ACCESSIBILITY OF THE FUNCTIONAL GROUPS OF CHITOSAN PROBED BY FT-IR SPECTROSCOPY OF DEUTERATED AEROGELS AND XEROGELS

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Abstract

Transmission FT-IR spectroscopy allowed to monitor the deuteration of wafers of chitosan aerogel and xerogel by D_2O vapour at room temperature. The complete deuteration of the alcohol and amine groups of the aerogel (surface area 175 m^2 g^{-1} as measured by N_2 volumetry) confirmed the high accessibility of its functional groups. The xerogel (surface area 5 m^2 g^{-1}) was only partially deuterated in more severe conditions. The isotopic shift of the deuterated groups allowed to confirm or revise some attributions of infrared bands of chitosan.

Introduction

Most infrared spectroscopic studies of chitosan were aimed at the determination of the deacetylation degree of chitin-based materials [1-9] or were directed to the characterisation of polysaccharides in hybrid materials [10-13]. Albeit these works provided a sound vibrational characterisation of the polymer, the preparation of the samples was unfitted for the study of the reactivity of the functional groups by FT-IR spectroscopy of adsorbed probe molecules, a powerful technique widely applied to the study of inorganic solids or, less frequently, high-surface area polymers [14-17]. The wafers of dried polysaccharides used for former spectroscopic studies presented a very small surface area. In this way, a too small fraction of functional groups was accessible for a significant study of their interaction with probe molecules.

The aim of the present paper is to show the unique possibilities offered by supercritical drying to prepare chitosan samples in which a large fraction of uronic groups is accessible. In the CO_2 supercritical extraction, the shrinkage induced by capillary evaporation is prevented and the solid prepared in this way, known as an aerogel, retains a high degree of dispersion. Indeed, very high surface areas have been reported for polysaccharide aerogels: values close to 300 m^2 g^{-1} for alginate aerogels [18] and higher than 100 m^2 g^{-1} for chitosan aerogels [19]. Due to such a high dispersion of the polysaccharide, a large fraction of the functional groups are accessible to probe molecules [19].

In this work, the accessibility of the functional groups of chitosan films has been monitored by FT-IR spectroscopy. The deuteration of the polysaccharide functional groups by D_2O vapours has been used as a probe reaction..

Materials and Methods

Sample preparation

Chitosan (from squid pen, Mahtani Chitosan PVT Ltd.) characterized by a degree of acetylation (DA) lower than 1.5 %, as measured by NMR and IR [2, 7] spectroscopy, and a weight-average molecular weight of 200,000 g/mol, measured by light scattering, was purified as follows: the polymer was dissolved at 1% (w/w) in a stoechiometric amount of aqueous acetic acid. After complete dissolution it was filtered successively on 3, 1.2, 0.8, 0.45 and 0.2 μm membranes (Millipore). Chitosan was precipitated with diluted ammonia up to a constant pH 9 of the solution and separated by centrifugation. The precipitate was repeatedly washed with deionised water and centrifugated until a neutral pH was achieved, then it was freeze-dried.

An aqueous solution was obtained by dissolving 0.2 g purified chitosan in 10 mL 0.055 M solution of acetic acid. This solution was spread out at room temperature in a Petri dish and gently covered by a 4 M NaOH solution. The gel was stored in the alkaline solution for 2 hours, and then filtered and washed with deionised water until pH 7.

Hydrogel drying

Xerogel films were obtained after drying the hydrogel on glass plates in an oven at 50°C. To obtain the aerogel films, intermediate alcogels were formed by immersion of the hydrogel films in a series of successive ethanol-water baths of increasing alcohol concentration (10, 30, 50, 70, 90, and 100 %) during 15 min each. The aerogel films were obtained by drying the alcogels under supercritical CO_2 conditions (74 bars, 31.5 °C) in a Polaron 3100 apparatus.

Evaluation of textural properties

Nitrogen adsorption/desorption isotherms were recorded in a Micromeritics ASAP 2010 apparatus at -196 °C after outgassing the sample at 50 °C under vacuum until a stable 3.10^{-5} mbar pressure was obtained with no more pumping. The surface areas were evaluated by the BET method. The average size of the polysaccharide fibrils was evaluated from the surface area by the formula $D = 4/(\rho S)$, where D is the fibril diameter in μm, S the surface area in $m^2\ g^{-1}$, and ρ the volumic mass of chitosan in g/cm^3. The surface/volume ratio allows to evaluate the fraction of geometrically exposed monomers. For these calculations, the material was assumed to present the properties ascertained for crystalline chitosan, viz. a volumic mass of 1.23 $g\ cm^{-3}$, an area per monomer of 0.439 nm^2 and a volume per monomer of 0.196 nm^3 [20].

Infrared spectroscopy

Suitable wafers were cut from the chitosan films and placed into a home-made all-silica cell provided with KBr windows for transmission IR measurements. The wafers were supported by a gold case. The cell allows evacuation, heat treatment and gas dosage. FT-IR spectra were recorded on a Bruker Vector 22 spectrometer, equipped with a DTGS detector. Adsorption of D_2O was carried out on wafers evacuated in situ at 80 °C (residual pressure lower than 10^{-6} mbar).

Results and Discussion

Textural properties of the materials

For the purpose of this article, a xerogel is defined as a dried gel which has considerably shrinked during drying. An aerogel is instead a dried gel which has retained most porous volume of the parent hydrogel [21]. Drying a hydrogel can result in a xerogel or an aerogel according to the drying method used. When the solvent, usually water, is evaporated, the capillary tension at the vapour-liquid interface brings together the secondary units of the gel. As a consequence, a xerogel with low porosity is formed. Alternatively, the solvent can be exchanged with a secondary solvent, usually CO_2 or low-molecular weight hydrocarbons, which is in turn compressed and heated beyond its critical point and evacuated as a supercritical fluid, in conditions in which no gas-liquid interface exists. In this way, the supercritical drying avoids the problems related with capillary tension and

prevents the pores of the material to collapse. The aerogel formed retains in the dry state an image of the dispersion of the wet gel.
Several previous spectroscopic studies on polysaccharide gels have been carried out on cryogels produced by freeze-drying. The ice formed by freezing the solvent can be sublimated with no capillary tension and most volume of the hydrogel can be retained. However, in many cases the pressure of growing ice crystals significantly alters the internal structure of the gel during freezing [22, 23].
The surface area of a chitosan cryogel, as measured by N_2 adsorption at -196 °C, is 5 m^2 g^{-1}, the same value measured for the evaporatively-dried xerogel used in this study. This value of surface area corresponds to an average diameter of the chitosan fibres about 0.7 μm and indicates that less than 0.3 % monomers are exposed at the surface.
The surface area of the supercritical-dried chitosan aerogel used in this study is 175 m^2 g^{-1}. Such a surface area corresponds to an average diameter of the chitosan fibrils of 18 nm and indicates that nearly 10 % monomers are exposed at the surface.
On the basis of their respective degree of dispersion, it can be expected that deuteration reactions would be more hindered by diffusion inside the material in the case of the xerogel than in the case of the aerogel.

Deuteration of the aerogel

The IR spectra of the outgassed aerogel before and after exposal to doses of D_2O vapour are reported in Figure 1 from 3800 to 1800 cm^{-1} and Figure 2 from 1700 to 600 cm^{-1}.
The O-H and N-H stretching bands form a broad envelope with main bands at wavenumbers around 3435, 3290 and 3180 cm^{-1}. The queue of the envelope towards lower wavenumbers extends until 2500 cm^{-1}, indicating a high level of hydrogen bonding of the functional groups.

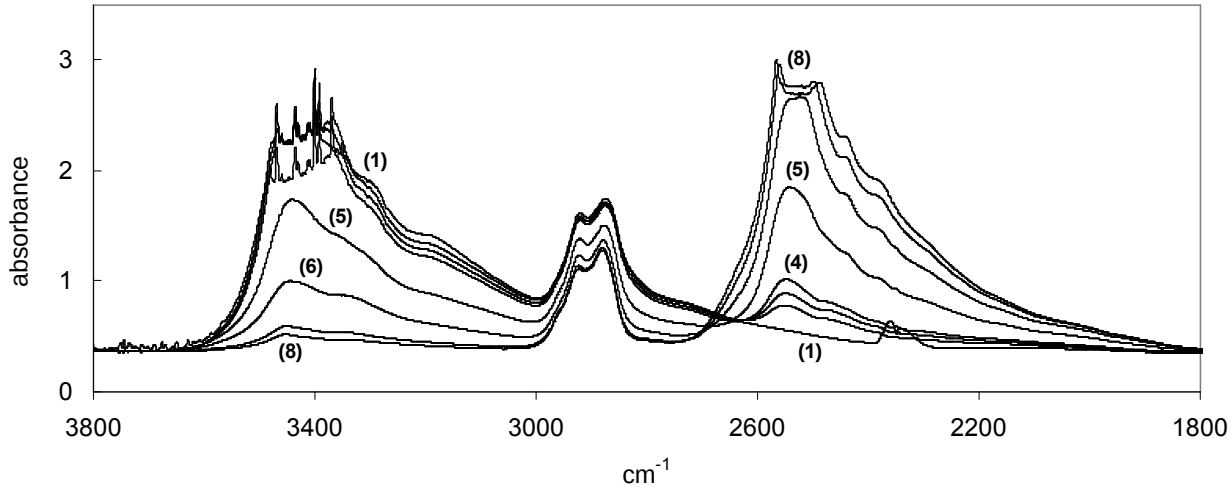

Figure 1. IR spectra of chitosan aerogel outgassed at 353 K (1) and exposed to D_2O pressure of 0.01 (2), 0.24 (3), 0.33 (4), 3.7 (5), 9.7 (6), 12 (7), and 13 (8) mbar. Wavenumber domain 3800-1800 cm^{-1}.

The admission of D_2O vapour brings about a progressive decrease of the intensity of all O-H and N-H bands. At a D_2O pressure of 13 mbar, the O-H and N-H bands have virtually disappeared, indicating a nearly complete deuteration of the alcohol and amine groups of the aerogel. The residual intensity at 3435 cm^{-1} corresponds to less than 0.5 % residual OH groups.
In close parallel with the decrease of the absorbance of the proton-bearing groups, the bands of the deuterated groups appear and grow at wavenumbers with maxima at 2529, 2432 and 2372 cm^{-1}. The isotopic shifts are in the region expected after the variation of reduced masses between protonated and deuterated species.
It can be observed that the envelope of the protonated groups, when allowance is made for spectra saturation, retains the same overall shape at all levels of deuteration. In the same way, the relative intensities of the bands of the deuterated groups remain the same during their rise of intensity. The

homothetical variation of the absorbance of the alcohol and amine bands indicates that D_2O is such an effective deuterating agent that no differences can be observed among the reactivities of the O-H and N-H groups.
The C-H stretching vibrations at 2914 and 2865 cm^{-1} are unaffected by deuteration, as expected on the basis of the low reactivity of the aliphatic C-H bonds.
The IR spectra in the wavenumber domain 1700-600 cm^{-1} for the aerogel, outgassed and exposed to doses of D_2O vapour, are reported in Figure 2. The NH_2 scissoring mode of the primary amine is observed at 1593 cm^{-1}, and the very low intensity of the amide band at 1655 cm^{-1} indicates a high degree of deacetylation of the sample.

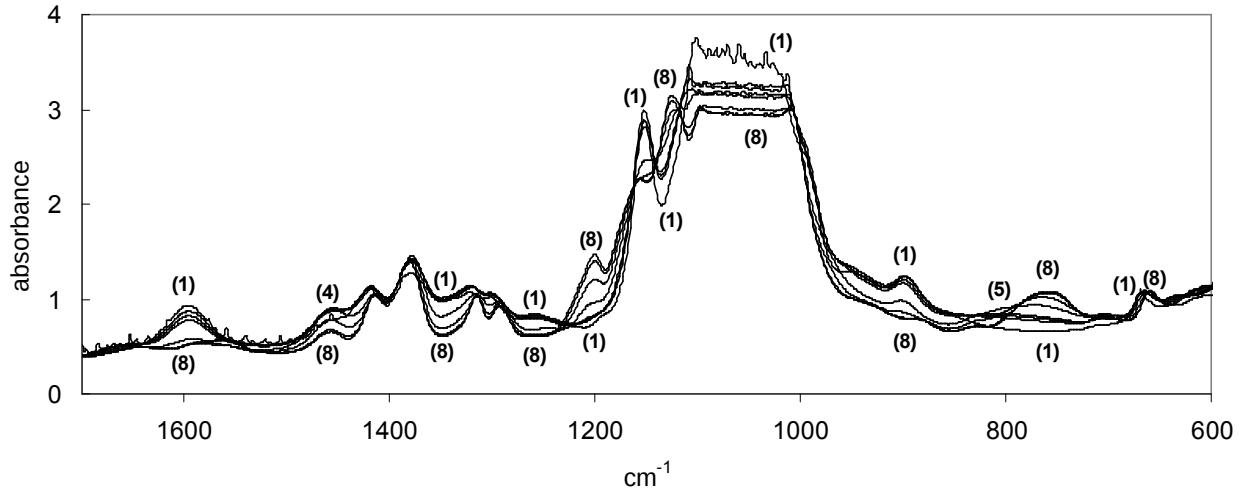

Figure 2. IR spectra of chitosan aerogel outgassed at 353 K (1) and exposed to D_2O pressure of 0.01 (2), 0.24 (3), 0.33 (4), 3.7 (5), 9.7 (6), 12 (7), and 13 (8) mbar. Wavenumber domain 1700-600 cm^{-1}.

The NH_2 scissoring band at 1593 cm^{-1} disappears with deuteration. The isotopic shift of this vibration has been calculated for other amines and allows to attribute to ND_2 scissoring the band which appears at 1197 cm^{-1} [24, 25]. It has to be observed that the rise of the band of the deuterated group does not exactly mirror the decrease of the band of the protonated group. The NH_2 scissoring band has virtually disappeared when the sample is at the equilibrium with less than 4 mbar D_2O vapour, whereas the ND_2 scissoring band is just appearing at such a pressure and grows at higher D_2O pressure. This trend suggests the presence of an intermediate NHD group, whose bending wavenumber should be intermediate between the wavenumbers of the NH_2 and ND_2 bands. It can be observed that the absorbance at 1453 cm^{-1} increases when the D_2O pressure rises until 3.7 mbar and decreases when the D_2O pressure increases further.
The band at 1453 cm^{-1} also includes a component whose intensity is not affected by isotopic exchange and which can be attributed to CH_2 scissoring. The intensity of the band at 1376 cm^{-1} is virtually unaffected by the isotopic exchange. This allows to discard its attribution to a C-OH stretching mode [13]. The attribution to the C-H bending of the methyne group is more likely, possibly with a contribution of CH_2 wagging [1, 24]. The bands at 1414, 1319 and 1295 cm^{-1} can be attributed to CH_2 bending or twisting modes [1, 11, 25]. These bands underwent an isotopic shift of some wavenumbers with no significant change of intensity with deuteration. This effect, observed in other instances, is an example of the way in which relatively distant substituents can affect the frequency of infrared vibrations [24].
The absorbance around 1345 and 1260 cm^{-1} strongly decreases with deuteration. Among the vibrations whose intensity can be strongly affected by the isotopic exchange, O-H in-plane bending or NH_2 twisting modes would be compatible with these wavenumbers [25, 26]. An ultimate attribution of these bands, as well as of the C-H bending bands, would demand the complete modelisation of the fundamental frequencies of chitosan.

The band at 1150 cm^{-1} has been differently attributed to the asymmetric stretching mode of the C-OC glycosidic bond or to the C-OH stretching of the secondary alcohol groups [1, 12]. The second interpretation has surely to be retained on the basis of the strong isotopic shift of the band. The difference of the reduced masses of the OH and OD groups allows to attribute to the corresponding C-OD stretching the band which appears at 1121 cm^{-1}, beyond a clear isosbestic point.
Strong C-O, C-N, and C-C stretching vibrations dominate the region between 1100 and 1000 cm^{-1}.
The intensity of the bands at 950 and 898 cm^{-1} rapidly decreases when D_2O vapours are admitted and they virtually disappear at high D_2O pressure. These bands have been attributed to, respectively, C-CH_3 wagging and ring stretching modes [1]. Such modes would not be affected by isotopic effects and, if they are present, they are overwhelmed by strong isotope-sensitive modes. NH_2 wagging vibrations are expected at these wavenumbers on the basis of calculations of other amines [24, 25]. The corresponding ND_2 wagging vibrations have been calculated at wavenumbers compatible with a very broad band which appears and grows with deuteration around 760 cm^{-1} [24, 25]. At intermediate wavelengths around 810 cm^{-1}, the absorbance increases with D_2O pressure up to 3.7 mbar and decreases for further pressure rise, possibly in correspondence with a NHD wagging vibration.
A significant isotopic shift, from 668 to 658 cm^{-1}, is also observed for a band which can be attributed to O-H out-of-plane bending [1, 26].
For ease of consultation, the attributions of the main IR bands of chitosan and deuterated chitosan are reported in Table 1.

Table 1. Main IR bands of chitosan and deuterated chitosan.

wavenumber cm^{-1}	chitosan	O-, N-deuterated chitosan
O-H stretching	3435	2529
N-H stretching	3290, 3180	2432, 2372
C-H stretching	2914, 2865	2914, 2865
NH_2 scissoring	1593	1197
CH_2 scissoring	1453	1453
CH_2 bending	1414	1412
CH bending, CH_2 wagging	1376	1376
CH_2 twisting	1319, 1295	1312, 1289
O-H bend, NH_2 twist	1254	?
C-OH stretching	1150	1121
C-O, C-N, C-C stretching	1100-1000	1100-1000
NH_2 wagging	950, 898	760
O-H out-of-plane bending	668	658

Deuteration of the xerogel
The IR spectra of the outgassed xerogel before and after exposal to doses of D_2O vapour are reported in Figure 3, from 3800 to 1800 cm^{-1}, and Figure 4, from 1800 to 400 cm^{-1}. The spectrum of the outgassed xerogel is nearly indistinguishable from the spectrum of the outgassed aerogel. However, its modifications upon exposure to D_2O vapours are much less intense. Exposal to 17 mbar D_2O (curve 4 in Figure 1) brought about a very limited formation of O-D and N-D groups, as visible in the 2600-2200 cm^{-1} region.
Several cycles of outgassing and admission of D_2O at the same pressure (17 mbar) were needed to move further towards the equilibrium of isotopic exchange (curves 5-10 in Figure 1). Nevertheless, the decrease of the O-H and N-H stretching bands and the growth of the O-D and N-D stretching bands were much more limited than in the case of the aerogel and the isotopic exchange was far from complete. It can be observed that a limited degree of isotopic exchange was also achieved in the pioneering experiments of chitin deuteration by Pearson and coworkers [1].

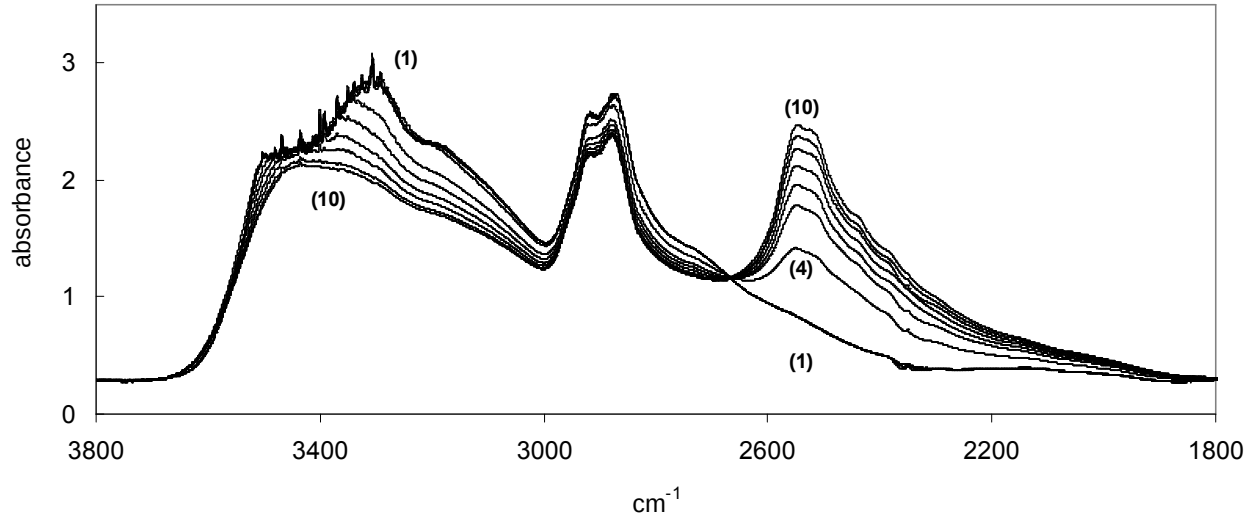

Figure 3. IR spectra of chitosan xerogel outgassed at 353 K (1) and exposed to D_2O pressure of 0.14 (2), 1.3 (3), and 17 (4) mbar and at repeated cycles of evacuation and exposure to 17 mbar D_2O (5-10). Wavenumber domain 3800-1800 cm^{-1}.

The shape of the envelopes of bands corresponding to protonated and deuterated species are very similar in the xerogel and the aerogel. It seems likely that a surface rim of the xerogel is completely exchanged and that the diffusion of D_2O inside the xerogel demands a longer time that the duration allotted to the IR experiment.

The low accessibility of the xerogel to the deuterating agent is confirmed by the examination of the lower-wavenumber part of the spectra, reported in Figure 5. Albeit most of the characteristic effects of deuteration observed in the spectra of the aerogel can be identified in the spectra of the xerogel, all of them corresponds to partial transformations. The evolution of the NH_2 scissoring mode at 1593 cm^{-1} and the corresponding ND_2 scissoring at 1197 cm^{-1}, as well the evolution of the NH_2 wagging at 950 and 898 cm^{-1} and the ND_2 wagging around 760 cm^{-1} indicates a partial deuteration of the amine group. In the same way, the evolution of the alcoholic C-O stretching at 1150 cm^{-1} confirms a partial deuteration of the alcohol groups.

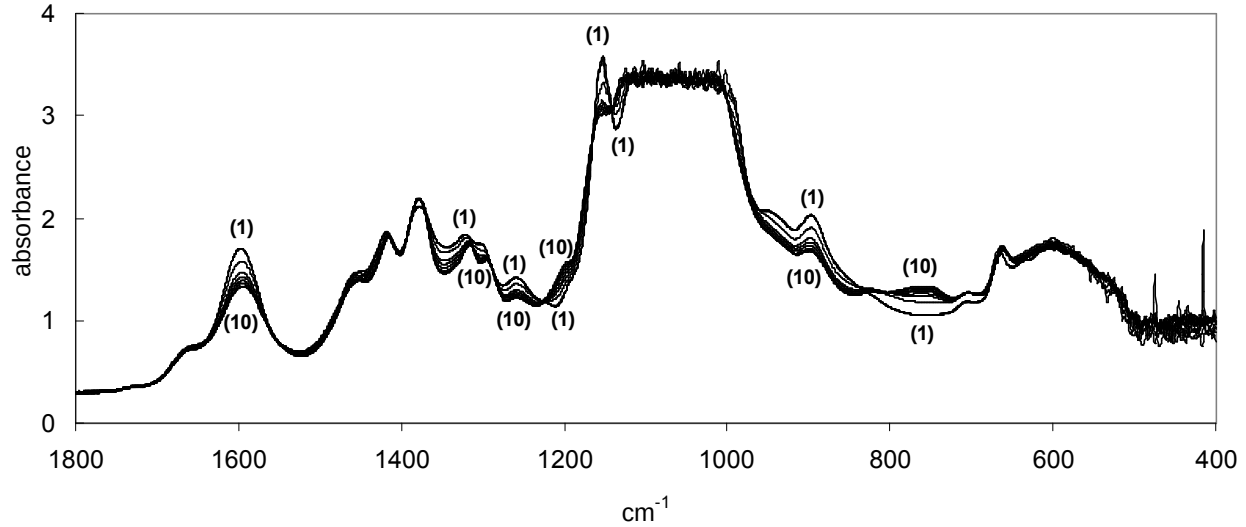

Figure 4. IR spectra of chitosan xerogel outgassed at 353 K (1) and exposed to D_2O pressure of 0.14 (2), 1.3 (3), and 17 (4) mbar and at repeated cycles of evacuation and exposure to 17 mbar D_2O (5-10). Wavenumber domain 1800-400 cm^{-1}.

A significant feature of the spectra of the xerogel as well as of the aerogel is the very limited contribution of the bands of molecular D_2O or H_2O. Only a shoulder at 2510 cm^{-1} could be attributed to adsorbed molecular D_2O. Considering that the activity of D_2O at 17 mbar and 25 °C is nearly 0.7, the adsorption of molecular water appears to be surprisingly low for a material considered as highly hydrophilic [27, 28].

Conclusions

The different reactivity of organic groups towards deuteration represents a useful tool for the identification of the spectral vibrations. The isotopic exchange of deuterium for hydrogen in alcohol or amine groups allows to differentiate them from the less reactive C-H groups or from the unreactive C-O-C or C-C bonds.
Moreover, the extent of isotopic exchange allows to evaluate the accessibility of the material to the deuterating agent. Under this respect, the aerogel and xerogel of chitosan differ in a striking manner. While the aerogel can be easily and completely deuterated by exposal to D_2O vapours, the xerogel can only be partially deuterated in more severe conditions. In this way, the deuterating agent is a probe of the accessibility of the materials to small polar molecules, and the difference observed indicates at which point the chitosan aerogel can be more effective than a xerogel for applications in catalysis or adsorption.

References

[1] F.G. Pearson, R.H. Marchessault, C.Y. Liang, J. Polym. Sci. 18 (1960) 101.

[2] T. Sannan, K. Kurita, K. Ogura, Y. Iwakura, Polymer 19 (1978) 458.

[3] G.K. Moore, G.A.F. Roberts, Int. J. Biol. Macromol. 2 (1980) 115.

[4] M. Miya, R. Iwamoto, S. Yeshikawa, S. Mima, Int. J. Biol. Macromol. 2 (1980) 323.

[5] A. Domard, Int. J. Biol. Macromol. 9 (1987) 333.

[6] A. Baxter, M. Dillon, K.D.A. Taylor, G.A.F. Roberts, Int. J. Biol. Macromol. 14 (1992) 166.

[7] Y. Shigemasa, H. Matsuura, H. Sashiwa, H. Saimoto, Int. J. Biol. Macromol. 18 (1996) 237.

[8] J. Brugnerotto, J. Lizardi, F.M. Goycoolea, W. Argüelles-Monal, J. Desbrières, M. Rinaudo, Polymer, 42 (2001) 3569.

[9] M.L. Duarte, M.C. Ferreira, M.R. Marvão, J. Rocha, Int. J. Biol. Macromol. 31 (2002) 1.

[10] X. Qu, A. Wirsén, A.C. Albertsson, J. Appl. Polym. Sci. 74 (1999) 3186.

[11] A. Pawlak, M. Mucha, Thermochim. Acta, 396 (2003) 153.

[12] T. Wang, M. Turhan, S. Gunasekaran, Polym. Int. 53 (2004) 911.

[13] J. Saraswathy, S. Pal, C. Rose, T.P. Sastry, Bull. Mater. Sci. Indian Acad. Sci. 24 (2001) 415.

[14] J. Ryczowski, Catal. Today 68 (2001) 263.

[15] H. Knözinger, S. Huber, J. Chem. Soc. Faraday Trans. 94 (1998) 2047.

[16] R. Buzzoni, S. Bordiga, G. Ricchiardi, G. Spoto, A. Zecchina, J. Phys. Chem. 99 (1995) 11937.

[17] A. Zecchina, G. Spoto, S. Bordiga, Phys. Chem. Chem. Phys. 7 (2005) 1627.

[18] R. Valentin, K. Molvinger, F. Quignard, F. Di Renzo, Macromol. Symposia 222 (2005) 93.

[19] R. Valentin, K. Molvinger, F. Quignard, D. Brunel, N. J. Chem. 27 (2003) 1690.

[20] K. Ogawa, T.Yui, K. Okuyama, Int. J. Biol. Macromol. 2004.

[21] A. Pierre, G. Pajonk, Chem. Rev. 102 (2002) 4243.

[22] R. Kocklenberg, B. Mathieu, S. Blacher, R. Pirard, J.P. Pirard, R. Sobry, G. Van den Bossche, J. Non-Cryst. Solids 225 (1998) 8.

[23] D. Serp, M. Mueller, U. von Stockar, I.W. Marison, Biotech. Bioeng. 79 (2002) 243.

[24] M.G. Giorgini, M.R. Pelletti, G. Paliani, R.S. Cataliotti, J. Raman Spectrosc. 14 (1983) 16.

[25] L.A.E.B. de Carvalho, L.E. Lourenço, M.P.M. Marques, J. Mol. Struct. 482 (1999) 639.

[26] J. Coates, in R.A. Meyers, Ed., Encyclopedia of Analytical Chemistry, Wiley, Chichester, 2000, 10815.

[27] D.R. Rueda, T. Secall, R.K. Bayer, Carbohyd. Polym. 40 (1999) 49.

[28] S. Despond, E. Espuche, N. Cartier, A. Domard, J. Polym. Sci. Pol. Phys. 43 (2005) 48.