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Regenerated chitin from phosphoric acid solutions

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Concentrated phosphoric acid at a minimum concentration of 75% readily dissolves α-chitin at room temperature. A maximum concentration of 3% (w/v) can be obtained. The initially high viscosity of these solutions decreases quickly during the first 12 h. After one week in phosphoric acid, chitin yields mainly a sugar phosphate. Under the same experimental conditions, *N*-acetyl-D-glucosamine, the monomer of chitin, has been shown to be esterified at its anomeric position by a phosphate group. During the first hours in a phosphoric acid solution, regenerated chitins are not modified chemically although their molecular weights are reduced. Concentrated phosphoric acid is shown to be useful solvent for the preparations of regenerated chitins having an average DP from 1000 to 100, © 1997 Elsevier Science Ltd

INTRODUCTION

The dissolution of chitin, a poly β(1–4)*N*-acetyl-D-glucosamine, provides a starting point for the production of films or fibres from this natural polymer. Consequently, there has been a number of studies on the dissolution of chitin. Aqueous solvents are generally unsuitable, with the exception of saturated lithium iodide and thiocyanate solutions. Chitin is soluble in concentrated mineral acids or in a number of anhydrous carboxylic acids. Organic solvents have also been studied (Roberts, 1993).

Hackman (1962) studied the action of concentrated mineral acids on chitin. There is rapid dissolution in 10 M hydrochloric, sulfuric and phosphoric acid solutions at room temperature with a rapid hydrolysis of the polymeric chain. It was reported that sulfuric acid can bring about sulfation of the hydroxyl groups (Nagasawa *et al.*, 1971). It was found that the turbidity of chitin solutions in phosphoric acid was greater than that of the other acidic solutions. This turbidity indicated a lower rate of hydrolysis of chitin in phosphoric acid. Concentrated phosphoric acid has been used for a long time as a solvent for cellulose, a polysaccharide which has a structure and properties close to those of chitin. In particular these solutions have been used for the determination of the intrinsic viscosity of cellulose solutions (Vink, 1967). Chitosan has also been dissolved in phosphoric acid (Omura *et al.*, 1991); (Hasegawa *et al.*, 1993). The experimental conditions used in these studies lead mainly to the formation of oligomers.

To our knowledge there is only one patent indicating the possibility of using phosphoric acid as a solvent for chitin (Capozza, 1975). We have shown recently that concentrated phosphoric acid can easily dissolve chitin within 20 min at room temperature under vigorous stirring, yielding viscous solutions with polymer concentrations in the range of 1–3% (w/v) (Vincendon, 1995). Chitin has been shown to remain as a polymer although the DP values decrease with the time spent in the solution.

The present paper investigates different mechanisms of chitin dissolution concentrated phosphoric acid and the possible concomittant chemical modifications of the polymer chain.

EXPERIMENTAL

Chitin powder (100–500 μm) from crab shells used in this work was a gift from Aber Technologies (France). Chitin solutions were prepared by stirring the chitin powder at high speed in 85% phosphoric acid at room temperature for 40 min. The viscous solution was then filtered on a no. 2 sintered glass funnel. Generally, no solid or gel was left on the funnel. Regenerated chitin from phosphoric acid solutions was obtained by precipitation in a cold 0.1 M NaOH solution. The precipitate was washed with water until neutral, then with ethanol and dried.

NMR measurements: ^{1}H NMR spectra were recorded on a Bruker AC 200 spectrometer operating at 200 MHz. ^{13}C NMR spectra were recorded on

a Brucker AM 400 spectrometer operated at 100 MHz.

Polarimetry measurements: performed on a 1% (w/v) concentration solution on a Jouan spectropolarimeter at a wavelength of 490 nm.

Intrinsic viscosities of chitin in *N,N*-dimethylacetamide/5%LiCl solution, were determined at 25°C according to the work of Terbojevich *et al.* (1988). The initial solution was filtered through a no. 3 sintered glass funnel and the viscosity measured with an Ubbelhode viscosimeter.

RESULTS AND DISCUSSION

N-Acetyl-D-glucosamine as a model for the chitin monomer

In order to obtain information about the nature of chitin chains in concentrated phosphoric acid. *N*-Acetyl-D-glucosamine was first investigated in the same medium. When dissolved in D_2O, *N*-acetyl-D-glucosamine rapidly reached the anomeric equilibrium: $\alpha = 79\%$, $\beta = 31\%$ (see Table 1). The ^{13}C NMR spectrum obtained in a 85% phosphoric acid solution, 30 min after the dissolution (Fig. 1A), shows an anomeric equilibrium $\alpha = 61\%$ and $\beta = 39\%$ which is shifted towards the β form. There is a noticeable solvent effect on the chemical shift of all the signals. The evolution of the *N*-acetyl-D-glucosamine ^{13}C NMR spectrum in phosphoric acid, is shown in Fig. 1. Distinct new ^{13}C signals are observed after 3 h in the spectrum of Fig. 1B, that indicates the formation of a new species in equilibrium with the two anomeric forms of the free sugar. The signal intensity of this intermediate species decreases after 15 h (Fig. 1C), yielding two new forms, clearly identified in the anomeric resonance region at 90 ppm. In Fig. 1D, obtained 1 wk after its dissolution, the *N*-acetyl-D-glucoamine shows only two isomers of a new derivative, the *N*-acetyl-D-glucosamine 1-phosphate (**3**) ($\alpha = 70\%$, $\beta = 30\%$). Thus, the *N*-acetyl-D-glucosamine is stable in concentrated phosphoric acid at room temperature, and reacts with the acid to yield a monophosphate at the anomeric position. The intermediate species identified during the reaction, has been assumed to be the glucofuranosyl oxazolinium ion (**2**) which was identified in the case of the dissolution of *N*-acetyl-D-glucosamine in anhydrous hydrofluoric acid (Bosso *et al.*, 1986). Four signals (indicated by an arrow) in the ^{13}C NMR spectrum of Fig. 1B are particularly characteristic of this ion at: 15 ppm for CH_3-C^+, 81.4 ppm for the glucofuranosyl C4, 114.4 ppm for C1 and 180.4 ppm for the C^+.

The ^1H NMR spectrum of *N*-acetyl-D-glucosamine, in deuterated phosphoric acid, shows a lower resolution, because of the high viscosity of the solution which induces a dipolar broadening of proton signals. The evolution of the ^1H NMR spectra, over a period of several hours, indicates through the variation of the H1 anomeric chemical shift, the esterification of the corresponding hydroxyl group.

Nature of the chitin chains in phosphoric acid solutions

Chitin rapidly dissolves in concentrated phosphoric acid at room temperature. Phosphoric acid should have a minimum concentration of 75% to obtain a clear and viscous solution, in a reasonable time. When these

Table 1. NMR chemical shift of *N*-acetyl-D-glucosamine and chitin in concentrated phosphoric acid solutions

Carbon signal	C=0	C-1	C-5[a]	C-3[a]	C-4	C-6	C-2	CH_3
N-Acetyl-D-glucosamine (D_2O)								
α 79%	173.5	90.0	73.0	69.8	69.2	60.75	53.2	21.0
β 21%	173.7	94.0	75.0				55.85	21.2
N-Acetyl-D-glucosamine (H_3PO_4/30 min)								
α 61%	177.5	90.6	73.6	71.0	70.2	61.2	55.15	21.0
β 39%	178.0	94.4	75.3				57.9	
N-Acetyl-D-glucosamine (H_3PO_4,15 h)	180.45	114.4[a]	81.45[a]	72.2	70.25	63.45[a]	57.9	15.0[a]
	178.85	94.5	75.7	71.2	70.15	61.35	57.35	21.0
	178.2	92.85	75.4		70.05		57.25	21.5
	177.85	90.7	73.7		68.6		54.95	
		89.2	72.75		68.2			
N-Acetyl-D-glucosamine (H_3PO_4, 1 wk)								
α 70%	179.0	89.2	71.0	71.0	70.0	61.2	54.9	20.9
β 3.0%		9.8	75.6	72.2			57.3	21.0
Chitin (H_3PO_4,15 h)	178.0	99.8	73.6	71.5	78.0	60.0	55.5	21.0
Chitin (H_3PO_4, 1 wk)	179.9	92.75		72.1	70.8	61.2	34.9	21.0
		89.25		72.1	70.		57.3	
		88.95						

[a]Assignment may be reversed.

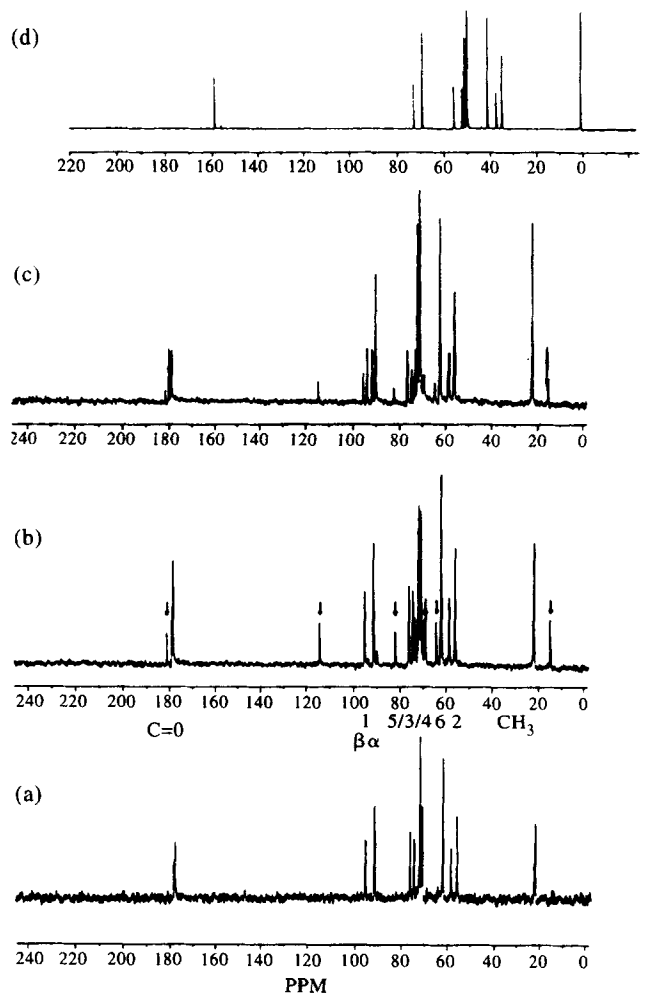


Fig. 1. 50 MHz ^{13}C NMR spectra of *N*-acetyl-D-glucosamine in 85% phosphoric acid solution: (a) 30 min after its dissolution; (b) 3 h (arrows indicate the glucofuranosyl oxazolinium signals); (c) 15 h; (d) 1 wk.

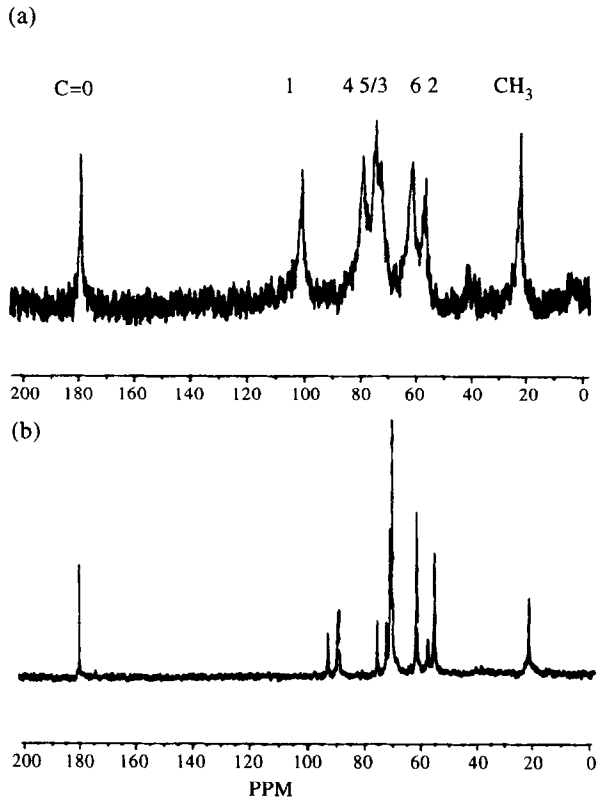
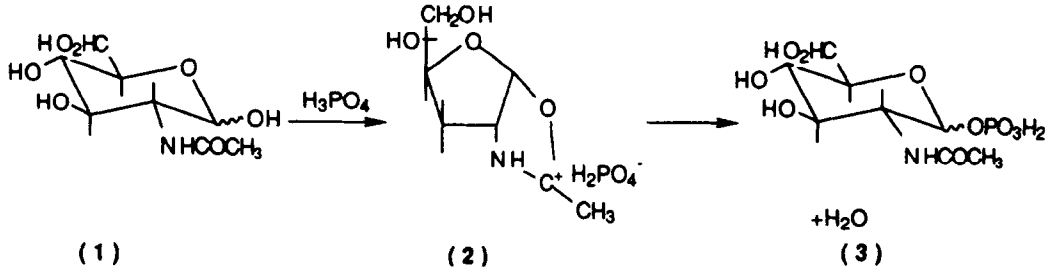


Fig. 2. 100 MHz ^{13}C NMR spectra of chitin in 85% phosphoric acid solution ($c = 1\%$) at room temperature: (a) 12 h after dissolution; (b) 1 wk after dissolution.

solutions are stored at room temperature they show a large decrease of their viscosity, particularly during the first 12 h (Vincendon, 1995). This is the time necessary to obtain the ^{13}C NMR spectrum shown in Fig. 2A. The resolution and the signal-to-noise ratio are low, because of the low polymer concentration on one hand ($c = 1\%$, w/v) and the high viscosity of the solution on the other. Nevertheless, this NMR spectrum clearly shows the presence of the chitin polymer chain, indicated by the eight charasteristic signals of the *N*-acetyl-D-glucosamine repeating unit. The chemical shift values of the carbon nuclei signals (Table 1) are in good agreement with those of chitin, obtained in non degrading solvents such as saturated lithium thiocyanate aqueous solution or *N*,*N*-dimethylacetamide containing lithium chloride (Gagnaire *et al.*, 1982). The presence of *N*-acetyl signals, which indicates that after 12 h in phosphoric acid no or few *N*-acetyl groups have been cleaved, should be noted.

The ^{13}C NMR spectrum shown in Fig. 2B was obtained for the same solution 1 wk after the dissolution. The increased resolution observed on this spectrum is likely due to the large decrease of the viscosity. This NMR spectrum presents signals at chemical shift values entirely different from those of chitin (see Table 1). It is possible to correlate most of these signals with those of the *N*-acetyl-D-glucosamine-1-phosphate



(1) (2) (3)

Scheme 1

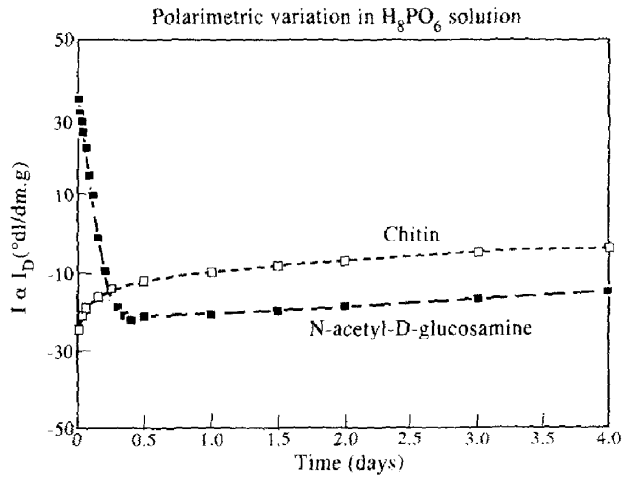


Fig. 3. Variation of the optical activity of *N*-acetyl-D-glucosamine and chitin as a function of time, in concentrated phosphoric acid.

(3). It can be concluded that after a long period in concentrated phosphoric acid at room temperature, chitin is hydrolyzed and yields mainly a monosaccharide phosphate.

$\alpha]_D$, of the solution, as shown in Fig. 3. Chitin in solution shows a negative $[\alpha]_D$ value which varies with time. This variation can arise either from *in situ* generation of degradation products or from a conformational change of the chains. It has already been observed that chitin, in the non degrading solvent *N,N*-dimethylacetamide/LiCl gives an evolution of the $[\alpha]_D$ value; this was attributed to a conformational change of the chains (Austin *et al.*, 1982). The *N*-acetyl-D-glucosamine optical activity variation is also shown on the same figure. It shows a large decrease which originates from esterification of the anomeric hydroxyl group. The final negative value $[\alpha]_D = -15°$, is due to the *N*-acetyl-D-glucosamine-1-phosphate in the anomeric ratio: $\alpha = 70\%$ and $\beta = 30\%$. This negative value is slightly different from that obtained for hydrolyzed chitin, $[\alpha]_D = -4°$. It can be explained by the presence of several compounds in the hydrolyzed chitin solution, *N*-acetyl-D-glucosamine-1-phosphate being the major one. The initial large variation of $[\alpha]_D$ presented by the chitin solution is more probably due to an order/disorder transition induced by a statistical cleavage of the chains, rather than to the formation of traces of sugar phosphates.

Characterization of regenerated chitins

Chitin in phosphoric acid solution can easily be regenerated by precipitation in a non solvent: water, alcohols, acetone or by dialysis against running water. We generally used a cold 0.1 M NaOH solution as a precipitation medium. The yield of regenerated polymer after washing and drying is given in Table 2. As expected, the yield decreases as the time spent in solution increases. After

Table 2. Yield of regenerated chitin from phosphoric acid solutions and their phosphorus and nitrogen contents

Time (h)	Yield (%)	Phosphorus content (%)	Nitrogen content (%)
0	100	0.08	6.68
1	89	0.16	6.59
3	84	0.15	6.4
5	80	0.17	6.44
10	72	—	—
15	65	0.17	6.18
24	45	—	—

1 h in phosphoric acid, 89% of the polymer is recovered, whereas after 24 h this value decreases to 45%. The unrecovered part corresponds to soluble oligomers in the experimental conditions used for the precipitation. In practice, a 2% (w/v) solution obtained in 40 min, was regenerated under fibres through a syringe needle in acetone used as a non-solvent.

The regenerated polymer was first chemically characterized by the determination of phosphorus and nitrogen contents. The results are given in Table 2. There was a small increase in the phosphorus content for chitin dissolved in phosphoric acid, however this increase seems to be independant of the time spent in the solution. As *N*-acetyl-D-glucosamine under the same experimental conditions, is esterified on the anomeric position, it is suggested that a phosphate group is linked on the anomeric chain end of chitin. The nitrogen variation is an indication of the *N*-acetyl cleavage. It is rather constant over the first 5 h spent in phosphoric acid.

The regenerated chitin is soluble in the complex solvent *N,N*-dimethylacetamide/LiCl, which has been used for its characterization. Figure 4 shows the ^1H NMR spectrum, in deuterated dimethylacetamide solution, of regenerated chitin after 12 h in phosphoric acid. This NMR spectrum is identical to that already published for natural chitin (Vincendon, 1985). It should be particularly noted that the NH signal at

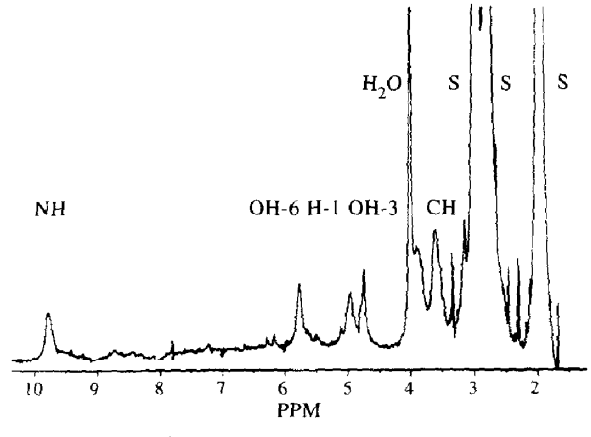


Fig. 4. 200 MHz ^1H NMR spectrum (60°C) of regenerated chitin after 12 h in concentrated phosphoric acid solution (solvent: deuterated *N,N*-dimethylacetamide/LiCl).

9.8 ppm, which reveals the presence of the *N*-acetyl group on the chitin chain after 12 h spent in phosphoric acid solution.

The ^{13}C NMR spectrum obtained on the same sample shows six signals which were assigned to the ring carbon of the *N*-acetyl-D-glucosamine repeating unit of chitin. The *N*-acetyl signals, C=O and CH_3, were not observed because of overlapping with the solvent signals. The spectrum of a sample regenerated after 24 h in phosphoric acid is shown in Fig. 5. In this spectrum, small extra signals were observed (labelled by an arrow in Fig. 5). For this time spent in solution, the hydrolysis of the chitin chains has reached such a level that the chain ends become visible.

The IR spectrum of regenerated chitin was identical to that of the starting material, and typical of the α form ($\nu_{C=O}$ = 1650 and 1625 cm^{-1}).

There was a large decrease in the chitin average molecular weight during the maturation time in phosphoric acid, which is indicated by the observed decrease in viscosity of the solution (Vincendon, 1995). In order to obtain a quantitative determination of this phenomenon, the intrinsic viscositics of the regenerated chitins were measured. The evolution of the intrinsic viscosity of different regenerated chitins in the non degrading solvent *N*,*N*-dimethylacetamide/5% LiCl is shown in Fig. 6. Using the Mark–Houwink relationship with constants $a = 0.69$ and $k = 2.4 \times 10^{-3}$ (Terbojevich *et al.*, 1988) it has been possible to determine the average degree of polymerization of the regenerated samples: DP values varying from 1300 to 110 are commonly obtained.

In conclusion, this study shows that it is possible to use concentrated phosphoric acid as a solvent for chitin. Solutions of chitin in phosphoric acid can be used directly to characterize this polymer by means of physicochemical methods (NMR, polarimetry, viscosimetry). These solutions also allow (at a minimum concentration of 2%, w/v) to regenerate fibres of chemically unmodified chitin. The acidic nature of this solvent causes a continuous decrease in the molecular weight as a function of the time spent in the solution. Thus the regeneration of chitins having various DP is possible. If the solutions are kept at room temperature for less than 5 h, the main chemical modification is a small increase in the phosphorus content, which mainly arises from the esterification of the anomeric end-chain.

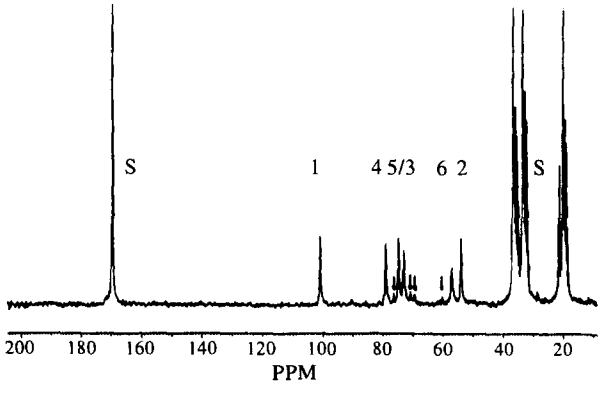


Fig. 5. 100 MHz ^{13}C NMR spectrum (60°C) of regenerated chitin after 24 h in concentrated phosphoric acid solution (solvent: deuterated *N*,*N*-dimethylacetamide/LiCl.

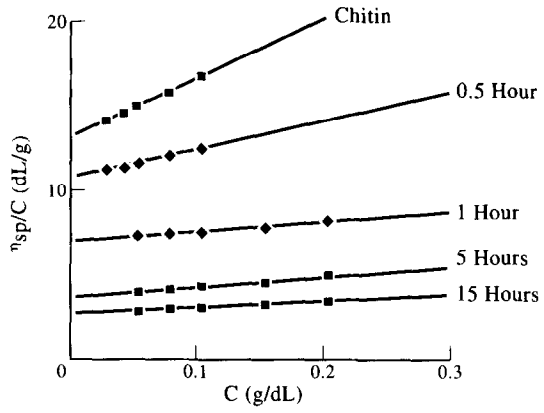


Fig. 6. Variation of the intrinsic viscosity of regenerated chitins with time in phosphoric acid (solvent: *N*,*N*-dimethylacetamide/5% LiCl).

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