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Alkaline *N*-Deacetylation of Chitin Enhanced by Flash Treatments. Reaction Kinetics and Structure Modifications

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ABSTRACT

A new method for N*-deacetylation of chitin is proposed in which a polymer almost free of* N*-acetyl groups is obtained by flash treatment. The reaction is carried out in 40% NaOH solution for 30–270 s at 140–190°C, using saturated steam.*

Flash treatment was found to proceed faster and with a higher activation energy for the deacetylation reaction ($E_a = 36$ kcal mol^{-1}) compared with the traditional treatment ($E_a = 11$ kcal mol^{-1}). X-Ray diffractometry, CP-MAS ^{13}C-NMR and FTIR spectroscopy show that the flash treatment induces structure modifications; in particular, higher crystallinity indexes and specific area values are observed together with changes in the local and chain conformation.

INTRODUCTION

Regeneration of amino functions from acetamidodeoxy carbohydrates can be performed under acid and basic conditions (Hanessian, 1972);

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unfavourable steric effects frequently hinder the reaction (Thompson & Wolfron, 1963). Despite numerous attempts, *N*-acetyl groups could not be removed by acid reagents without inducing hydrolysis of the polysaccharide backbone. In the presence of alkali, polysaccharide chains were found to undergo degradation because of the high concentrations of reagents and prolonged reaction times required to obtain a complete deacetylation. The low reactivity of chitin against the deacetylation reaction was ascribed to the *trans*-arrangement of acetamido groups in the monomeric unit with respect to the hydroxyl group OH-3 (Muzzarelli, 1977).

Several alkaline methods have been proposed, most of them involving the use of sodium or potassium hydroxide as well as anhydrous hydrazine–hydrazine sulfate (Dmitriev *et al.*, 1975). More recently, a new method for deacetylation has been presented in which tiophenol is added to prevent degradation and to exert a catalytic effect (Domard & Rinaudo, 1983). However, the deacetylation reaction was performed under heterogeneous conditions leading to sparsely as well as non-uniformly accessible block copolymers of *N*-acetyl-D-glucosamine and D-glucosamine residues, whose physicochemical properties appeared quite different with respect to those of chitosan randomly deacetylated under homogeneous conditions (Kurita *et al.*, 1977).

In the present work, *N*-deacetylation of chitin was performed by alkaline treatments in the presence of saturated aqueous vapour. The aim of the work was to compare the kinetics of flash *N*-deacetylation reactions with those of traditional alkaline treatments. The most favourable conditions for the modification of the morphology and supermolecular structure of the substrate to make the resulting chitosan more accessible to chemical reagents and hence more suitable for uniform derivatization along the polymer chain were also investigated.

EXPERIMENTAL

Material and methods

A commercial sample of crangon α-chitin in flake form was supplied by Chitobios (Italy). Flash treatment was performed in a EC 300 Deltalab pilot reactor (CNRS Patent). The system consists of a reactor (nominal capacity 1 litre) used for heating and compression, and a cyclone (50 litres) which collects the exploded material after decompression.

In a typical run, a sample of 30 g of chitin was impregnated with 500 ml of 40% (w/w) aqueous NaOH solution *in vacuo* and then stored at 4°C for 16 h and eventually flashed.

Flash treatments were carried out in the range of 140–190°C for 30–270 s; the material was exploded and released into the cyclone by instantaneous decompression (1–2 s) of the reactor. The solid chitosan (chitosan A) collected after the explosion was repeatedly washed with water, held submerged in 10% (v/v) methanol–HCl (37%) mixture for 16 h, neutralized with triethylamine, washed with methanol and finally dried *in vacuo* at 50°C.

Conventional *N*-deacetylation treatment was performed as follows: 500 mg of chitin was impregnated under stirring with 15 ml 40% (w/w) aqueous NaOH solution in a Pyrex flask equipped with screw cap. The flask was then placed in an oil bath at constant temperature (in the range of 80–110°C) for 0·5–30 h under stirring. At the desired time, the chitosan (chitosan B) was recovered by filtration and washed as for flash treatments.

Intrinsic viscosity

The viscosity of chitosan solutions in 0·1 M CH_3COOH–0·2 M NaCl was determined using a suspended level Ubbelohde viscometer at 25·0 ± 0·1°C. Dilutions were performed directly in the viscometer. Intrinsic viscosity $[\eta]$ values were obtained by means of the Huggins equation (Huggins, 1942).

Water retention values (WRV)

The WRV values were determined by submerging samples in excess distilled water. After 20 h the excess water was removed by filtration through a glass filter (G4) and then centrifuged for 10 min at 4000 rpm. The values were then calculated from the equation (Ferrus & Pages, 1977):

$$\text{WRV}\,(\%) = \frac{W_1 - W_0}{W_0} \cdot 100$$

where W_1 and W_0 are the weights of samples after centrifugation and drying to constant weight at 105°C, respectively.

X-Ray diffractometry

X-Ray powder patterns were recorded using Ni-filtered Cu Kα radiation from a Siemens (Munchen) 500 D diffractometer equipped with a scintillator counter and a linear amplifier.

The crystallinity index (CrI) was determined according to the method proposed for cellulose (Segal *et al.*, 1959) and applied to chitosan

(Struszczyk, 1987) by using the equation:

$$\mathrm{CrI} = \frac{I_{110} - I_{am}}{I_{110}} \cdot 100$$

where I_{110} is the maximum intensity (arbitrary units) of the (110) lattice diffraction and I_{am} is the intensity of amorphous diffraction in the same units at $2\theta = 16$.

The apparent crystal size D_{app} (110) of chitosan in the direction perpendicular to the (110) crystal plane was calculated with the aid of the Scherrer equation (Klug & Alexander, 1969):

$$D_{app}(110) = \frac{k\lambda}{\beta_o \cos\theta}$$

where β_o (in radians) is the half-width of the reflection corrected for instrumental broadening; k constant, indicative of crystallite perfection was assumed to be 1; λ is the wavelength of radiation used; θ is one half of the Bragg angle.

^{1}H- and ^{13}C-NMR spectra

^{1}H-NMR measurements were performed on 4% (w/v) 0·1 M CH_3COOD solutions in D_2O. The spectra were recorded at 200 and 300 MHz on Bruker (Karlsruhe) AC-200 and CXP-300 spectrometers, respectively. The residual HOD was suppressed by selective presaturation. The spectra of the most viscous samples were recorded on solutions of added DCl (pH = 2·0).

The acetyl content was determined from the ratio between the integral of CH_3 and those of H-2 glucosamine signals on spectra recorded both at 23° and 80°C in weak and strong acidic media, respectively.

CP-MAS ^{13}C-NMR spectra were obtained with a Bruker CXP-300 spectrometer at 75 MHz. The cross polarization was 1 ms while the repetition time and the ^{1}H 90° pulse were 4 s and 4·75 μs, respectively. The chemical shifts were measured with respect to tetramethylsilane (TMS), with benzene as a secondary substitution reference (128 ppm). Scans (1000–3000) were taken; the rotational speed was about 3·4 kHz.

Infrared spectra were obtained with a Bruker IFS 66 FTIR spectrometer using both KBr pellets and deuterated films. The films were prepared by drying in polyethylene moulds (0·5 cm deep, 2·8 cm diameter) 2 ml 0·1 M CH_3COOH solution of 4 mg of polysaccharide and by exposing the film to D_2O vapour. The spectra were either recorded

directly, or after regeneration (by 0·1 M NaOH solution for 16 h at room temperature, further rinsing in distilled water and drying at room temperature). Between 16 and 100 scans were taken with a resolution of 2 cm^{-1}.

RESULTS AND DISCUSSION

Kinetics

The deacetylation of chitin was carried out by the traditional treatment with 40% (w/w) aqueous NaOH as catalyst, as well as by flash treatment with the same concentration of alkali but in the presence of saturated steam. Whilst in the former case the temperatures explored were in the range 80–100°C, in the latter case they were higher, reaching values up to 180°C. The higher temperatures and correspondingly high steam pressures resulting from the explosion step, led to a marked enhancement of the reaction rate. Thus, the reaction times associated with the flash treatment were much shorter than those of the traditional treatment.

The time course of the chitin deacetylation carried out according to the above procedure is shown in Table 1, where the degree of deacetylation is shown as a function of the reaction time. The most significant data are also depicted in Figs 1 and 2. The trends of the two reactions appear quite similar, however the durations involved are of the order of hundreds of seconds for the flash treatment (Fig. 1) and tens of hours for the traditional one (Fig. 2). Due to the complexity of the heterogeneous reacting system the reaction rate could not be easily expressed in terms of a strict kinetic equation with a definite rate constant and reaction order, so the influence of temperature on the deacetylation rate has been investigated only on the basis of the initial rates as evaluated from the initial slopes, of the lines shown in Figs 1 and 2. The logarithms of the initial rate data were plotted against the reciprocal of the temperature $(1/T)$ and the results depicted in Fig. 3 were obtained. As can be seen from this figure, in both cases a rather good linearity was found, making it possible to estimate activation energy values of 36 ± 1 and 11 ± 1 kcal mol^{-1} for the flash and traditional treatments, respectively.

The dependence of the reaction rate on the temperature was also investigated taking into account the inverse of the times corresponding to 75% deacetylation ($t_{3/4}$), instead of the initial rates. Plotting $\ln t_{3/4}$ versus $1/T$ resulted in lines of similar slope to that obtained with the initial rate data (Fig. 3) indicating that the second approach leads to analogous values of E_a.

TABLE 1
Degree of Deacetylation (Per Cent) of α-Chitin Obtained in the Presence of 40% (w/w) Aqueous NaOH by Flash or Traditional Treatment at Different Temperatures and Various Times

Temperature (°C)	*Time (s)*								
	30	*60*	*90*	*120*	*150*	*180*	*210*	*240*	*270*
Flash treatment									
140	—	—	—	—	—	—	74·8	—	—
150	—	65·4	—	—	75·8	—	—	—	—
160	—	74·0	—	—	—	82·8	—	—	—
170	68·5	—	—	84·3	—	86·8	—	87·7	—
180	76·8	84·9	88·5	—	—	91·8	—	93·8	94·6
190	85·3	91·0	94·6	—	—	—	—	—	98·0
Temperature (°C)	*Time (h)*								
	0·5	*1*	*2*	*3*	*4*	*6*	*8*	*24*	*30*
Traditional treatment									
80	—	59·8	—	—	—	—	76·1	82·0	—
90	—	64·9	—	—	—	—	—	—	96·1
100	—	69·3	72·4	—	77·3	80·2	—	90·4	—
110	68·0	71·1	—	77·7	80·3	—	—	93·9	—

The large difference between the activation energy values determined for flash and traditional treatments clearly indicates that the mechanism involved in the two cases is somewhat different. It must be inferred that factors associated with the pressure and explosion step markedly modify the reaction mechanism with respect to that involved in the traditional treatment.

Viscosity measurements

Both traditional and flash treatments caused depolymerization of chitosan due to glycosidic bond hydrolysis and the 'peeling' reaction. Intrinsic viscosity values of flashed chitosan A decreased with increasing temperature or time of treatment (Fig. 4).

Likewise, $[\eta]$ values in the range 4·0–6·0 (dl/g^{-1}) were found for chitosan B treated at 80–100°C in an air atmosphere, thus indicating the presence of oxygen as a prerequisite for degradation in alkaline medium.

Experiments in the presence of oxygen scavangers like polysulphide or antraquinone compounds are in progress.

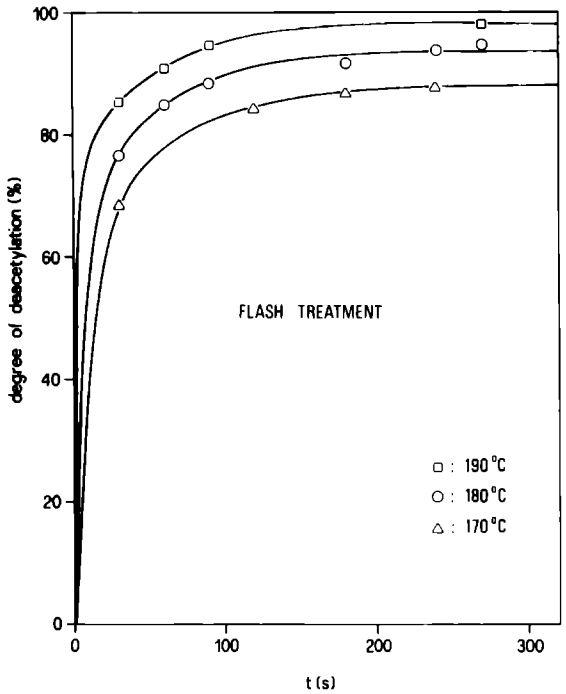

Fig. 1. Time courses of α-chitin deacetylation performed by flash treatment (product; chitosan A).

X-Ray diffraction and surface area measurements

Figure 5 shows diffraction patterns of chitin, flashed deacetylated chitin (chitosan A) and traditionally deacetylated chitin (chitosan B) materials. Both treatments result in the disappearance of most of the chitin equatorial reflection. Chitosan A showed a higher CrI than chitosan B (65 versus 50%) as well as a larger apparent crystal size (21·1 vs 12·3 Å). Differences between the main reflections of chitosan A and B were also observed.

The penetration of alkali into the chitin crystallites with cleavage of the acetyl groups conceivably modifies the initial order of the chitin molecules. However, it seems that this order was not completely destroyed and/or was partially recovered during the regeneration step, and probably during the heating step.

These results are similar to those obtained for lignocellulosic materials (Marchessault *et al.*, 1983) for which the increase of crystallinity observed after steam explosion treatment was explained as an effect equivalent to that of the heat annealing of thermoplastics.

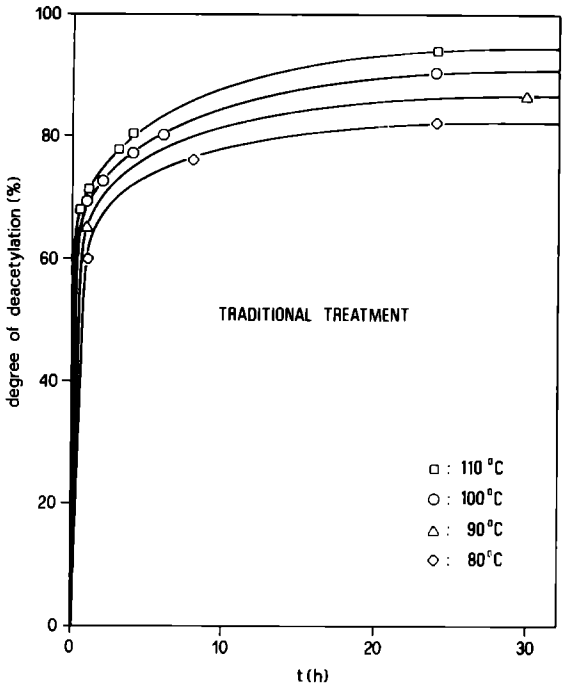

Fig. 2. Time courses of α-chitin deacetylation performed by traditional treatment (product; chitosan B).

The relative surface areas can be deduced from the WRV values (Table 2). These areas increase on going from chitin to chitosans with values for chitosan A being consistently higher than those for chitosan B. Although alkali treatment of polysaccharides generally induces an increase in accessibility, in alkali flash treatments the explosion step would seem to provide the most important contribution.

CP-MAS ^{13}C-NMR spectroscopy

The CP-MAS ^{13}C-NMR spectra of the flash treated chitin (Fig. 6(A)) clearly reflect the different extents of *N*-acetylation achieved at different temperatures, especially through the reduction in intensity of the signal of the methyl-acetamido-carbon (at $23 \pm 0{\cdot}5$ ppm).

Signals attributable to individual carbons of chitosan (Saito *et al.*, 1982) are essentially 'singlets' for products obtained at temperatures below 150°C. For products obtained at 160–170°C, because of the occurrence of a new signal emerging at $82 \pm 0{\cdot}5$ ppm, the C-4 signal consists of a doublet, which, in line with current interpretation of solid-phase NMR spectra of cellulose (Earl & Vanderhart, 1980), suggests the

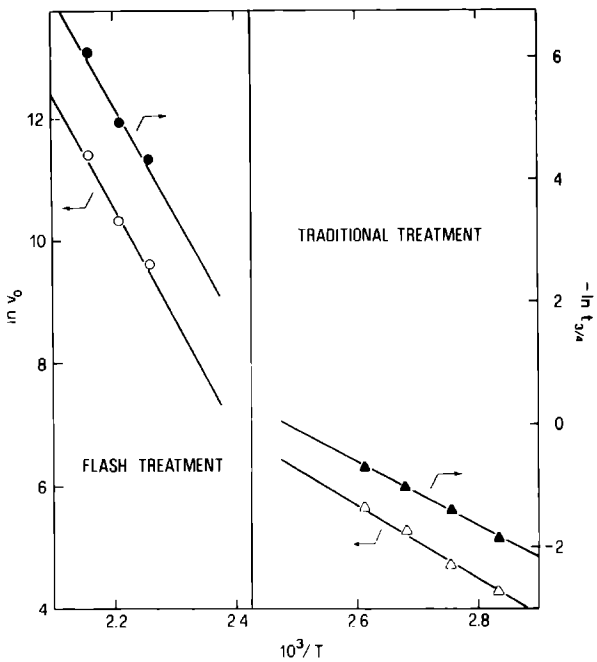

Fig. 3. Dependence of deacetylation rate on temperature. The percentage degree of deacetylation per hour v_0 is obtained from the initial slopes of the reaction rate data shown in Figs 1 and 2; $t_{3/4}$ is the time for 75% deacetylation.

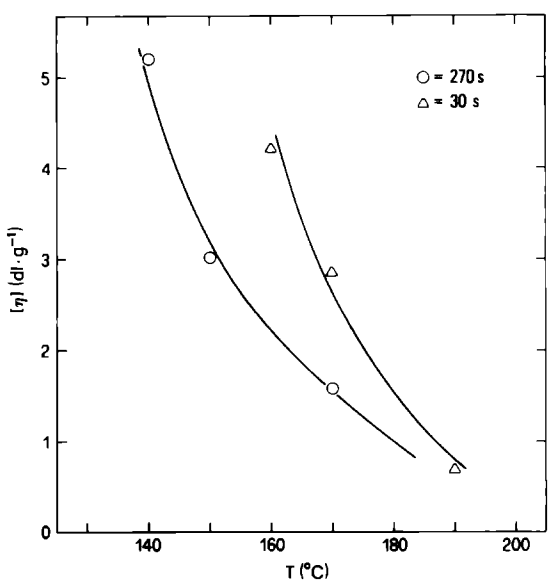

Fig. 4. Intrinsic viscosity of chitosan A solution as a function of temperature at different times of treatment.

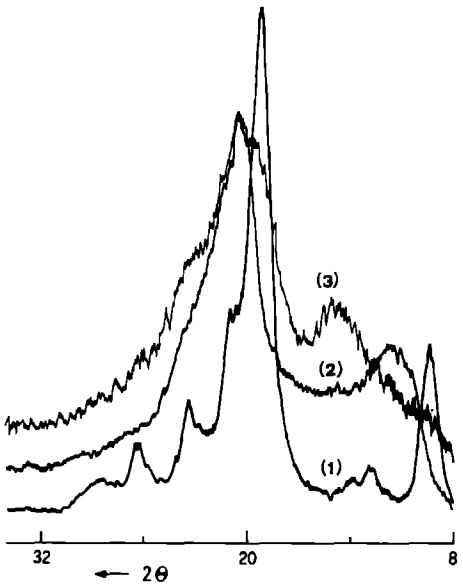

Fig. 5. X-Ray diffractograms of: (1) α-chitin, (2) chitosan A and (3) chitosan B.

TABLE 2
Water Retention Values of α-Chitin and Chitosans A and B

Samples	*Water retention values* ($cm^3\ 100\ g^{-1}$)
α-Chitin	18·4
Chitosan A	68·4
Chitosan B	37·4

coexistence of at least two distinctly different chain conformations and/or intermolecular chain aggregations. By analogy with the thermal behaviour of chitin (Ogura *et al.*, 1982), the chain arrangement suggested by the split of the C-4 signal for flash treatments above 150°C, may be attributed to a glass transition that allows chains in the amorphous region to attain a greater freedom of motion and 'relax' in a conformation controlled more by intramolecular than intermolecular interactions.

As shown in Fig. 6(B), signals of chitosan A are sharper than those of chitosan B, indicating an overall higher degree of order, which has also been suggested by X-ray and FTIR analysis. By increasing the degree of deacetylation, a shift in the C-2 signal occurs; this, together with signal

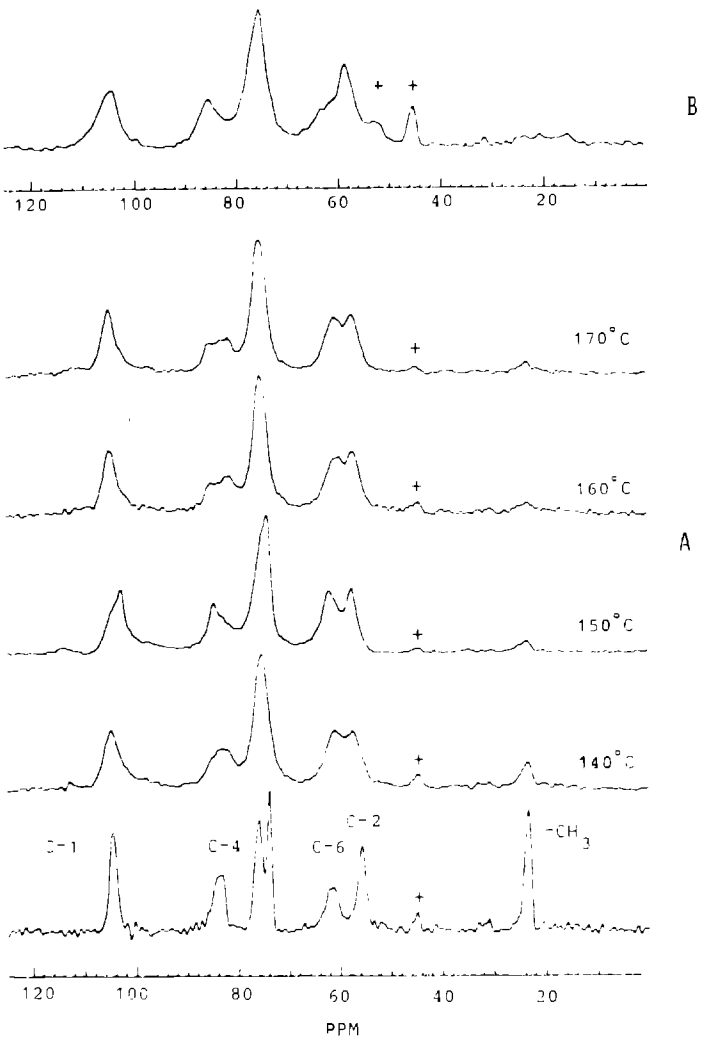

Fig. 6. CP-MAS ^{13}C-NMR spectra of chitosans A and B.

broadening, causes the C-2 and C-6 signals to merge into one large resonance. The splitting of the C-4 signal is not seen for chitosan B treated at 100°C, even if it cannot be ruled out in the sample prepared at 110°C.

FTIR spectroscopy

For the same degree of deacetylation the FTIR spectra of chitosans A and B were similar to each other, but quite different from that of the

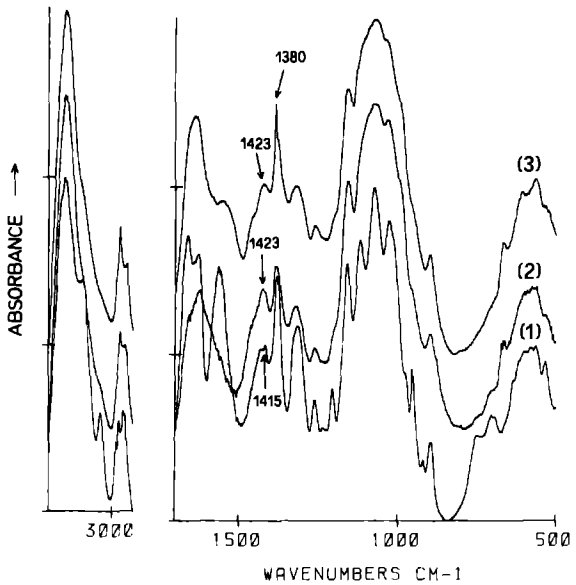

Fig. 7. FTIR spectra of: (1) α-chitin, (2) chitosan A and (3) chitosan B.

parent α-chitin (Fig. 7). As expected *N*-deacetylation is associated with a progressive weakening of the bands occurring at 1665 cm^{-1} (amide I vibrational mode) and at 3265 and 3100 cm^{-1} (NH amide bond stretching) (Samuels, 1981). Furthermore, the vibrational mode of amide II at 1550 cm^{-1} shifted to about 1560 cm^{-1}, and a new band appeared progressively at 3363 cm^{-1} due to the stretching of NH_2 groups (Darmon & Rudall, 1950).

The absence of strong absorptions above 3500 cm^{-1} in both the spectra of chitosan A and B inferred that there are no free OH groups. Both OH-3 and CH_2OH are involved in intra- and intermolecular hydrogen bonds as can be deduced from the features at 3480, 3400, 3270 and 3100 cm^{-1} for chitin and at 3454 cm^{-1} for chitosans A and B in the spectra recorded using the KBr pellet technique (Pearson *et al.*, 1960).

The region of 1500–1200 cm^{-1} in the IR spectra of carbohydrates is related to the local symmetry (Mathlouthi & Koenig, 1986), and the band around 1429 cm^{-1} was assigned to CH_2 bending and was dependent on the most favourable orientation of the primary OH group. This band appeared in chitosans A and B at 1423 cm^{-1} and at 1415 cm^{-1} in the parent chitin. On going from chitin to chitosans A and B the intensity of this band markedly decreased. An intensity decrease was

also observed with increasing temperature treatment for both chitosans A and B. As suggested by the NMR spectra, this may be ascribed to the occurrence of several conformations induced both by the new arrangement of deacetylated chains of chitosan and by the rearrangement of hydrogen bonds, at least in the amorphous regions of the polysaccharide.

The vibration mode of cellulose at 1372 cm^{-1} and the vibration of chitin and chitosan at 1379 cm^{-1} have been assigned to CH bending with some OH-bending contributions (Vasko *et al.*, 1972; Mima *et al.*, 1982). This band should reflect conformational changes (Hatakeyama *et al.*, 1976). The ratio of the intensities at 1372 and 2900 cm^{-1} was suggested as an index of crystallinity for cellulose (Nelson & O'Connor, 1964). This band occurred at 1379 and 1382 cm^{-1} for chitin and chitosan A and B respectively, and the absorbance ratios $a_{1379\,cm^{-1}/2900\,cm^{-1}}$ and $a_{1382\,cm^{-1}/2920\,cm^{-1}}$ decreased from 1·20 to 0·91 and 0·30 on going from chitin to chitosans A and B, reflecting the decrease in order as found by X-ray and NMR measurements.

The deuterium exchange of relatively loosely bound hydroxyl hydrogens of chitosan A and B films resulted in a drastic decrease in the intensity of intermolecular hydrogen bond bands, but the high frequency band at 3454 cm^{-1} of intramolecularly bonded hydroxyls of crystalline regions was less affected (Fig. 8). In addition, the band at 894 cm^{-1} assigned to a vibrational mode involving C-1 and the four atoms attached to it, was very sensitive to deuteration and therefore to the nature and extent of hydrogen bonding.

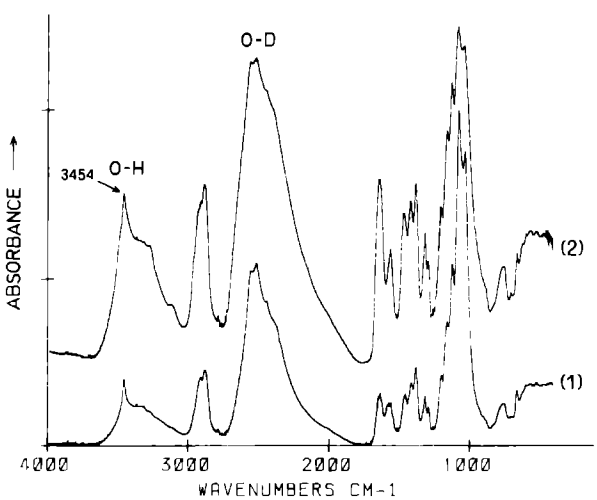

Fig. 8. FTIR spectra of deuterated: (1) chitosan A and (2) chitosan B.

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