodynamically poor solvent. Negative deviation from additivity of parameter A with increasing chitosan concentration in blend with PEO and PVA indicates some compatibility of the components in solutions by ionic complex formation.

References

1. R.A.A. Muzzarelli, Ch. Jeanieaux, G.M. Gooday, "Chitin in Nature and Technology", Plenum Publ. Co., New York 1986.
2. A.F. Roberts, "Chitin Chemistry", The Macmillan Press Ltd., London 1986.
3. Proceedings of the 6th International Conference on Chitin/Chitosan, Gdynia (1994), pp. 15,31.
4. T. Seo, H. Ohtage, T. Unishi, T. Iijima, *J. Appl. Polym. Sci.* **58**, 633 (1995).
5. W.M. Kulicke, Th. Griebel, M. Bouldin, *Polym. News* **16**, 39 (1991).
6. K. Aoi, A. Takasu and M. Okada, *Macromol. Rapid Com.* **16**, 757 (1995).
7. A. Wrzyszczyński, L. Xia Qu, L. Szosland, E. Adamczak, L.A. Linden and J.F. Rabek, *Polymer Bulletin* **34**, 439 (1995).
8. M. Mucha, *Macromolecular Chem. Phys.* **198**, 471 (1997).
9. K.R. Holme, L.D. Hall, R.A. Speers, M.A. Tung, **225**, 307 (1992).
10. A-L. Kjoniksen, B. Nyström, T. Nakken, O. Pahngrén and T. Tande, *Polymer Buletin*, **38**, 71 (1997).

POLYELECTROLYTE COMPLEX FORMATION WITH CHITOSAN

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Abstract

A commercial sample of chitosan, i.e. a highly deacetylated chitin, was characterized physicochemically by means of potentiometry and viscometry. From the potentiometric titration curves the degree of deacetylation was determined to be 75% and an acidity constant of $pK_a^0 = 6.2$ was found for the amino groups. Based on the $[\eta]$ - M relation, the molecular weight was determined viscometrically to be $3,59 \times 10^5$ g/mol.

The complex formation behaviour of the chitosan-HCl solutions at pH=3 and pH=6 was investigated by using a combination of polyelectrolyte titration techniques, i.e. potentiometry, conductometry and turbidity. The stoichiometry at the titration endpoint was investigated as a function of the polyanion added. Significant deviations from a 1:1 stoichiometry were obtained when using Na-polyacrylates at pH=3 because of the partial deionization of the carboxylic groups. Light scattering measurements showed significant deviations between the packing density and mass of the polyelectrolyte complex particles as a function of the degree of conversion when using Na-polystyrenesulfonate as polyanion but not in the case of Na-polyacrylate.

The results clearly indicate that the conformation of the chitosan-HCl is of predominant importance with regard to the resulting polyelectrolyte complex stoichiometry but not the degree of dissociation of the amino groups.

Up to a degree of conversion of 20% the generation of new similar shaped complex particles is characteristic for the PAA-chitosan system in contrast to the PSS-chitosan system, where aggregation and compaction of the primary complex particles is of relevance, too.

Keywords: chitosan, polyelectrolyte complexes, light scattering, stoichiometry

Materials and methods

Materials: The components used are given in Table 1. The Na-polyacrylates, PAA I and II were obtained by Fluka[®], Na-polystyrene sulfonate (PSS) by Aldrich[®]; chitosan was a commercial product. All polyelectrolytes were used without further purification.

Table 1

Polyelectrolyte	Molecular weight (g/mol)	Elemental analysis
PAA I	63,000*	--
PAA II	235,000*	--
PSS	70,000 ^x	--
Chitosan	359,000**	degree of deacetylation: 73.5%

* molecular weight determined by GPC (M_w)

** molecular weight determined by viscosimetry (M_η)

^x molecular weight given by manufacturer

Methods

Titration experiments: The titration experiments (chitosan characterization, polyelectrolyte complex formation with chitosan) were carried out by using a combination of potentiometry, conductometry, and turbidimetry.^{1,2}

To the excess component (chitosanhydrochloride, 0.04% w/w) a defined solution of an oppositely charged titrant component was slowly added under stirring at a flow rate of 5ml/h to maintain equilibrium conditions. The changes in turbidity, conductance, and pH were automatically registered as functions of the titrant's volume (Figure 1 and 3).

Static light scattering: Static light scattering investigations of polyelectrolyte complexes consisting of chitosanhydrochloride and PAA II as well as chitosanhydrochloride and PSS were performed with a motorized goniometer (SEM-633 by SEMAtech) at 632.8 nm at a scattering angle α' with $30^\circ < \alpha' < 150^\circ$. The samples were placed in a temperature-controlled measuring cell.

The preparation procedure involved a filtration of every sample through a 200 nm microfilter in order to remove particulates from the solutions investigated.

The initial concentration of the aqueous chitosanhydrochloride solutions was chosen with 0.04% w/w regarding to the titration conditions. The structural parameters of symplex particles were determined at several degrees of conversion of complex formation in 1m NaCl solution at pH=3 as well as pH=6 by using Zimm plot.

Results and discussion

- Chitosan characterization

In *Figure 1* the potentiometric back titration of a chitosanhydrochloride solution is given together with the turbidimetric titration curve. The first equivalence point of the pH-curve directly corresponds to the degree of dissociation $\alpha=0$. The second equivalence point ($\alpha=1$) is in good agreement with the flocculation point; that is the maximum of the turbidity curve. From the NaOH-consumption between $\alpha=0$ and $\alpha=1$ the degree of deacetylation is directly determined to be 75%- a result in good agreement with the elemental analysis (73.5%).

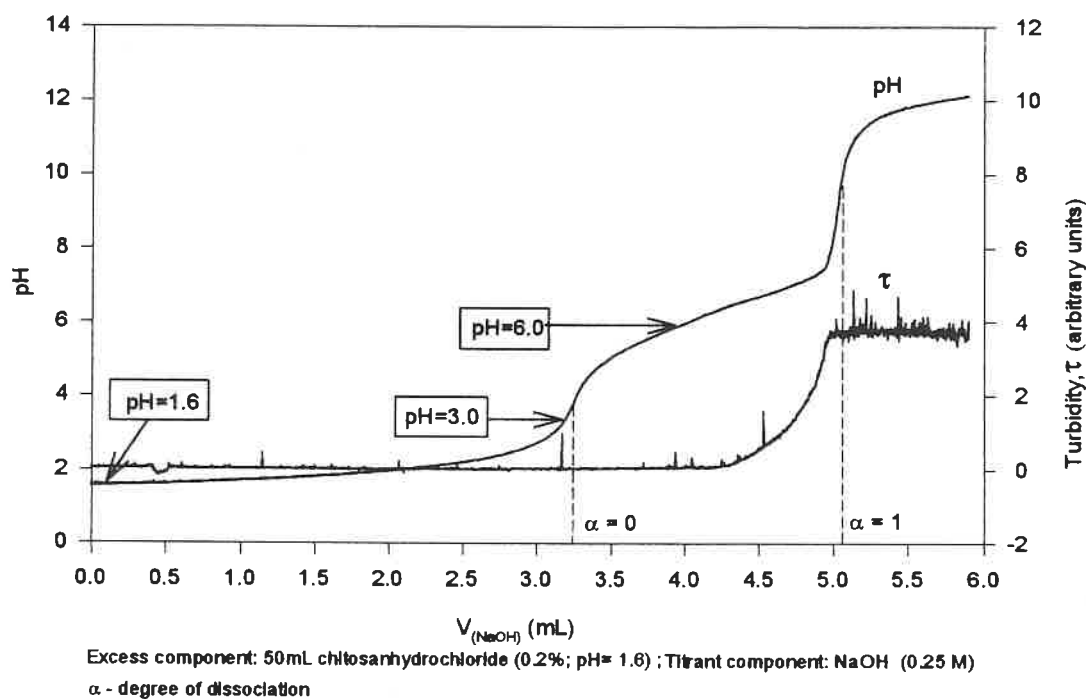
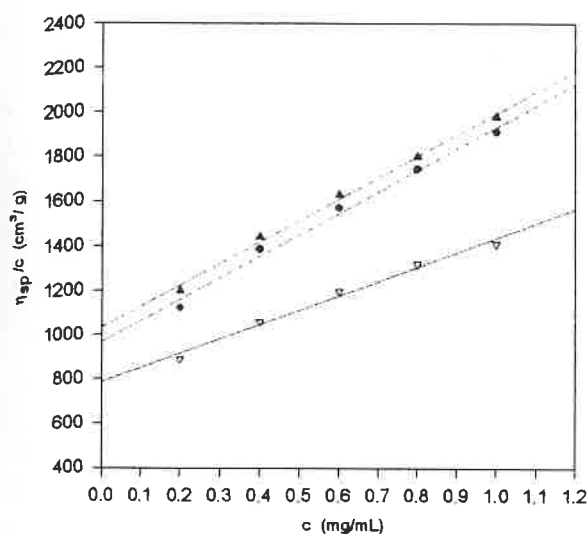


Figure 1 Back Titration of Chitosanhydrochloride

The intrinsic acidity constant (pK_a^0) of the amino groups was determined by using the Henderson-Hasselbalch equation $pK_a = pH + \log(1-\alpha/\alpha)$ with $pK_a^0 = pH$ at $\alpha=0.5$ at an ionic strength above 0.5 mol/l. The resulting $pK_a^0=6.2$ is in good agreement with literature data³.

For a more comprehensive viscometric characterization the Huggins-plot of the chitosanhydrochloride solution at pH=3 was compared with the plot at pH=6 as well as the plot of the so called "Lee-solvent" (4m urea / 0.2m acetic acid / 0.1m sodium chloride)⁴ (*Figure 2*). In all cases the Huggins constants are in the same order of magnitude, that means, in a range between 1 ± 0.1 demonstrating a quite similar solution behaviour. Using the Kuhn-Mark-Howink-Sakurada parameters K

and a for the "Lee-solvent"⁴ the molecular weight was determined via the $[\eta] - M$ relation to be 359,000 g/mol. In general, the results show no significant influence of the degree of dissociation on the hydrodynamic behaviour of the chitosan solution.



Polymer	Solvent	$[\eta]$ (cm ³ /g)	k_{η}
Chitosan 0.1%	HCl (pH 3) - 2.40 10 ⁻² M NaCl	965.9	1.03
Chitosan 0.1%	HCl (pH 6) - 2.60 10 ⁻² M NaCl	1034.0	0.90
Chitosan 0.1%	4M Urea - 0.2M Acetic Acid - 0.1M NaCl	784.6	1.06

$$[\eta] = 784.6 \text{ cm}^3/\text{g}$$

$$\Rightarrow M = 3.59 \cdot 10^5 \text{ g/mol}$$

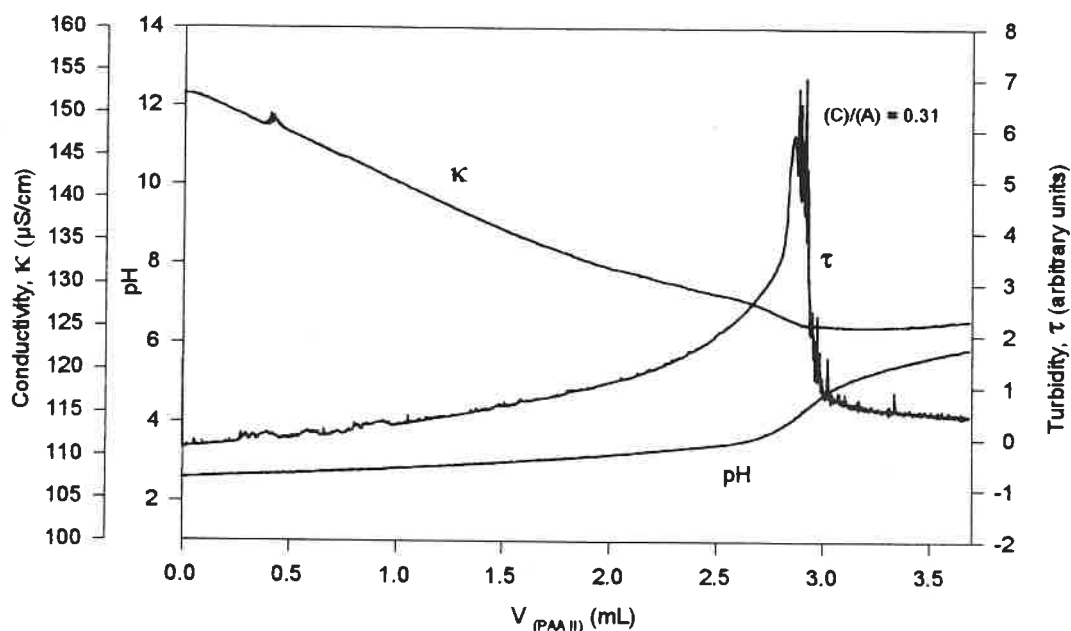
$$k = 8.93 \cdot 10^{-2} \text{ cm}^3/\text{g}$$

$$a = 0.71$$

Figure 2 Determination of the Intrinsic Viscosity $[\eta]$

-Polyelectrolyte complex formation with chitosan

The complex formation behaviour of the aqueous chitosanhydrochloride solutions at pH=3 and pH=6 was investigated by adding two kinds of polyanions, i.e. the "weak" acid Na-polyacrylate in contrast to the "strong" acid Na-polystyrenesulfonate. The stoichiometry of the complexes was determined by the molar ratio of cationic to anionic functional groups $[C/A]$ at the titration endpoint. *Figure 3* demonstrates a good agreement between the turbidimetric and electrochemical endpoint, i.e. the break point between the steep slope and the flatter one of the conductometric titration curve and/or the equivalence point of the pH-curve.



Excess component: 50 mL chitosanhydrochloride (0.04% w/w, pH= 3) ;

Titrat component: PAA II - (1% w/w)

(C)/(A) - Molar ratio of cationic to anionic functional groups

Figure 3 Symplex-Titration

Table 2 summerizes the complex stoichiometries at the titration endpoints as a function of the polyanion used, the pH and ionic strength of the system. The given data show no significant influence of the molecular weight nor of the concentration of added low molecular salt.

Table 2

pH=3

pH=6

Titrat	NaCl (mol/l)	[C]/[A]	Titrat	NaCl (mol/l)	[C]/[A]
PAA I	---	0.26	PAA I	0.1	0.89
	0.1	0.27		1	0.92
	1	0.22			
PAA II	---	0.31	PAA II	0.1	0.98
	0.1	0.30		1	0.95
	1	0.23			
PSS	---	1.14	PSS	0.1	1.44
	0.1	1.24		1	1.14
	1	1.12			

Turning to the "weak"-acid Na-polyacrylate a strong dependence on the pH of the solution under investigation is observed. Deviations from the 1:1 stoichiometry were observed at pH=3 due to protonation (that means, inactivation with regard to polyanion-polycation complex formation) of the carboxylic groups. At pH=6, where the amino-groups are half-neutralised and the carboxylic groups nearly completely dissociated, a 1:1 stoichiometry was observed.

Using the "strong"-acid Na-polystyrenesulfonate a nearly 1:1 stoichiometry was detected at pH=3 as well as pH=6. These data show that only the degree of dissociation of the polyanion influences the stoichiometry but not the degree of dissociation of the amino groups of chitosan.

Light scattering measurements were carried out to gain additional insight into the dependence of individual parameters of the complex aggregates on the degree of conversion. A Zimm plot of the chitosan-solution without added polyanion could not be realized. The scattering plots were strongly curved and thus could not be extrapolated to zero angle. This behaviour is not unusual and is particularly found for systems with large and more compact particles such as polysaccharides, e.g. sepharose⁵.

For the chitosan-polyanion complexes the Zimm-procedure was used to obtain the particle mass and the radius of gyration. Having these two parameters the packing density of the particles can be determined. In Figure 4 and 5 the particle mass and packing density of the polyelectrolyte complex particles is given as a function of the degree of conversion.

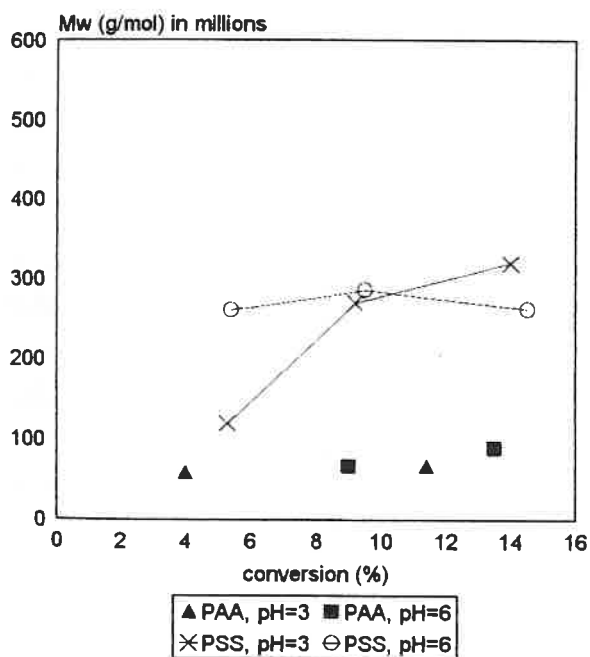


Figure 4 Dependence of the particle mass of chitosan-polyanion complexes on conversion

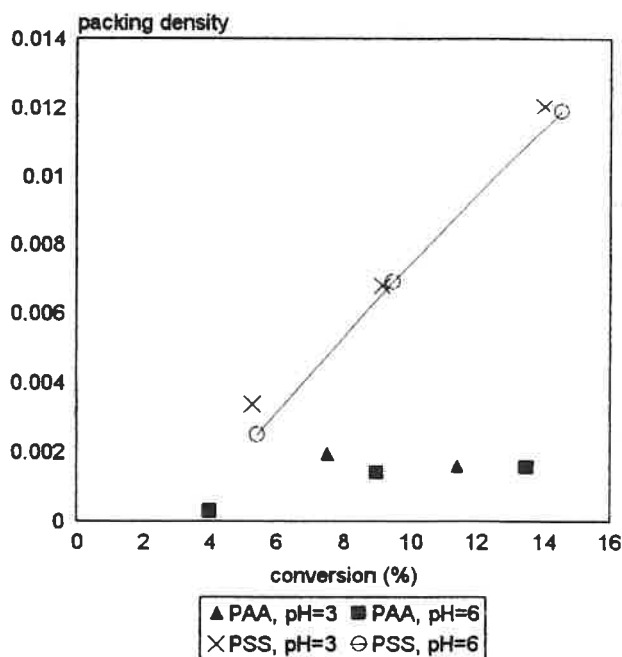


Figure 5 Dependence of the packing density of chitosan-polyanion complexes on conversion

For all chitosan-Na-polyacrylate complexes the particle mass is quite constant and varies between 50 and 100×10^6 g/mol irrespective of pH. The particle density of these aggregates is 0.0015 ± 0.0005 up to a degree of conversion of 20%. In contrast, the chitosan-Na-polystyrenesulfonate complexes show a strong dependence on the degree of conversion. The packing density increases from 0.003 up to 0.012 and the particle mass from 40 up to 300×10^6 g/mol. These data demonstrate significant differences with regard to the generation of new particles as a function of the degree of conversion between the "weak"-acid Na-polyacrylate and the "strong"-acid Na-polystyrenesulfonate, whereas the pH of the system has essentially no influence.

The dissymmetry z , the quotient of extrapolated values of scattering intensities at 45° and 135° , varies for the systems investigated between 3 and 7. This range of dissymmetry underlines the presence of polydisperse systems with relatively large, complex particles.

Conclusions

Our viscometric and potentiometric investigations show no significant influence of the degree of dissociation on the conformation of chitosan in solution.

Results from titrating a chitosan-hydrochloride solution with "weak"-acid Na-polyacrylate events deviations from 1:1 stoichiometry only at pH=3 due to the partial deprotonization of the carboxylic groups but not at pH=6 where the amino groups are partially deprotonated. Independent of the pH and consequently of the resulting complex stoichiometry, the chitosan-Na-polyacrylate complex aggregates show similar low packing densities and particle masses in the order of magnitude of 50 to 100×10^6 g/mol. Nevertheless up to a degree of conversion of 20% the generation of new similar shaped particles is the dominant process during complex formation.

Using the "strong"-acid Na-polystyrenesulfonate as titrant-component, a 1:1 stoichiometry was observed independent of the degree of dissociation of the chitosan as well as the ionic strength of the whole system. Contrary to the above discussed system, the packing densities as well as the particle masses drastically increase with increasing degree of conversion. This process can be understood by an aggregation and compaction of the primary as well as newly generated complex particles.

The results given here demonstrate clearly the eminent role of chitosan conformation in solution and nonrelevance of the degree of dissociation of the amino groups with regard to the resulting stoichiometry of polyelectrolyte complex formation.

Light scattering measurements show that, independent of the resulting stoichiometry, different aggregation processes can be realized, which are mainly controlled by the strength of Coulombic forces.

References

- 1 J. Kötz, K.-J. Linow, B. Philipp, L.-P. Hu, O. Vogl,
1986 *Polymer* **27**(10) 1574
- 2 J. Kötz, I. Neels, B. Philipp, M. Diamantoglou
1991 *Das Papier* **45** (5) 226
- 3 Ye. Ye. Skorikova, G.A. Vikhoreva, R.I. Kalyuzhnaya, A.B. Zezin,
L.S. Gal'braikh, V.A. Kabanov
1988 *Vysokomol. Soedin., Ser A*: **30**, 44
- 4 Lee, V.F. *University Microfilms*
(Ann Arbor) 1974, 74/29446
- 5 G. Berth, H. Dautzenberg, G. Rother,
1994 *Carbohydrate Polymers* **25** 187-195

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SYNERESIS IN CHITIN GELS

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Abstract

Chitin gels were obtained by N-acetylation of chitosan in an acetic acid-water-propanediol solution. The effect of the solvent on the syneresis of these gels was investigated. These studies were performed on samples immersed in various solvents such as demineralized water, aqueous alcoholic solutions, DMAc-LiCl. The sensitivities of these gels to ionic strength and pH were also investigated as a function of the acetylation degree. In aqueous alcoholic solution or in demineralized water, syneresis was less important for samples of low acetylation degree, due to electrostatic repulsion of numerous residual protonated amine groups along the polymer chains. When the ionic strength increases, syneresis increases for both low- and high-acetylations. The effect of ionic strength on gelation and acetylation was also studied. An increase of the ionic strength of the acetylating medium provides an increase of gelation time, a decrease of syneresis, and a decrease of the final acetylation degree. Ionic strength probably hinders the organization of the acetylated segments, favouring an organization of the polymer chains in random coils. The composition of the solvent at equilibrium of the volume determined by GC was also studied.

The syneresis was also observed to be less important with increasing the concentration in relation with the increase of number of reticulation linkages. Finally, a fitting theoretical law to $M_t = (M_0 k_1 + k_2 t) / (k_1 + t)$ gave a good representation of the syneresis process.

Keywords : Chitin, Chitosan, Gelation, Syneresis, Degree of Acetylation

Introduction

N-acetylchitosan gels were largely studied, especially the influence of different parameters on their formation (1-4). The chitin gels we study are obtained by N-acetylation of chitosan in an acetic acid-water-propanediol solution (5). This solvent, never used before, allows an improved study of gelation by its relatively high viscosity offering relatively low kinetics of gelation. This kind of gels belonging to the family of hydrogels, are very interesting for various biomedical applications (6, 7). The high water content, the soft and rubbery consistency, and the diversity of the mode of interactions could allow their use as drug delivery systems or for

the uptake of undesirable products. Measurements of syneresis are of great importance for the possible applications of these gels. This paper gives the results of the syneresis behaviour of chitin gels with respect to acetylation degrees, pH, ionic strength and solvent. A kinetics law, recently proposed for another system (8), gave a good representation of the syneresis process.

Materials and Methods

The gels were obtained by acetylation of chitosan (from Aber Technologies, lot n°A32E03, $M_v = 470\,000$ g/mol) with a residual acetylation degree of 2.4% (NMR) and a water content of 10.4% (TGA).

Formation of the gels : an aqueous acetic acid (0.5%) solution of chitosan (1%) was prepared with 1,2-propanediol. The acetylating solution was slowly added to the solution of chitosan under stirring and the gel was transferred into a mould. Two molar ratios R 2.5 and 10 (anhydride over free amine) were studied.

For the study of the influence of the ionic strength on gelation, a known amount of KCl was dissolved in the aqueous alcoholic chitosan solution before the introduction of the acetylating solution.

Syneresis monitoring : a known amount of gel with a cylinder form (≈ 20 g) was immersed in 100 ml solution (study of the influence of the ionic strength) and 200 ml (study of the influence of the pH) in a thermostated bath at $22 \pm 0.5^\circ\text{C}$. pH was maintained within ± 0.2 unity by addition (if necessary) of NaOH (0.1M) or HCl (0.1M). Syneresis were measured by weighing the gel periodically, the surface of the gel was wiped with paper.

Gas Chromatography analysis : the composition of the solvent after syneresis was determined on a chromatograph DELSI 700 with a CP WAX 52 CB capillary column from Chrompack coupled to a catharometer detector. Oven temperature : 110°C

Flow gas : Helium

^1H NMR spectroscopy : ^1H NMR spectra were recorded on a BRUKER AC 250 (250 MHz for ^1H). Chitin gels were studied in D_2O after hydrolysis by HCl (20% w/w) at 298 K. The samples obtained in absence of salt with molar ratios equal to 2.5 and 10, present degrees of acetylation equal to 92 and 98 % respectively (5).

Results and Discussion

Effect of ionic strength on gelation and acetylation degree

The influence of the ionic strength on gelation was studied on two samples prepared with molar ratios $R = 2.5$ and 10. The added salt was KCl. We observed that an increase of ionic strength of the acetylation media induced an increase of the gelation time (independently of R) and a decrease of the mechanical properties of the obtained gels, compared to the gels prepared without added

salt. The salts in solution screen the charges distributed along the polymer chains which organize themselves in relatively dense random coils, less favourable to the organization of the acetylated segments, responsible of gelation. For the same reasons, syneresis, resulting from the reorganization of the acetylated segments, decreases when the ionic strength increases (Figure 1a, 1b). The ionic strength is more effective for low molar ratios for which acetylation degrees are lower. With the latter samples, the residual free amine groups are more numerous, and are mainly in the ammonium form NH_3^+ . This form, contrarily to the acetylated form, is sensitive to ionic strength for two major reasons : the screening and the dehydration of the charges.

The acetylation degrees of the samples obtained in salt solution are lower ($R = 2.5$; $\text{DA} = 78\%$, instead of 92% in absence of added salts⁽⁵⁾). The random coil conformation due to the presence of salts can also induce a lesser access of the free amine groups to the acetic anhydride, thus reducing the acetylation degree.

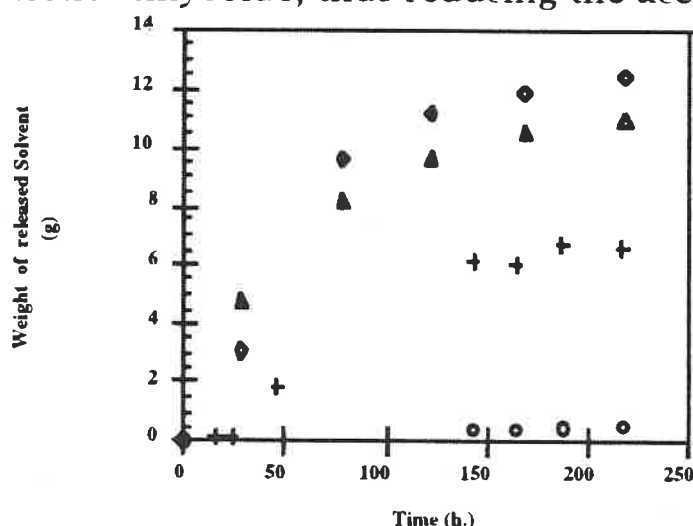


Figure 1a : Syneresis of a low acetylated chitin gel obtained with added salts in acetylating medium

▲ Water/Alcohol ♦ (KCl) = 0.04 M + (KCl) = 0.08 M ○ (KCl) = 0.11 M

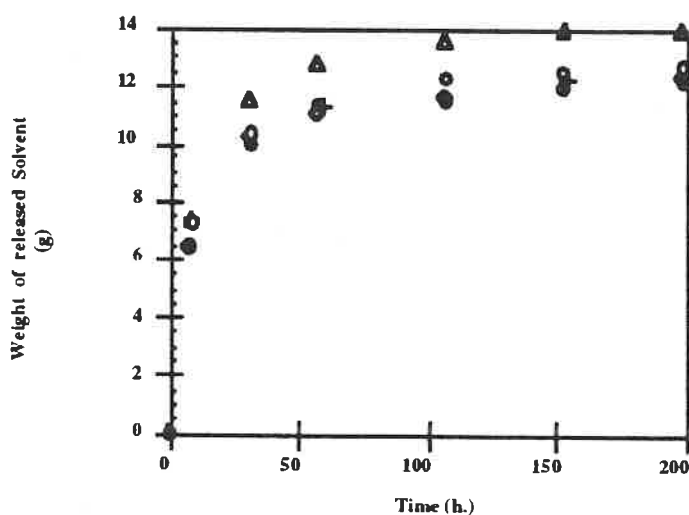


Figure 1b : Syneresis of a high acetylated chitin gel obtained with added salts in acetylating medium

▲ Water/Alcohol ♦ (KCl) = 0.04 M + (KCl) = 0.08 M ○ (KCl) = 0.11 M ● (KCl) = 0.2 M

Behaviour of chitin gels in various solvents

The stability of a polyelectrolyte gel in solution results from the balance between several competing thermodynamic forces such as *i*) osmotic pressure of free counterions in the gel but also the charges beared by the chains, *ii*) molecular interactions between solvent and polymer molecules, *iii*) network elasticity, and *iv*) Debye-Hückel interaction of ions (9, 12). This balance can be changed by addition of salt to the solvent. This results in an increase of the osmotic pressure and collapse of the gel into a smaller volume compared to the initial one. If the gel is immersed in a solvent of the same nature as the one of the gel formation, solvent molecules may be exchanged between the bath and the gel. Thus, we observed that the lower syneresis rates are obtained in water-propanediol and demineralized water (without added salts) (Figure 2). In these two solvents, the syneresis rate is lower for weakly acetylation degrees (Figure 2a), because of the higher level of residual protonated amine residues (hydrophilic) in relation with the higher elasticity of the network due to the less numerous acetylated segments which bring a certain stiffness to the network. In high acetylation chitin gels, the hydrophobic character due to the acetylated segments, dominates and the screening effect of the charges as well as the dehydrating effect of the salts on the hydrophilic groups is not so effective (Figure 2b) as for weakly acetylated gels. In DMAc-LiCl, solvent of the native chitin, the syneresis degree of the low acetylated sample is slightly higher than for the highest acetylated sample. The structure of the latter is closer to the native chitin and the interactions with the solvent are more favorable.

A progressive increase of the maximum syneresis was observed by increasing the salt concentration (KCl) (Figure 2a), as is generally the case for ionic gels (9). When a gel is immersed in a bath without salts, the osmotic pressure inside the gel is higher than in the bath, due to the ions belonging to the chains (NH_3^+) and to the counterions (CH_3COO^-). Thus, the gel expands in order to equilibrate the osmotic pressure inside and outside the gel. The expansion depends on the number of residual charges along the polymer chains, and then on the acetylation degree. At low acetylation degrees, the osmotic pressure in the gel is higher than at high acetylation degrees, providing a greater expansion of the gel (Figure 2a and b), in relation with the length of the chain segments between reticulation points. When salts are added in the bath (KCl), the osmotic pressure in the bath is higher than in the gel. Thus, a part of this salt ions migrates to the gel, providing a lower difference between the osmotic pressure inside and outside the gel. The gel will adjust its volume so that the osmotic pressure is equilibrated, but the depletion is higher than without salts.

At high ionic strength, the volume at the plateau is fairly the same for the low and the high acetylation. The osmotic pressure in the

bath is so high that the influence of the residual charges beared by the chains (NH_3^+) and the counterions (CH_3COO^-) in the gel becomes negligible. Thus, the difference of behaviour between high- and low-acetylated gels is smoothed (Figure 2a and b).

All the salts do not give the same effects on syneresis (10-12). Another kinds of salts should be studied such as NaCl (10) or polyions (12).

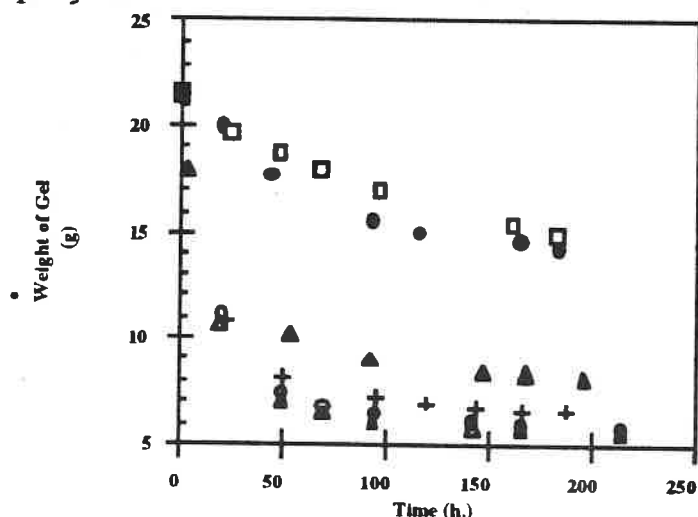


Figure 2a : Syneresis of a 92% acetylated chitin gel in various solvents

● Water . + (KCl) = 0.02 M ○ (KCl) = 0.04 M ▲ (KCl) = 0.1 M
□ Water/Alcohol (50/50 w/w) ▲ DMAc-LiCl (5%) solution

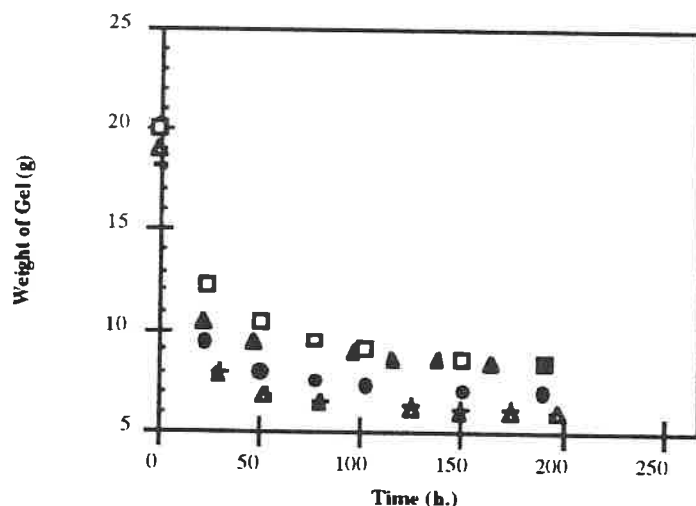


Figure 2b : Syneresis of a 98% acetylated chitin gel in various solvents

● Water . + (KCl) = 0.02 M ○ (KCl) = 0.04 M ▲ (KCl) = 0.1 M
□ Water/Alcohol (50/50 w/w) ▲ DMAc-LiCl (5%)

The composition of the solvent after syneresis, at the equilibrium volume of the gel, was determined by GC. The composition of the solution in water and alcohol is the same as the initial one (50/50 w/w) when the syneresis medium is not modified. Furthermore, it is independent of the acetylation degree. The amount of water in solution increases (91% w/w) when syneresis occurs in demineralized water (without added salts). But, the concentration

of added salt (KCl) does not seem to modify the composition of the solvent.

Syneresis of agar gels has been recently studied (8). It has been shown that it obeys to the following law :

$$M_t = (M_0 k_1 + k_2 t) / (k_1 + t)$$

where M_t is the weight of the gel at the time t and M_0 the weight at the initial time. k_1 and k_2 are parameters depending on gelation and syneresis conditions. The influence of chitosan concentration, acetylation degree of the gels, pH and ionic strength on these parameters were also studied.

Influence of pH on the behaviour of chitin gels in solution

The external pH has an important effect on the balance of forces that determine the equilibrium volume in hydrogels (12). As the pH is lowered to the acidic region, the amine functions of the glucosamine residues become protonated, thus increasing the charge density of the network and the osmotic pressure inside the gel (Figure 3a). At pH 2, there is a combined effect of increasing ionic strength due to the peculiarly high concentration of H^+ . Thus, the volume of the gel at pH 2 is lower than the one at pH4, although the chain protonation is not significantly higher.

Varying the acetylation degree appears to affect the extent of the volume. First, an increase of the acetylation degree involves a decrease of the concentration of ionisable groups and lowered the osmotic pressure of the gel. Second, the overall hydrophobicity due to the acetylated sequences, increases. These effects generally contribute to decrease the amount of entrapped solvent (Figure 3a and b). As network hydrophobicity increases, a greater degree of ionisation of the chains is required to enable the transition. This increase of ionisation degree is obtained by decreasing the pH.

On the contrary, increasing the pH induces the deprotonation of the glucosamine residues and changes the rotational freedom of the amine groups which become more flexible (13). This increase in flexibility should facilitate the association of the hydrophobic sequences of the polymer chains, resulting in a phase separation of the polymer from the water phase to collapse. Furthermore, the formation of hydrogen bondings between the deprotonated amine groups reinforces the collapse of the gel at basic pH. These phenomena are peculiarly true for low acetylation degrees where the residual amine groups are still relatively numerous. Above pH4, the deprotonation is responsible of the shrinking of the gel, below pH4, the high osmotic pressure of the bath constrains the gel to collapse. The effect of pH on high acetylation degrees is more negligible (Figure 3b).

Hysteresis was observed in swelling/deswelling cycles of a low acetylation sample (Figure 3a). With pH increasing from 2 to 4, we observed an increase of the volume (+25%), corresponding to a decrease of the ionic strength of the bath. Varying pH from 10 to 4

induces a lower increase of the volume (+10%). At basic pH, the organization of the hydrophobic acetylated segments, and the formation of hydrogen bondings between the amine groups which are not protonated induces the formation of reticulation points. The latter are sensitive to pH variations. Thus, decreasing pH causes their disappearance, but the hydrophobic interactions, independent of pH, remain and hinder the swelling of the gel.

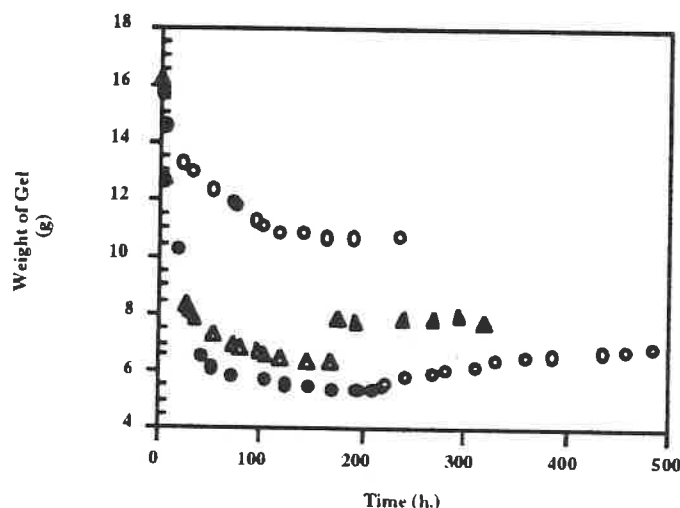


Figure 3a : Syneresis of a 92% acetylated gel in various solutions

△ pH = 2 ● pH = 10 ▲ pH increase from 2 to 4 ○ pH decrease from 10 to 4

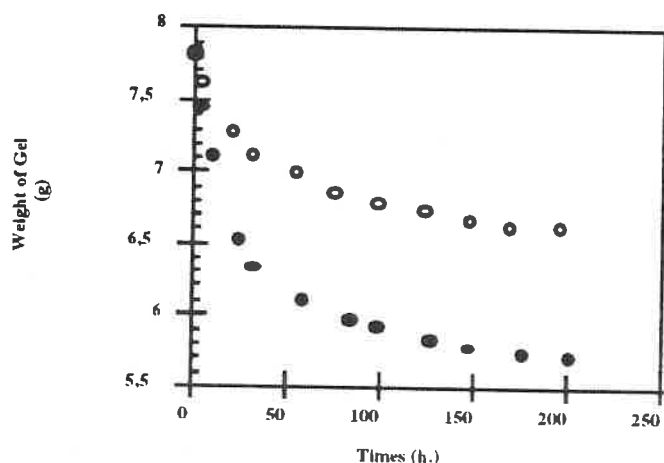


Figure 3b : Syneresis of a 98% acetylated chitin gel in various solutions

○ pH = 4 ● pH = 10

Influence of the chitosan concentration on the syneresis

We observed that increasing chitosan concentration induced increase of the equilibrium volume. When the chitosan concentration increases, the charge density in the gel increases, resulting in an increase of the osmotic pressure inside the gel. In order to equilibrate this pressure, the gel will expand. Furthermore, the free volume in the gel at high chitosan concentration decreases, providing a lower mobility of the solvent in the gel.

Conclusion

The ionic strength of the acetylating medium influences the mechanical properties, gelation and Degree of acetylation (DA) of the gels. Syneresis of chitin gels obtained by acetylation of chitosan in water-propanediol solution, depends on the nature of the solvent in which the gel is immersed. A slight increase of the ionic strength induces the increase of syneresis, pecularly for low acetylation degrees, whereas at high ionic strength, syneresis becomes independent of the acetylation degree. The effect of pH on syneresis brings out the importance of the protonation of the amine groups depending on one hand, on pH values and the existence of reticulation points between the glucosamine residues (NH₂) at basic pH, and on another hand between acetylated segments .

References

1. S.Hirano, and Y.Ohe, *Agr. Biol. Chem.*, 39 (1975) 1337-1338
2. S.Hirano, and Y.Ohe, H.Ono, *Carbohydr. Res.*, 47 (1976) 315-320
3. G.K.Moore and G.A.F.Roberts, *Int.J.Biol.Macromol.*, 2 (1980) 73-77
4. G.K.Moore and G.A.F.Roberts, *Int.J.Biol.Macromol.*, 2 (1980) 78-80
5. L.Vachoud, N.Zydowicz and A.Domard, *Carbohydr. Res.* 1997 in press
6. Y.Hirota, S.Tanioka, T.Tnigawa, Y.Tanaka and R.Ojima, "Advances in Chitin Science", A.Domard, C.Jeuniaux, R.Muzzarelli; G.Roberts Eds, Jaques-André Publisher, 407-413 (1996)
7. A.Domard, B.Grandmontagne, T.Karibian, G.Sparacca and H.Tournebise, France Patent PCT/FR96/01106 (1996)
8. K.Nagasaka and S.Taneya, *Nippon Shokuhin Kagahin Kogaku Kaishi*, 43 (1996) 1176-1182
9. R.Rydzewski, *Continuum Mech. Thermodyn.*, 2 (1990) 77-97
10. I.Ogawa, H.Yamano and K.Miyagawa, *J. Appl. Polym. Sci.*, 54 (1994) 1971-75
11. T.V.Budtova, N.G.Belnikevitch, I.E.Suleimenov and S.Ya Frenkel, *Polymer*, 34 (1993) 5154-56
12. R.A.Siegel and B.A.Firestone, *Macromol.*, 21 (1988) 3254-3259
13. K.Kataoka, H.Koyo and T.Tsuruta, *Macromol.*, 28 (1995) 3336-3341