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Carbohydrate Polymers **23** (1994) 211–219

0144-8617/94/$07.00

Chitosan carboxylic acid salts in solution and in the solid state

S. Demarger-Andre[a,b] & A. Domard[a,*]

[a] *Laboratoire d'Etudes des Matériaux Plastiques et des Biomatériaux (URA CNRS n° 507), Université Claude Bernard Lyon I, 43, Boulevard du 11 Novembre 1918, 69622 Villeurbanne Cedex, France*
[b] *COLETICA, 32, Rue Saint Jean de Dieu, 69007 Lyon, France*

(Received 18 November 1993; accepted 14 December 1993)

When chitosan is associated to simple carboxylic acids, such as formic, acetic, etc., electrostatic interactions occur, resulting in a salt formation, to a greater or lesser degree. Fourier transform infra-red (FTir) spectroscopy and potentiometric techniques were used to characterize the nature of such interactions, in aqueous solutions and in films. The evolution of solid state samples during storage and upon dehydration was studied, by FTir spectroscopy and transmission X-ray diffraction. Results showed that the carboxylic acid content of the films progressively decreased in relation with the physicochemical parameters of the acids (pK_a, solubility, etc.). These results were compared with those obtained with a strong acid such as HCl or a complex carboxylic acid (lactic). In X-ray diffraction experiments, three crystalline structures of chitosan were found: a hydrated form, independent of the chemical nature of the salt, and two dehydrated forms depending on the chemical structure of chitosan.

INTRODUCTION

Chitosan, a poly [β-(1 → 4)-linked 2-amino-2-deoxy-D-glucose] is obtained by *N*-deacetylation of chitin, one of the most abundant naturally occurring polysaccharides with cellulose. It has found many applications in the fields of cosmetics (Lang & Clausen, 1989), wound healing (Malette & Quigley, 1982; Sapelli *et al.*, 1986), dietetics (Kobayashi *et al.*, 1979; Jennings *et al.*, 1988), waste-water treatment (Axberg *et al.*, 1980), etc., and is used in various physical forms.

The primary amino function of chitosan can easily be protonated by dilute acids such as hydrochloric, formic, acetic or butyric acid; the antitumor activity of the latter carboxylic acid has been reported (Nudelman *et al.*, 1992).

Due to the β-(1 → 4) linkages between the residues constituting the chain, chitosan has good film and fiber forming properties which lead to an important part of its applications. Films are easily obtained by evaporation of dilute acid solutions of the polymer (Saitô *et al.*, 1987; Chandy & Sharma, 1989). Therefore, it seemed interesting to characterize the acid–base properties of chitosan in relation to the stability of the films formed with various acids.

The aim of the present work was to study the association of chitosan and various carboxylic acids, namely formic, acetic, butyric and valeric. Interactions occurring in aqueous solutions were characterized by potentiometry (pH-metry), while Fourier transform infra-red (FTir) spectroscopy and X-ray diffraction were used to analyze solid materials and more especially their evolution with time.

MATERIALS AND METHODS

Materials

Partially *N*-deacetylated chitin (batch BGL25, supplied by Aber Technologies, France) was fully deacetylated according to a method elaborated by Domard and Rinaudo (1983). The degree of *N*-acetylation was controlled by FTir spectroscopy (Miya *et al.*, 1980). This chitosan of 0% acetyl content had a viscosity average degree of polymerization ($\overline{DP_V}$) of 4900, measured in a 0·2 M acetic acid/0·1 M ammonium acetate buffer, and using the viscosity law described by Roberts and Domszy (1982).

*To whom correspondence should be addressed.

The carboxylic acids (formic, acetic, butyric and valeric) were all of reagent grade (purity >99%).

The concentration of aqueous hydrochloric acid and sodium hydroxyde was 0·1 M.

Chitosan carboxylate solutions and films

Aqueous 50 mM solutions of the various acids studied, were prepared and titrated with NaOH, by pH-metry.

Chitosan was dissolved in a stoichiometric amount of a given acid, to form the corresponding chitosan salt solution of concentration 0·5 mM, as previously described (Domard, 1987).

Films were obtained by spreading such solutions onto glass microscope slides and drying them in open air or under vacuum at 70°C. The films were easily removed from their supports; no particular treatment was required.

Techniques

pH-Metry measurements were performed on a Tacussel Minisis 8000 pH-meter, equipped with a standard calomel and a glass electrode (Tacussel), after 1 min of stirring followed by 1 min of rest of the solution. The pH was measured as a function of α, the dissociation coefficient of the ammonium function ($-NH_3^+$).

FTir transmission spectra of the films were recorded on a Perkin-Elmer 1760 spectrophotometer (10 scans).

Thermogravimetric Analysis (TGA) was performed on a TGA 2950 Thermogravimetric Analyzer (Du Pont Instruments) coupled with a Thermal Analyst 2000 computer (Du Pont Instruments). The flow gas was helium.

For transmission X-ray diffraction analyses, spectra were plotted in the range $2\theta = 3°$ to 38°, using Cu K_α radiation, by means of a Siemens D500 X-ray diffractometer (results of Fig. 5) and a Phillips diffractometer set up by the Physical Spectrometry laboratory of Grenoble, France (results of Fig. 7). In the latter case, the chitosan films were heated with hot air; the temperature was controlled by means of a thermocouple placed near the samples.

RESULTS AND DISCUSSION

The behavior of chitosan in the presence of various acids was studied in aqueous solution and with films obtained by evaporation of the corresponding solution.

Solution studies

As reported previously (Domard, 1987), when a complexation occurs between chitosan and, for example, copper(II) ions, the pH is lowered and the equivalent point of a potentiometric titration of the complex is shifted beyond $\alpha = 1$, the neutralization degree in the absence of metal ions.

Sakurai *et al.* (1984) titrated chitosan formate and butyrate salt solutions and observed a difference between the two end points, the chitosan butyrate end point occurring earlier than the formate one. They concluded that butyric acid and the polysaccharide yield a true complexation process, as opposed to pure electrostatic interactions in the case of formic acid.

The neutralization of chitosan/carboxylic acid solutions should therefore enable us to determine the nature of the interactions existing between the two components. Figure 1 shows the comparison of the titration, by 0·1 M NaOH, of various equimolar (0·5 mM) chitosan/carboxylic acid solutions with that of chitosan hydrochloride, the latter being representative of a purely electrostatic interaction. Carboxylic acids of increasing chain lengths, namely formic, acetic, butyric and valeric, were compared to hydrochloric acid.

We notice in Fig. 1 that all the curves are similar and in particular have the same equivalent point, close to pH 7·1. The interactions between chitosan and the various carboxylic acids are therefore of the same type as those occurring in the case of hydrochloric acid, which implies that they are purely electrostatic. No particular complexation is observed and only chitosan salts can be supposed to be formed in solution. These results contradict the reports of Sakurai *et al.* (1984) who stated that chitosan forms a true complex with

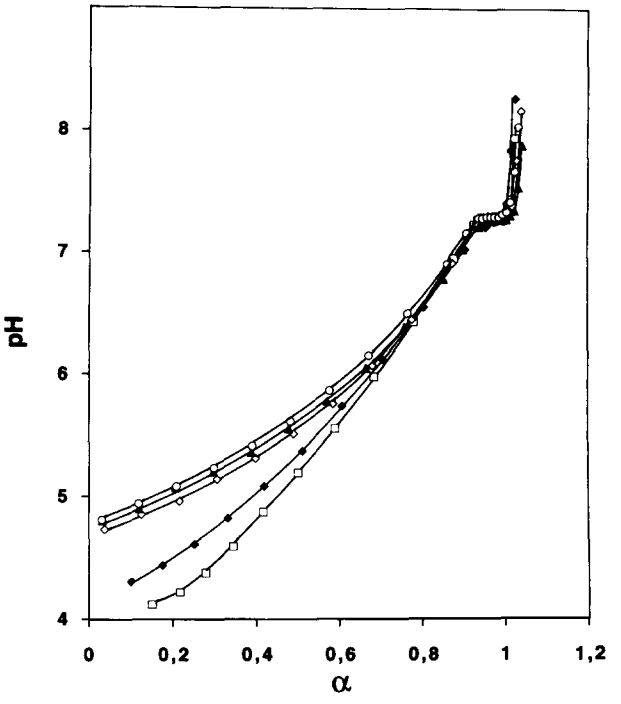

Fig. 1. pH variations as a function of α, the degree of neutralization, in titrations (by 0·1 M NaOH) of 0·5 mM chitosan salt solutions (○, valerate; ▲, butyrte; ◇, acetate; ◆, formate; □, hydrochloride).

butyric acid. In fact, to study the phenomenon, they used solutions obtained by redissolving films of the corresponding chitosan salts. FTir studies on films, discussed in the following section, will confirm the results of the present study and allow us to explain the behavior observed by Sakurai and his co-workers.

In Fig. 1, we also notice a variation of the initial pH values (pH_i) of the solutions, which largely depend on the counterions. As shown in Table 1, it is interesting to point out that the order of pH_i's is directly related to that of the pK_a's of the corresponding carboxylic acids. In the case of butyric or valeric acid salts, the H^+ ion concentration is approximately three and five times lower than with formic and hydrochloric acids, respectively. We must remember that the pK_0 of chitosan is close to 6·5 (Domard, 1987). This means that, contrarily to classical polyamines like polylysine, the amino function of chitosan is a weak base and so we are typically in the presence of salts corresponding to weak acids and a weak base. These salts are only partially formed and in equilibrium with a significant part of the acids remaining in the undissociated form. This presents a great interest for biological applications since in this case chitosan would be considered as an acid vector for sustained release acid delivery systems. These results are also interesting for applications involving chitosan solutions which, traditionally, are relatively acidic. Since the counterion affects the apparent pK_a of chitosan salts, the pH of such solutions may be raised to 5 or more, simply by partial protonation of the polysaccharide with butyric or valeric acid. One can easily conceive that undesired reactions in biological media due to the acidity of some chitosan salts, may thus be minimized.

Chitosan solutions are usually the starting material in the production of films (Sakurai *et al.*, 1984; Saitô *et al.*, 1987) and membranes (Chandy & Sharma, 1989). Therefore, films were prepared from the chitosan carboxylate solutions in order to perform FTir and X-ray analyses.

Studies in the solid state

FTir spectroscopy

Figure 2 shows the FTir spectra of air-dried films, obtained from solutions of chitosan salts (hydrochloride, formate, acetate and butyrate). The following observations can be made.

Firstly, the purely electrostatic nature of the interaction between chitosan and the acid is confirmed. Indeed, on the spectra a of Fig. 2, chitosan $-NH_3^+$ bands are observed at 1514 and 1615 cm^{-1}, but no apparent $-NH_2$ signal (1598 cm^{-1}) is found and the acids are essentially present in their carboxylate form, $-COO^-$ (1550 cm^{-1}). The peak resolution and assignments were controlled by analyses of second derivatives of the spectra and subtractions (the following spectra were subtracted to those of chitosan salts: chitosan NH_2, chitosan NH_3^+, carboxylic acid COOH, carboxylate ion COO^-; results not shown). The authors were thus able to confirm that the interactions between chitosan and the acid occur via the $-NH_3^+$ and $-COO^-$ functions, no significant chitosan $-NH_2$ bond being apparent.

The second observation arising from the FTir results concerns the drying of chitosan carboxylate films. As the films are dried, chitosan $-NH_3^+$ (1514, 1615 cm^{-1}) and carboxylic acid $-COO^-$ (1550 cm^{-1}) bands regress progressively as shown in II, III and IV in Fig. 2. In the case of acetic acid (III in Fig. 2), all the ir absorption bands of the acid have disappeared after 6 months, and the FTir spectrum obtained is identical to that of pure chitosan in its free amino form. On the contrary, in the case of chitosan butyrate (IV in Fig. 2) and chitosan valerate films (spectrum not shown but similar to IV in Fig. 2) the loss of acid content remains incomplete even after 6 months of drying. This can be attributed to their boiling points (bp) which are higher than the bp of acetic acid (~118°C, 165°C and 187°C for acetic, butyric and valeric acids, respectively). A second important parameter is the solubility of the carboxylic acid, which decreases rapidly with the increase of molecular weight. However, contrarily to freshly prepared films, all 6-month-old chitosan carboxylate films showed a similar characteristic: they became insoluble in pure water. On the other hand, whatever the drying time, the chitosan hydrochloride films remained soluble. This must be related to the fact that, in the case of weak acids, the chitosan $-NH_3^+$ content considerably decreases upon drying, and the proportion of $-NH_2$ form, which is thus regenerated, is sufficient to induce the insolubility of chitosan. We can situate the limit of insolubility for a proportion of regenerated $-NH_2$ function over 45% (Domard, 1987).

To determine whether the water content had an effect on the progressive loss of carboxylic acid in dried chitosan salt films, the authors compared two drying processes (Fig. 3): (A) the films were stored in open dishes, at room temperature, and (B) the films were dried *in vacuo* at 70°C. Since acid removal is accompanied by a conversion of the $-NH_3^+$ chitosan function to $-NH_2$, the evolution of the $-NH_3^+$ band (1514 cm^{-1}) was studied as a function of time. To do so, its absorbance was related to the 1155 cm^{-1} band,

Table 1. Relationship between the pK_a's of various carboxylic acids and the pH_i's of the corresponding chitosan salt solutions

Acid	Chemical structure	pK_a	pH_i
Hydrochloric	HCl	Strong acid	4·12
Formic	HCOOH	3·75	4·31
Acetic	CH_3COOH	4·76	4·73
Butyric	CH_3–$(CH_2)_2$–COOH	4·81	4·80
Valeric	CH_3–$(CH_2)_3$–COOH	4·82	4·81

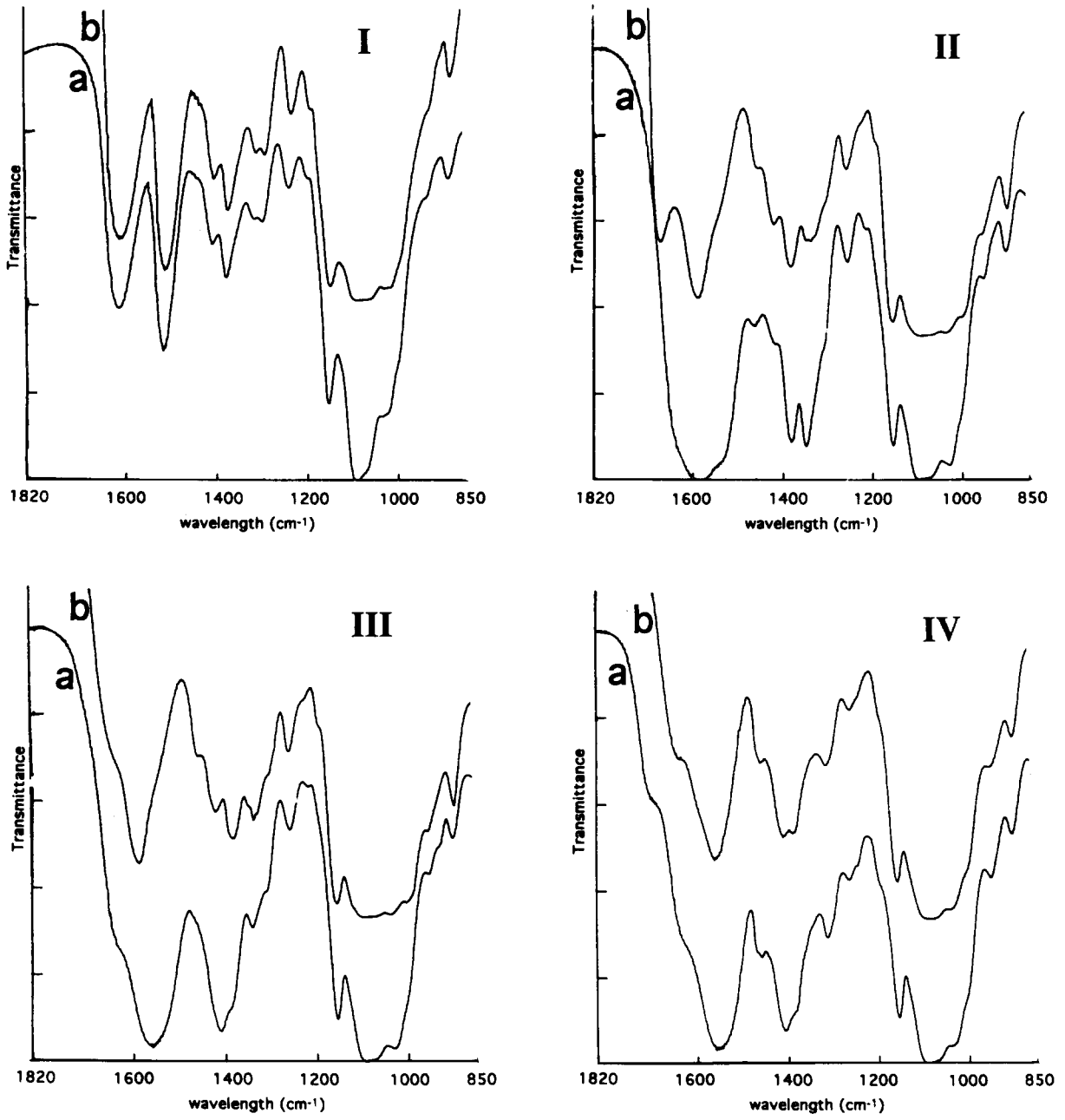

Fig. 2. FTir spectra of films of chitosan salts obtained with the following acids: I, hydrochloric; II, formic; III, acetic; IV, butyric. (a), After 15min drying; (b) after 6 months storage.

which is specific to chitosan (ether band) and independent of the amine content. The water contents of the films were measured by TGA; the method consisted in heating the films from room temperature (T_{room}) to 250°C using a 10°C/min ramp. After 66 h drying the water contents are 11·64% (method A) and 8·58% (method B) for chitosan hydrochloride films, and 6·38% (method A) and 4·98% (method B) for chitosan acetate films. Chitosan hydrochloride was used as a reference, since its chemical structure remains unchanged upon drying. The rapid initial increase of the ratio A(1514)/A(1155), i.e. of the $-NH_3^+$ proportion, certainly arises from partial removal of residual water, corresponding to the evaporation of free water adsorbed on the films. Indeed, the absorbance at 1155 cm^{-1} is affected by the interference of water absorption bands; these bands being more important in the air-dried cases, the ratio A(1514)/A(1155) will be lower.

Figure 3 shows that while the structure of chitosan hydrochloride is stable with time both in the presence and the absence of water, a chitosan acetate film evolves with time, depending on its water content. In this case the $-NH_3^+$ proportion decreases very rapidly in the first hours of drying, after which a plateau is reached where

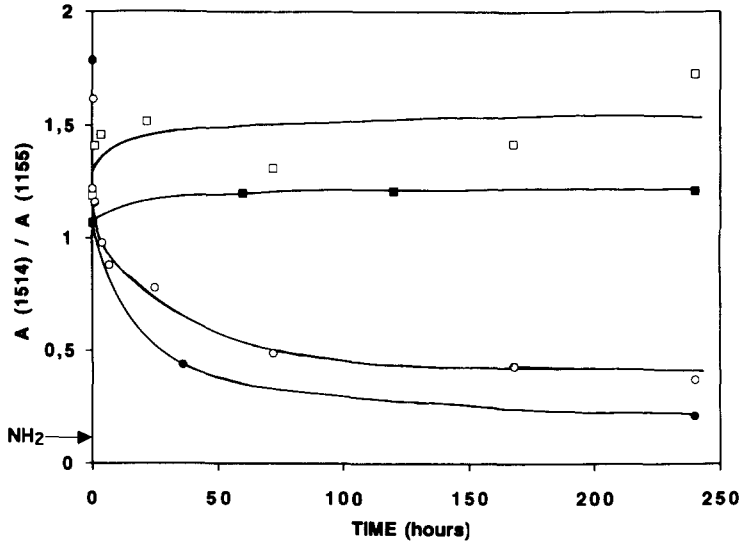

Fig. 3. Variation of the absorbance ratio A(1514)/A(1155) as a function of time, for various chitosan salt films: chitosan hydrochloride, dried *in vacuo* (□) and in open air (■); chitosan acetate, dried *in vacuo* (○) and in open air (●).

$-NH_3^+$ is slowly converted to $-NH_2$. The curve of Fig. 3 comprises two parts: the first part shows a rapid decrease of the ratio A(1514)/A(1155) and would concern a domain of high water content of the films. This will promote salt hydrolysis and therefore acid removal, causing a regeneration of free amino functions (A(1514)/A(1155) decrease). These $-NH_2$ functions being less hygroscopic than $-NH_3^+$, they will favor water evaporation. As the water content of the films decreases, the salt hydrolysis will slow down until a plateau is reached (the second part of the curve) where the $-NH_3^+$ functions are only very slowly converted to $-NH_2$. Together with the interference of water infrared bands on the A(1514)/A(1155) ratio, this dependence of salt hydrolysis on the water content of the films explains the different kinetics of acid removal in vacuum and air-dried films; in the former case, the water content of the films is lower, inducing a slower conversion process than in the latter case. So, as seen on Fig. 3, a film dried in air reaches the plateau sooner than one dried *in vacuo* (after 2 days, approximately 75% of the $-NH_3^+$ content has been removed).

We have seen that the water content of the films is related to the $-NH_3^+ \rightarrow NH_2$ conversion and vice versa. These facts allow us to understand why, in the case of HCl, where the chitosan salt form is stable and hygroscopic, the water content remains both constant and higher than that of a salt formed with the weaker acid. Moreover, a TGA experiment shows that a chitosan hydrochloride salt can easily lose its bound water near 130°C, but this water is always completely recovered upon cooling to T_{room}.

Generally, in the presence of water, we may consider the following equilibrium:

$$RNH_3^+ + A^- \overset{k}{\rightleftharpoons} RNH_2 + HA$$

where RNH_2 represents chitosan, HA the acid. With a strong acid such as hydrochloric acid, the equilibrium constant k is very low and the above salt hydrolysis reaction will not occur. In addition, HCl is very soluble in water, which explains that the ammonium chloride form is favored even at very low water contents, and is retained when a chitosan hydrochloride film is dried. This structure is certainly most interesting for the storage of protonated chitosan. It is also very soluble in water, yet its dissolving leads to very low pH's, which may be a disadvantage for certain biological applications.

For a weak acid such as acetic acid, calculations allowed the authors to evaluate that approximately 11·5% of the acid is initially present as HA in the medium. Furthermore, acetic acid is much less soluble in water than HCl and its boiling point is relatively low (118°C). These factors allow acetic acid to be easily removed upon drying.

For weaker acids like butyric or valeric acids, the above equilibrium is even more shifted to the right, but in these cases the boiling points of the acids are higher and their solubility in water is low. Their removal upon drying of chitosan salt films thus occurs at a lesser degree than with acetic acid. The role of water is important since, inside the film, the greater the water content, the more molecules will be concerned by the above equilibrium, and hence the loss of weaker acids will be greater. This system could be interesting for the sustained delivery of acid, especially if chitosan is partially reticulated (therefore insoluble in water). The acid removal may also be considered as a drawback in cases like acetic acid, which, in some circumstances, leads to secondary reactions, in particular in living media.

It is important to note that, for chitosan salts of weak acids which are volatilizable in ordinary conditions, the loss of acid will render it difficult to prepare solutions of

known concentrations from solid forms. This allows us to explain, in particular, the abnormal behavior observed by Sakurai *et al.* (1984); more precisely the fact that the end point obtained with butyric acid was below that of formic acid. Indeed, they studied the titration of solutions obtained by redissolving dried films.

It could be particularly interesting to consider chitosan salts obtained with weak, solid, soluble and non-toxic acids, for which the chemical structure of the films is stable during storage while in aqueous media progressive delivery of the acid is possible.

An example of such a system is that obtained with lactic acid, an α-hydroxy acid. Indeed, the chemical structure of a chitosan lactate film is stable upon storage, despite the good solubility and relatively low boiling point (~120°C) of the acid. This must be due to the presence of hydrogen bonding occurring between the acid and chitosan hydroxy groups adjacent to the reacting functions. Chitosan lactate can be stored as films, and lactic acid, very soluble, will be released in the presence of water. We therefore have a chitosan salt capable of delivering progressively an antibacterial acid (Cherrington *et al.*, 1992). However, in solution such a system yields very low pH's, due to the pK_a of lactic acid (3·08). This could be overcome by partially neutralizing the initial chitosan salt solution in order to obtain the desired pH (<6).

The behavior shown in II in Fig. 2 concerns the case of chitosan formate, which does not undergo the usual salt hydrolysis discussed above for the other carboxylic acids. Indeed, when a film prepared with chitosan and formic acid is dried, the chitosan $-NH_3^+$ and the formate $-COO^-$ bands regress, but a new peak also appears at 1673 cm^{-1} (this phenomenon occurs whether the film is dried in air or *in vacuo*; II in Fig. 2 shows the first case). It is known (Packer & Vaughan, 1958) that formic acid, upon dehydration, yields esters (with alcohols) or amides (with amines). The former show a peak at 1725 cm^{-1}, which does not appear in our spectra. Since, contrarily to other carboxylic acids, formic acid does not give an anhydride upon drying, we may conclude that an amide has been preferably formed between chitosan and the acid (usual formamides absorb around 1675 cm^{-1}). A mechanism for this reaction is proposed in Fig. 4, and the authors have noticed that the more the films are dried, the greater is the intensity of the 1673 cm^{-1} band, i.e. the more formamide is yielded. This result is not very surprising, since formic acid very often behaves differently to other carboxylic acids in organic reactions.

The FTir studies allowed the authors not only to confirm the purely electrostatic nature of the interactions occurring when chitosan is placed in the presence of carboxylic acids, but also to show that a chitosan carboxylate salt in the solid state evolves with time, depending on the pK_a's, boiling points, solubilities and possible interactions other than electrostatic ones (for example H-bonds).

X-ray diffraction

Ogawa *et al.* (1992) studied the polymorphism of chitosan membranes obtained from chitosan/carboxylic acid solutions. They found that chitosan samples showed differences in their crystallographic characteristics, depending on the preparation method, especially between, as called by the authors, completely and incompletely dried films. Their experimental conditions were somewhat different to those of the present study, but it seemed interesting to see if, in the present cases, aging of chitosan salt films had an effect on the structure of their crystalline phase.

The X-ray diffraction experiments were performed on films of chitosan hydrochloride, formate, acetate and the free amino form ($-NH_2$ chitosan). The spectra are reported in Figs 5 and 7, and the main observations are summarized in Table 2, where they are compared to results of Ogawa *et al.* (1992).

Three chitosan allomorphs are obtained; two of them

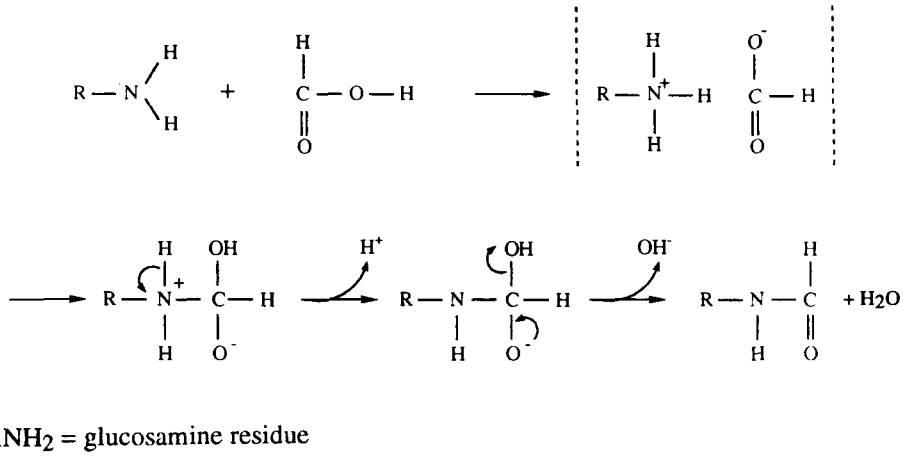

Fig. 4. Proposed mechanism for the formation of a formamide structure between chitosan and formic acid, upon drying of a chitosan formate film.

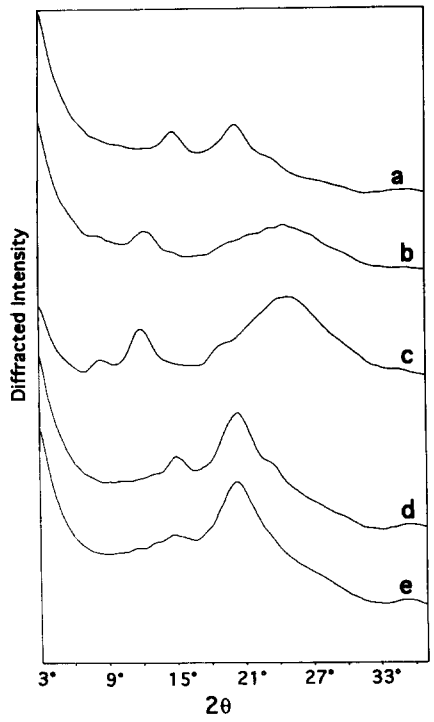

Fig. 5. Transmission X-ray diffraction spectra of various films of chitosan carboxylate salts. (a) Chitosan in its free amino form (NH_2); (b) chitosan hydrochloride (NH_3^+); (c) chitosan acetate, dried 15 min *in vacuo*, analyzed within 48 h; (d) chitosan acetate, analyzed after 6 months storage; (e) chitosan formate, analyzed after 6 months storage.

are now commonly known and correspond to the 'tendon' and 'annealed' forms described by Ogawa and other authors (Ogawa, 1991; Ogawa *et al.*, 1992; Domard & Cartier, 1992).

Before discussing the results, let us clarify the terms 'dry' and 'dehydrated' used below, to avoid ambiguities. Dry samples correspond to samples having aged in open air or under vacuum at 70°C, and whose spectra were recorded at T_{room} (i.e. the same conditions as for the FTir study). Dehydrated samples contain no water molecules in the crystal structure. A sample referred to as 'dry' is therefore not necessarily a dehydrated sample, especially for hygroscopic materials. Hence we must distinguish two types of experiments: those run at T_{room} on previously dried or non-dried films and those run at 130°C where true dehydration is obtained. Unless otherwise stipulated, the spectra discussed below were run at T_{room}.

The hydrated ('tendon') form of chitosan shows a ring at 2θ around 10° (or peaks at ~8° and 12°). It is observed with pure hydrated —NH_2 chitosan (Ogawa *et al.*, 1992: an incompletely dried chitosan film immersed in a NaOH solution), or hydrated chitosan salts. The latter include salts of weak acids dried only briefly (curve c in Fig. 5), and chitosan hydrochloride (curve b in Fig. 5, and curves a and c in Fig. 7) whose water content remains relatively high and constant with time. Therefore, the 'tendon' allomorph may be obtained with hydrated films, whatever the chemical structure of chitosan (—NH_2 or —NH_3^+).

The dehydrated ('annealed') form shows a peak at $2\theta = 15°$ (Ogawa *et al.*, 1992). It corresponds either to dry —NH_2 chitosan (curve a in Fig. 5), or —NH_2 chitosan heated in water before or during the X-ray analysis (not specified by the author, Ogawa, 1991) or dry chitosan salts of weak volatilizable acids (acetic acid, etc.) (curves d and e in Fig. 5). In the latter cases, however, as a result of salt hydrolysis or formation of a formamide, we do not observe a true dry —NH_3^+ form (see FTir study). Although it is quite easy to analyze hydrated samples, a true dehydrated chitosan is more difficult to obtain especially for the —NH_3^+ form, which is either too unstable (case of weak acids) or too hygroscopic. Yet, in order to further the study, it seemed important to show whether a true complete dehydration led to modifications of the crystallinity.

In a first step, to determine the conditions necessary

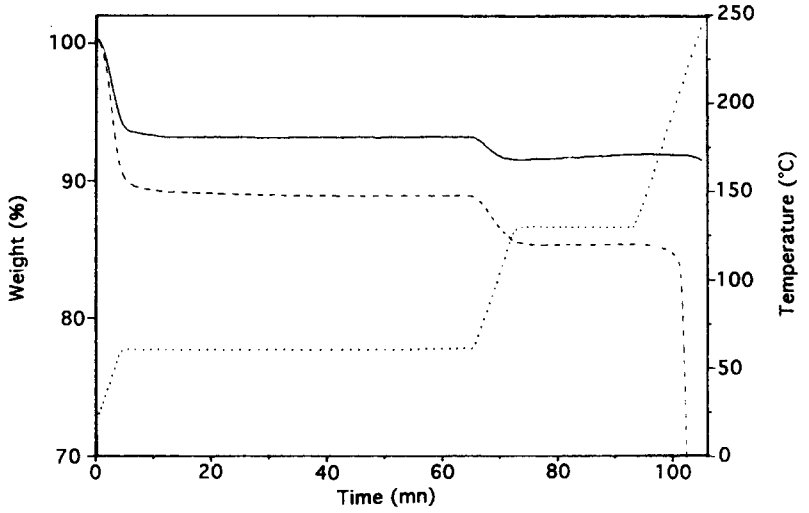

Fig. 6. Thermogravimetric analysis of films of chitosan hydrochloride (---) and chitosan in its free amino form (—). The heat treatment is shown by the dotted line (· · ·) and the heating ramps were 10°C/min.

for the dehydration of chitosan and control the behavior of the samples, the authors performed thermogravimetric analyses of a $—NH_2$ form as well as a true salt form — chitosan hydrochloride (Fig. 6). In both cases, it was observed that the water could be entirely removed only after heating (briefly) at 130°C. The experiments reported in Fig. 6 show the entire elimination of the usual two kinds of water: the free water at 60°C and the bound water at 130°C. Both samples had been initially dried *in vacuo* at 70°C for 1 h and then stored at T_{room} for several hours before the TGA measurements. They correspond to what is generally considered as dried samples. Figure 6 shows that they always contain ~8·5% and 14% of water for the $—NH_2$ and $—NH_3^+$ forms, respectively. The 60°C and 130°C isotherms show that the two kinds of water — free and bound — can be easily eliminated, and heating the films at 130°C for a relatively short time yields true dehydrated samples. Nevertheless, cooling down to T_{room} leads to fast recovery of this water (result not shown). Therefore, whatever the case, samples at T_{room} always contain water.

It then appeared interesting to study the crystallography of true dehydrated samples. To do so, the authors recorded the X-ray diffraction spectra of dried $—NH_2$ and $—NH_3^+$ forms at T_{room}, 130°C and after cooling down to T_{room} (1 h later). In the case of $—NH_2$ chitosan, there is no change upon heating and the crystalline form is then really a dehydrated form (Fig. 7). In the case of the $—NH_3^+$ film (Fig. 7), a new form giving bands at 8° and 13·5° appears for the experiment at 130°C and is converted to the initial form after cooling. This shows that a true dehydrated crystalline form corresponding to the salt exists but can only be obtained after complete elimination of water. It is also shown that in this case, the crystalline structure acts as a sponge-like system. On the other hand, it seems that the dehydrated form is more difficult to obtain with the salt than with the free amino form.

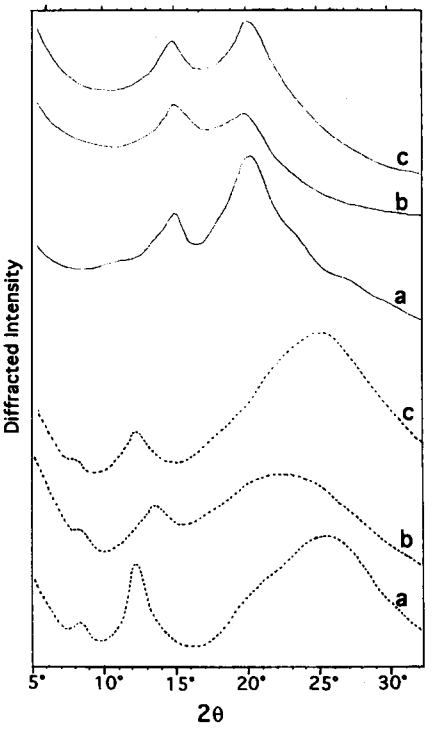

Fig. 7. Transmission X-ray diffraction spectra of films of chitosan hydrochloride (---) and chitosan in its free amino form (—). (a) At T_{room}, before any heating; (b) at 130°C; (c) at T_{room}, after cooling down from 130°C to T_{room}.

These results have shown the existence of one hydrated crystalline form, independent of the chemical structure ($—NH_2$, $—NH_3^+$) and dehydrated forms, different for the $—NH_2$ and $—NH_3^+$ structures.

The structure of chitosan salt crystals has been studied by Saitô *et al.* (1987) who proposed the following: Type I crystals, which contain no water molecules, correspond to a twofold helix and the length of the c-axis (fiber axis) is ~10 Å. Type II crystals include water molecules, and show a helical structure which is prob-

Table 2. Comparison between the results obtained by X-ray diffraction on the various solid state forms of chitosan

Sample	Diffraction peaks or spots used to compare the structures	Source
Chitosan in the NH_2 form		
Dry or dehydrated NH_2	15°	Ogawa, present study
Hydrated NH_2	10°	Ogawa *et al.* (1992)
Chitosan in a salt form		
Hydrochloride (hydrated)	8°, 12°	Present study
Hydrochloride (dehydrated)	13·5°	Present study
Acetate, dried briefly	8°, 12°	Present study
Acetate, dried briefly	10°	Ogawa *et al.* (1992)
Acetate, dried 6 months	15°	Present study
Acetate, dried 6 months	15°	Ogawa *et al.* (1992) ($\overline{DP_v}$ = 660, 1720)
Formate, dried 6 months	15°	Present study

ably four- or eight-fold, with a *c*-axis of ~41 Å. The lattice parameters (*c*-axis lengths) and the fact that the packing of four- or eight-fold helices is much looser than that of two-fold helices, show that the presence of water molecules in the crystal lattice considerably increases the distances between the chitosan chains. We can therefore partly understand why the chemical structure of hydrated chitosan does not influence the very loose crystalline structure, contrarily to dehydrated chitosan where a change in the chemical nature of the molecules, much more closely packed, will have a great influence on the chain arrangements within the crystals.

Finally, in Fig. 5 we notice an increase, upon storage, of the crystallinity of the chitosan films prepared with weak acids (the peak around 20° becomes sharper and the resolution of the spectrum as a whole is improved). A similar observation has been reported for glucosamine oligomers (Domard & Cartier, 1992), whose crystallinity increased considerably with the aging of samples in the solid state ('tendon' form), in particular in the presence of water.

CONCLUSIONS

Potentiometric and spectroscopic studies allowed the authors to show that the interactions between chitosan and carboxylic acids (up to C5) are purely electrostatic in aqueous solutions and the corresponding salts are formed, to a greater or lesser degree, depending on the strength of the acid. The pH's of such solutions vary with the pK_a of the acid used, and thus the pH of a chitosan solution may be controlled simply by using the appropriate acid as the counterion.

If such chitosan salt solutions are dried, films may be obtained whose chemical and crystalline structures depend on the drying procedure. Upon long-term storage, all films become more crystalline and anhydrous. For strong water-soluble acids such as HCl, the chitosan salt, in the solid state, is very stable upon storage, while with weak, volatilizable acids such as acetic and other acids, the films progressively lose their acid content, particularly in the presence of water.

We can thus conceive sustained release delivery systems where chitosan could vector useful carboxylic acids to a biological site (for example, butyric acid in cancer treatments (Nudelman *et al.*, 1992), or lactic acid in wound healing), without causing inflammatory responses due to low pH's, and then progressively deliver the acid.

ACKNOWLEDGMENTS

Chitosan was kindly supplied by Aber Technologies, France. The authors are grateful to D. Antoni (COLETICA), G. D'Assenza and Dr J.F. Legrand (Spectrométrie Physique, Grenoble) who recorded the X-ray diffraction spectra. Financial support from COLETICA (Lyon) and the ANRT is acknowledged.

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