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Study of molybdate ion sorption on chitosan gel beads by different spectrometric analyses

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Abstract

Molybdate ion uptake both by raw chitosan and by glutaraldehyde cross-linked chitosan beads was investigated. This study focused on the identification of sorption mechanisms by means of several analytical procedures such as infra-red and reflectance spectrophotometries and CP-MAS ¹³C NMR analyses. Although the amine functions of glucosamine residues remain the major sites of interaction with the metal species, other functional groups can also be involved. It is certainly the case with carbonyl functions provided by the glutaraldehyde structure in cross-linked sorbents. Due to the large size of the polynuclear hydrolysed molybdate species, the sorption may involve several monomer units, resulting in additional linkages between the polymer chains. This behaviour can be confirmed by the chemical shifts of the carbon atoms observed by CP-MAS ¹³C NMR on raw chitosan beads, showing that the carbon atoms supporting the amino sites are not the only atoms affected by molybdate ion sorption. Moreover, cross-linking promotes a partial reduction of molybdenum species in the presence of some unreacted aldehyde groups.

Keywords: Molybdate ions; Chitosan; Sorption; Infra-red spectrometry; Reflectance spectrometry; ¹³C NMR spectrometry

1. Introduction

Several works have been published over the last thirty years on the use of micro-organisms or biopolymers to collect and remove metal ions from dilute solutions, thus allowing the treatment of waste water or the recovery of valuable metals [1]. The aim of most studies is to optimise sorption performances, especially sorption isotherms. Some of them suggest uptake mechanisms which can involve adsorption, complexation, precipitation or ion exchange processes [1–3], but little

has been done on the identification of the sorption sites and the interpretation of the molecular interactions between the sorbents and the solute. This paper deals in particular with the study of the differences between the mechanisms involved in molybdate ion removal by raw chitosan and cross-linked chitosan beads.

Preliminary works on metal ion biosorption have been performed firstly on fungal or bacterial biomass, then on cell walls [4]. Various other studies have also investigated the interactions of the constituents of these cell walls with metal ions: alginate [5], chitin, chitosan [6,7]. Several functional groups are involved in metal ion immobilisation depending on the nature of the sorbent, the pH of the solution and the speciation of

metal ions (according to the concentration). These groups include hydroxide, phosphate, carboxylic or amino functions [8,9].

Chitosan is a well-known biosorbent of metal ions. Several studies have been carried out on its sorption capability, using native chitin or chitosan or more sophisticated forms resulting from a grafting of specific functional groups [10,11]. The study of uranium sorption by chitosan and modified forms of this polymer has shown that the grafting of new functional groups produces an increase in chitosan sorption performances through two mechanisms: direct contribution by the new sorbing sites and an improvement in the diffusion properties of the material [12]. The diffusivity of metal ions in raw chitosan is greatly reduced by its low porosity and high crystallinity [13]. Therefore, many works have concerned the technique of chitosan gel bead formation, to improve the accessibility of the solute to the centre of the sorbent particles [14–16], or to enhance component release from chitosan beads for pharmaceutical applications, using experimental methods quite similar to those used for alginate bead processing [5]. On the other hand, it is well known that chitosan can dissolve either in organic or mineral acids [7]. This is a restrictive parameter for industrial applications in waste water treatment. A cross-linking procedure can be used to reinforce the chemical and mechanical properties of chitosan gel beads [14,17], through different chemical reagents such as glutaraldehyde, and epichlorohydrine. In this work, we used glutaraldehyde for chitosan cross-linking. The reaction of this reagent with chitosan has been extensively discussed by Roberts [7]. It appears that this di-aldehyde can be bound to chitosan through either 1:1 or 1:2 linkages and then offers the possibility for a glutaraldehyde molecule to have a free aldehyde group.

Sorption performances of such systems have been extensively studied [14–17], but few data are available on the mechanisms and reactions between metal ions and sorption sites. The study of molybdate ion sorption by cross-linked chitosan beads has shown that uptake capacities as high as 750 mg/g (on a dry basis) could be obtained [16]. Draget et al., have studied chitosan cross-linking by molybdate ions [18]. The maximum uptake is reached at a pH near 3. This result was discussed in terms of metal ion speciation and sorbent protonation. The pK_0 (intrinsic pK) value of chitosan is close to 6.5 and is independent of the degree of acetylation, up to 25% [19].

Molybdate is an anionic form of molybdenum, usually encountered in aqueous systems under natural oxido-reduction conditions. It is easily hydrolysed in aqueous media to produce several polynuclear hydrolysed species, whose distribution depends on the total metal concentration and pH. At pH 3, several species co-exist, whatever the total molybdenum con-

centration [20]. The high level of uptake coincides with the presence of highly anionic and hydrolysed forms of molybdate ions. Thus, the protonated forms of chitosan are more effective in anionic molybdate recovery at pH 3: the electrostatic attraction improves the affinity of the sorbate for the biosorbent.

In this work, the study of molybdate ion sorption was performed using several analytical techniques such as: infra-red spectrophotometry, CP-MAS ^{13}C NMR analysis and surface reflectance, to identify the functional sites which are involved in molybdate ion uptake and the metal ion forms which are present on the sorbent. The presence of free-aldehyde sites (on glutaraldehyde) provides reductive sites which are able to react with molybdate ions through oxido-reductive processes.

2. Material and methods

2.1. Materials

Ammonium molybdate was provided by Fluka AG and infra-red spectroscopy grade potassium bromide was obtained from Carlo Erba. Chitosan was supplied by Aber-Technologies (France) (Lot N°A17G28). This polysaccharide, a poly(D-glucosamine) or poly(β -(1- \rightarrow 4)-2-amino-2-deoxy-D-glucopyranose), although it occurs naturally, is obtained by a deacetylation step (in caustic soda and at high temperature) from chitin, a polymer found in the cuticles of arthropods. Commercial chitosans generally appear to be partially acetylated and a number of complex steps are needed to achieve total deacetylation [7]. The distribution of molecular weights was determined by size exclusion chromatography on TSK GW columns using on-line double detection carried out by a Waters R410 differential refractometer and a Dawn-Wyatt multi-angle laser light scattering photometer. In the case of an eluent at pH 4.3 (0.15 M acetic acid/ammonium acetate buffer), the refractive index increment was found to be 0.172 (cm^3/g) [13]. The deacetylation percentage was determined by FTIR spectrophotometry on chitosan films using a Perkin-Elmer 1760 FTIR spectrophotometer. The spectra were treated according to the methods of Baxter et al. [21] and Miya et al. [22].

Chitosan beads were produced by means of a 2-step method well-known for alginate bead processing [5,14,17]. Chitosan (4% w/w) was first dissolved in an acetic acid solution (4% w/w), then introduced dropwise into a coagulating bath (sodium hydroxide 2.5 M). Falling into the alkaline solution, the beads kept their initial spherical forms and formed standard chitosan beads (diameter 1 ± 0.05 mm). Several rinsing baths removed the excess alkalinity. In order to strengthen the mechanical properties of the beads, the sorbents

were left to stand for 16 h in contact with a cross-linking solution of glutaraldehyde (2.5% w/w) with a 1:1 stoichiometric ratio between amino functions and aldehyde groups. After several rinsing steps, the beads were stored in demineralized water.

2.2. Procedures

2.2.1. Metal ion sorption

Chitosan beads (with a dry sorbent concentration of 170 mg/l) were continuously stirred in a solution containing varying initial concentrations of sodium molybdate. The pH was adjusted to 3 with either 1M H₂SO₄ or NaOH solutions. Experiments were performed at room temperature (ca. 20°C). When the equilibrium was achieved (24 h) the biosorbent was removed by filtration onto a 1.2 μm Whatmann filter membrane, washed with demineralized water and dried at room temperature.

2.2.2. Infra-red spectrometry

The biosorbent powders were ground with infra-red grade KBr to prepare pellets (0.1% w/w). They were analyzed in transmission with a Nicolet FTIR spectrophotometer 510 (50 scans, resolution: 2 cm⁻¹).

2.2.3. CP-MAS ¹³C NMR

¹³C NMR spectra were recorded on a AM 300 Bruker spectrometer, with a solids accessory. Experimental conditions were: ¹³C (75.470 MHz frequency); zircon cell (length: 2 cm; internal diameter: 5 mm). The technique used was a cross-polarization method (rotation speed: 5000 run/s, contact time: 5 ms; relaxation time: 5 s) running at the 'magic spinning angle' (spinlock). This procedure was modified to introduce a T.O.S.S.-A.U.R. sequence and reduce the spinning side bands (SSB) observed for each 5000 Hz step.

2.2.4. Reflectance spectrometry

The reflectance spectra of chitosan beads, placed in 2.5 ml polyethylene crystal cells, were obtained with a spectrometer (PHOTORESEARCH, USA) equipped with a Pritchard-style mirror aperture and a 128 silicon photocell array. The wavelength resolution was below 3.5 nm. The apparatus allows measurements with a solid angle precision of 1°. To avoid experimental artefacts, each datum was obtained through five cell fillings, on which three measurements were successively performed. The transmittance of the void cells was initially measured and used to correct the sample measurements. The texture of the material (shape and surface structure) and its effect on the cell packing involves shading and some significant variations in the measurement series. So as to avoid such uncertainty, experimental series were normalized using the following procedure: the reflectance R_λ (reflectance at λ wavelength) was corrected using the formula:

$$R'_\lambda = \frac{R_\lambda - R_{400 \text{ nm}}}{R_{\text{max}} - R_{400 \text{ nm}}}$$

3. Results and discussion

This work was performed using a chitosan sample whose characteristics are as follows: degree of acetylation, 87%; average molecular weights, MW_n, 125.000; MW_w, 191.000. Infra-red, NMR and surface reflectance spectrometries were used to characterize the modification of chitosan beads by the cross-linking reaction with glutaraldehyde and more interestingly to follow the influence of molybdate ion sorption on its structure. The main specific bands were identified and the metal sorption was characterized in relation with the variations observed in the spectra.

3.1. Infra-red spectrometry studies

The characterization of chitosan structure by infra-red spectrometry has been intensively studied. Although the assignments of several secondary transitions are still subject to controversy, the main absorption bands of the spectra are now clearly identified [13]. Moreover these spectra can be influenced by parameters such as the deacetylation percentage or crystallinity. Chitosan is essentially produced from chitin by a deacetylation reaction. The I.R. spectra of chitosan correspond to a convolution of specific signals specific to both carbohydrates and absorption due to amine and amide functions [23,24].

Within the range 4000–2000 cm⁻¹, hydroxyl and C–H groups can be identified, together with amide A and B absorption bands [7,21,22] but, these bands are neither indicative nor significant for the identification of metal sorption mechanisms. Most infra-red studies on metal sorption therefore concern to the wave-number range 2000–600 cm⁻¹. The most significant parts of the spectra in terms of metal ion sorption are located in the two regions within the 1800–1300 cm⁻¹ (amine/amide or ester/carboxyl absorption bands) and the 1000–700 cm⁻¹ (Mo–O absorption bands). Table 1 gives the main amine and amide bands. The partial overlapping of the bands explains the difficulty in exactly identifying each of them for varying experimental runs related to the deacetylation level or crystallinity. In the 1800–1600 cm⁻¹ range, new bands appear, possibly due to chemical modifications of chitosan resulting from reactions with, for example, aldehydes or carboxylic acids. Some of these observations were reported by Muzzarelli et al. [8] and Jansson-Charrier et al. [12] for the cases of reactions of chitosan with ascorbic or oxo-2-glutaric acids. Under such conditions a new band corresponding to C=O groups appears near 1700–1740 cm⁻¹.

The other wave-number range which is useful for the understanding of the mechanism of the molybdate ion sorption is the 1000–700 cm⁻¹ range. In this region, the spectra of components containing molybdate ions are characterized by the appearance of two bands representing stretching modes of M=O and Mo–O–Mo centered near 905–915 and 945–970 cm⁻¹ [6,25,26].

Fig. 1 shows the infra-red spectra of chitosan beads in the free amine form obtained before and after the cross-linking reaction by glutaraldehyde. The spectrum of the raw chitosan beads (RCB) is a standard chitosan spectrum in the free amino form, with the following transitions. A shoulder is first observed at 1670–1650 cm⁻¹. It corresponds to the overlap of an amide I band due to the residual acetylated residues and –OH bands corresponding to the pyranose structure [19]. A second band is observed at 1600–1590 cm⁻¹ and corresponds to the free amine form of the glucosamine residues. A third transition corresponding to the amide II band can be detected by the presence of a weak band near 1560–1520 cm⁻¹. The third band corresponds to the residual acetylated residues. The spectrum of the crosslinked chitosan beads (CCB) is also clearly identified by four bands in the region 1750–1500 cm⁻¹. These transitions can be interpreted by considering the following reaction:



The Schiff's reaction between amine functions and aldehyde groups appears to be the most representative reaction of chitosan cross-linking by glutaraldehyde [7]. A first shoulder appears near 1730–1720 cm⁻¹ and can be attributed to the C=O carbonyl groups of unreacted aldehyde functions of glutaraldehyde. The second band, which is the most important observed in this region for the CCB, appears near 1690–1640 cm⁻¹. It can easily be attributed to the –C=N– bond formed according to Eq. (1). The band corresponding to the free amine functions can be thus observed in the same region as for RCB but this band is much weaker due to a high level of consumption of these functions during the cross-linking reaction. The fourth transition, corresponding to the amide II band, can also be detected by

a small shoulder in the region 1560–1520 cm⁻¹. Some differences between the spectra of the two kinds of products can also be observed in the lower wave-number range but they are more difficult to interpret.

Fig. 2 gives the comparison between the spectra of CCB, and RCB beads after molybdate uptake at pH 3, from a 100 mg/l solution. Molybdate ion sorption involves significant changes in the structure of the polymers and therefore on their infra-red spectra. It is important to notice that in contrast with the cases described above, the spectra were recorded from systems placed in acidic media. Thus, the spectra of RCB in the region within 1700–1450 cm⁻¹ are similar to the spectra of chitosan alone, in the ammonium form, which has not been in contact with metal ions. Indeed, we essentially find two bands at 1620–1530 and 1520–1510 cm⁻¹ which can be easily attributed to –NH₃⁺ deformation bands. Simultaneously, the band specific to –NH₂ functions has largely disappeared, except for the presence of a slight shoulder near 1600–1590 cm⁻¹. This behaviour can be explained either by the partial formation of a complex with molybdate ions involving the free amino form or simply by the fact that, even in acidic media, a small proportion of the amine groups of chitosan are necessarily in the free amino form.

In the case of the complexation of chitosan with various other metal ions such as U^{VI}, Al^{III}, Cu^{II} and Ag⁺, at pH > 6, the band near 1630 cm⁻¹ has been shown to correspond to the overlapping of three bands [13]. The first corresponds to a residual amide I band at 1660–1655 cm⁻¹ due to the incomplete deacetylation of the polymer and also weak bands related to the presence of primary and secondary alcohol functions. A second band is located at 1600–1595 cm⁻¹ and corresponds to uncomplexed amino groups in the free amino form. The third band, centered near 1625 cm⁻¹, is specific to the interaction between chitosan and metal ions and has been observed for all the metals mentioned above. Under the same conditions, a band located at 1525 cm⁻¹ was also observed and attributed to an amide II type band characteristic of the interaction of metal ions and chitosan. In conclusion, depending on the involvement of either the ammonium or the free

Table 1
Assignments of the main nitrogenous functions involved in the sorption process onto chitosan

Band	Vibration mode	Wave-number (cm ⁻¹)
Amide I	–>C–O stretching mode conjugated with –N–H deformation mode	1650 ^a
Amide II	>N–H deformation mode conjugated with >C=N– deformation mode	1550–1590 ^a
Amide III	>N–H deformation mode conjugated with >C=O and >C=N– stretching mode	≈ 1300 ^a
Amine	>NH stretching mode	^b
	–NH ₂ free amine	1605–1580
	–NH ₃ ⁺	1550–1480

Depends on the acetylation degree (a) and protonation (b).

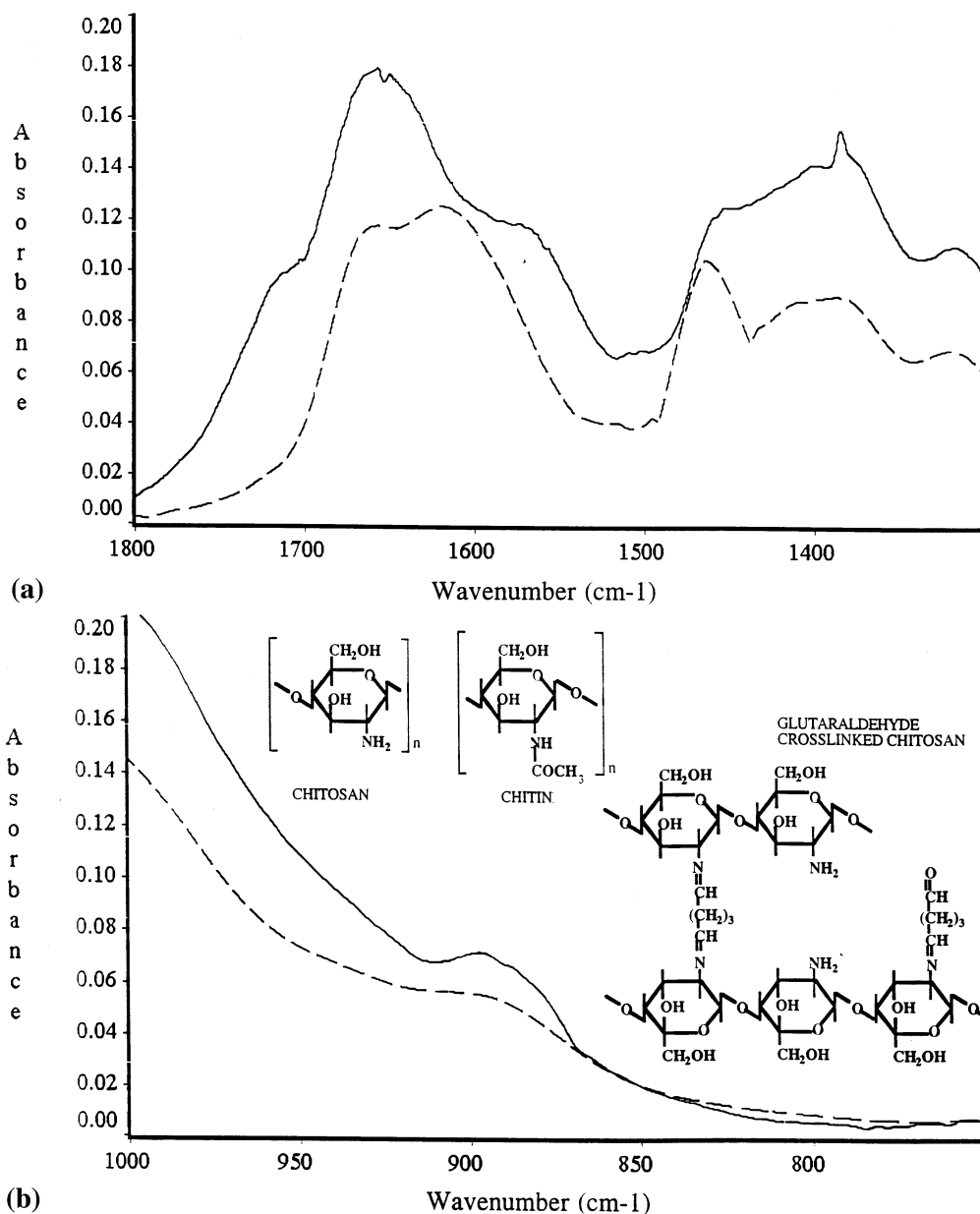


Fig. 1. Infrared spectra of raw chitosan beads (---) and cross-linked chitosan beads (—) in the free amino forms: (a) in the region 1800–1300 cm^{-1} ; and (b), in the region 1000–750 cm^{-1} .

amine form of the amine groups of chitosan chains, two kinds of mechanisms could be considered, leading to similar absorption bands. In fact, pH conditions are very different in the two types of experiments and the interactions with molybdate ions arise in acidic media and thus it is difficult to envisage a complexation with amino groups in the free amine form. Therefore, the hypothesis involving an initial interaction between the ammonium form and an anionic species of molybdenum appears to be the most probable: $\text{Mo}_7\text{O}_{21}(\text{OH})_3^{3-}$, $\text{Mo}_7\text{O}_{22}(\text{OH})_2^{4-}$, $\text{Mo}_7\text{O}_{23}(\text{OH})^{5-}$

In the case of CCB, the results are similar to those obtained with the RCB except concerning the band in the range 1730–1720 cm^{-1} , which is emphasized after

contact with molybdate ions. This behaviour, which is thus significant of the molybdate ion sorption on the CCB form, leads us to suppose that an additional mechanism of interaction may exist in the case of the cross-linked chitosan. As a consequence, in this case, free amine groups are not the only groups involved in the metal ion uptake. Carbonyl groups arising from unbound sites of glutaraldehyde can participate in molybdate ion uptake and the absorption band located near 1720 cm^{-1} could be assigned to the interaction between the metal and the carbonyl groups leading to a situation similar to ester bonds. This result can be easily confirmed by means of the subtraction of the spectra before and after interaction. This behaviour is consistent with previ-

ously reported results on uranium removal by chitosan and glutamate glucan, an oxo-2-glutaric substituted form of chitosan [12].

As shown in Fig. 3, Molybdate ion sorption is also characterized by the presence of a strong absorption band close to $890\text{--}900\text{ cm}^{-1}$, which is assigned to Mo=O stretching [25]. On the other hand, the metal uptake induces a significant increase in the band near 800 cm^{-1} , which is particularly emphasized in the case of the RCB. The presence of a band in this region, characteristic of the interaction between chitosan and molybdenum, is confirmed by the subtraction of spectra, as mentioned above. Similar observations were reported

by Miller and Sherrington from spectra of some synthetic polymers in the presence of molybdate ions [25]. This band was attributed to Mo–O–Mo stretching, with molybdenum appearing in either a pentavalent or an intermediate form. A comparison of the spectra with those of sodium or ammonium molybdate (the most representative form of molybdate ion after its sorption on chitosan amino sites) shows that a band is usually encountered at around $830\text{--}840\text{ cm}^{-1}$, while with molybdenum(V) chloride, two bands are observed near 977 and 735 cm^{-1} [26]. This behaviour reinforces the hypothesis of a possible reduction of sorbed molybdate ions on CCB.

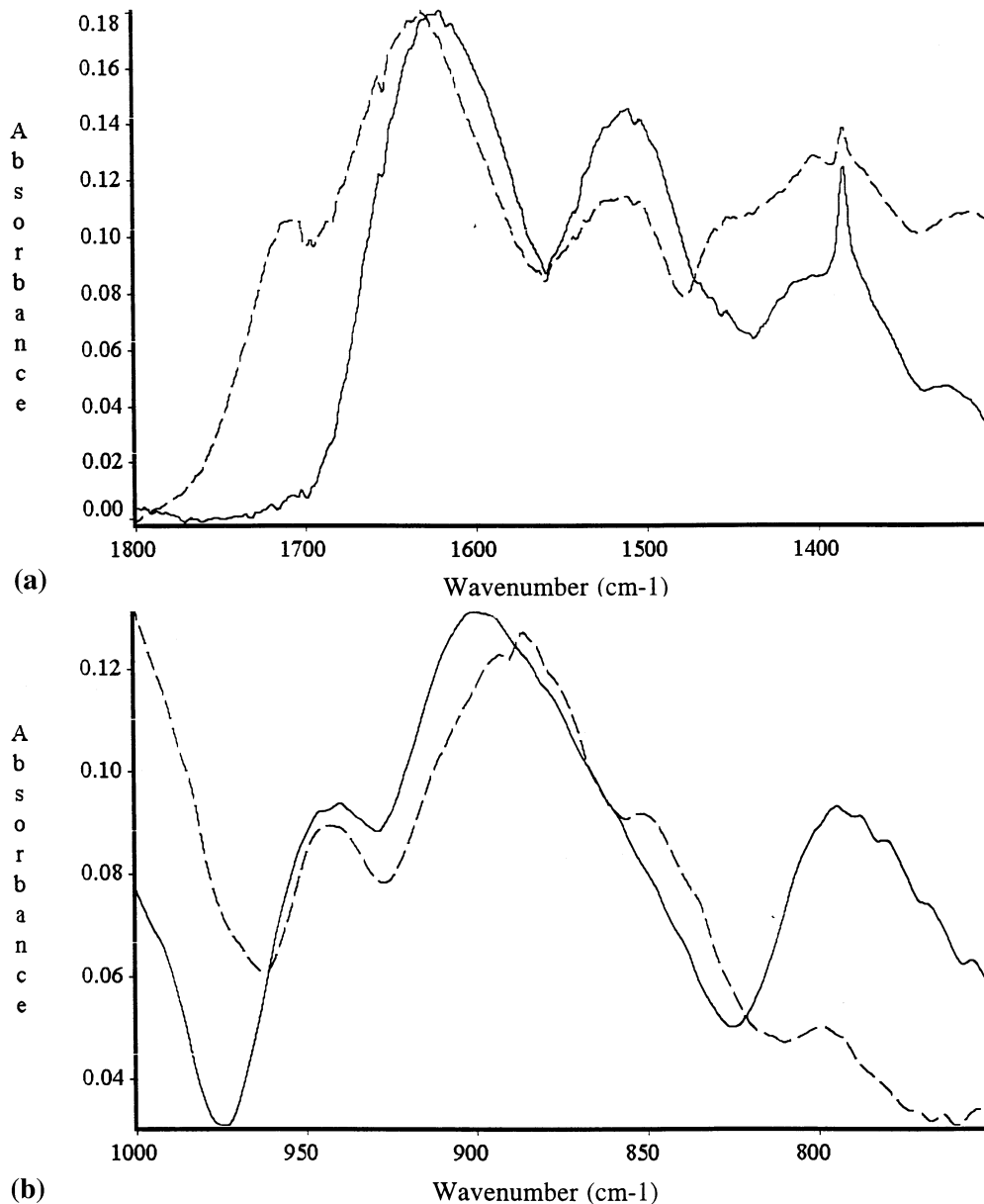


Fig. 2. Infrared spectra of chitosan beads after molybdate sorption (pH 3, initial molybdate concentration, 100 mg/l): raw chitosan beads (---) and cross-linked chitosan beads (—) in the regions: (a) $1800\text{--}1300\text{ cm}^{-1}$; and (b), $1000\text{--}750\text{ cm}^{-1}$.

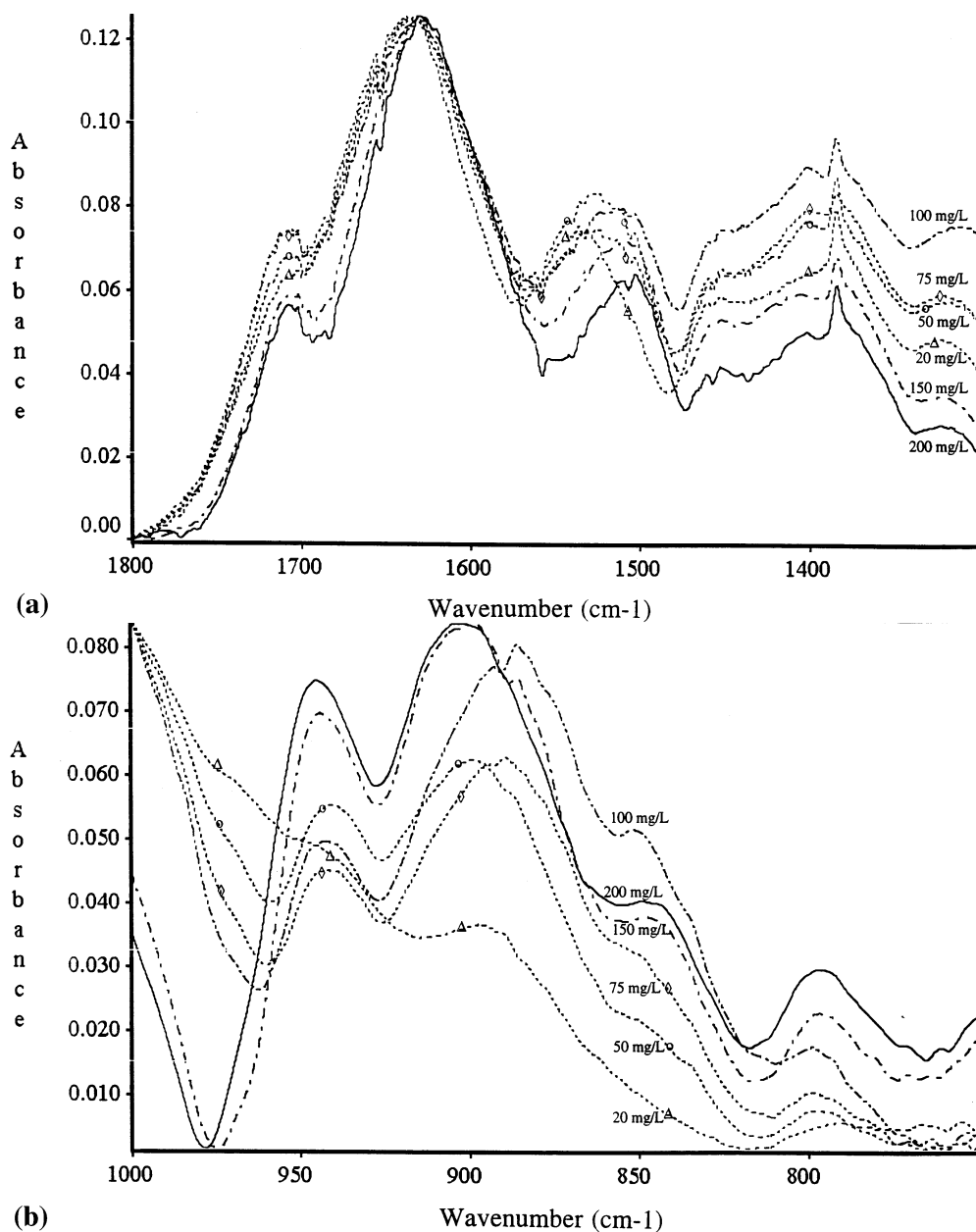


Fig. 3. Effect of increasing initial molybdate concentration on the infrared spectra of cross-linked chitosan beads (pH 3) in the regions: (a) 1800–1300 cm^{-1} ; and (b), 1000–750 cm^{-1} . 20 mg/l (- Δ -), 50 mg/l (-o-), 75 mg/l (- \diamond -), 100 mg/l (- - -), 150 mg/l (- - -), 200 mg/l (-).

3.2. ^{13}C NMR studies

^{13}C NMR spectrometry yields complementary information about the sorption sites which are modified by molybdate removal. Even if molybdate ion is mainly bound to amine sites, it appears that the carbon sites which support these amine functions are influenced by the modification of the amine group.

The assignments of the carbon signals for chitin and chitosan-based products are reported in a wide range of publications [27–29]. The exact location of the singlets may vary according to the NMR technique (solid or liquid analysis). While it is well known that liquid state NMR gives the best resolved patterns, dissolving the

samples could modify the structure and binding properties of chitosan versus molybdenum. Solid state analysis is therefore preferable. The location of the signals also depends on the crystallinity of the samples, as reported for cellulose materials or chitosan products [30,31], and on the sample state: dried or hydrated [32]. The main representative information may thus be difficult to deduce from shifts below 2 ppm.

Table 2 shows the assignments for β -D glucosamine, raw chitosan, chitosan beads and cross-linked chitosan beads, respectively. The signals observed on the spectra (Fig. 4) at 174 and 24 ppm correspond to the signals of $-\text{C}=\text{O}$ and $-\text{CH}_3$, respectively, which are present on the acetylated fraction of chitosan. This property is fre-

quently used to evaluate the deacetylation degree [33].

The NMR pattern of cross-linked chitosan beads is less well-resolved than that of its raw form (Fig. 4). This behaviour could be explained by a possible loss of crystallinity, as has been reported in several studies concerning the polymorphism of cellulose or chitosan materials [30–32]. Moreover the C-1 signal is broadened, C-4 and C-5 signals overlap and form a shoulder at the bottom of the C-3 signal. The C-2 and C-6 signals are also less well-resolved and it appears that the C-2 signal decreases. The baseline, in the 90–20 ppm region is significantly increased. Glutaraldehyde grafting produces new alkyl groups which cause the appearance of a new broad peak. Thus, additional signals appear at 92.2 and 141.8 ppm. These bands are related to the appearance of new bonds between amine functions and aldehyde groups. They can be attributed either to the modification of the vicinity of the carbon holding the amine groups (C- shifting: $-C=N-CH$) or to the existence of typical $>C=O$ bonds.

As shown in Fig. 4c, molybdate ion sorption on chitosan beads brings about some changes in the recorded NMR patterns. While no spectrum is obtained with CCB, a clear pattern is observed with RCB. The lack of resolution of CCB loaded with molybdate ions, shown by a wide line width, is attributable to the presence of a paramagnetic form of molybdenum which prevents a clear spectrum from being obtained. Mo(V) is known to give rise to this behaviour. This observation provides further evidence for the reductive mechanism proposed above. Chitosan–glutaraldehyde cross-linking produces reductive sites which are able to reduce the molybdate form to an intermediate form between Mo(VI) and Mo(V).

The comparison of NMR spectra for RCB before and after loading with molybdate ions shows that a better resolution is obtained after molybdate ion sorption (Fig. 4a and c), which could indicate an increase in the crystallinity of the material. More interesting are the modifications and shifts observed in the NMR patterns of the latter case. It appears that C-2 and C-6 signals are maintained near 60.6 and 56.9 ppm, but their relative intensities are increased. The C-1 signal is considerably shifted, from 105 to 98.5 ppm and is broadened in comparison with the original spectrum. A shoulder which appears at about 94.5 ppm could be related to a positive

(+ 8.7 ppm) shift of the C-4 signal (initially at 85.8 ppm), while the C-3/C-5 signals are difficult to interpret. A peak appears at 75.6 ppm, representative of the shifted (– 6 ppm) C-5 signal, and a shoulder at 72 ppm, represents the shifted (– 4 ppm) C-3 signal. It is interesting to note that these shifts are significantly higher than those observed when organic substituents are grafted onto a chitosan backbone [12]. Moreover it appears that the signals which are the most modified: C-3; C-4; and C-5 are those which are not directly involved in the sorption mechanism, although C-2 and C-6, which hold NH_2 and CH_2OH groups, the potential sorbing sites, are not significantly changed.

The modification of the spectra and chemical shifts of the C- signals relative to glucopyranose ring is therefore brought about by molybdate ion uptake on vicinuous chains: the molybdate ions interact with chitosan to form cross-links, as observed by Draget et al. [18] to explain chitosan gelation in presence of this anion. It is possible to suggest that the modification of the chitosan structure is caused by the formation of additional bonds between Mo and the sorbent which produce a constriction of the polymer network. The modification of the conformation of the polymer involves significant carbon shifts on the glucopyranose repeating unit [34], due to a modification of hydrogen bonding between the chains. So the shift of glucopyranose C- signals by molybdate–ion interaction with chitosan is not surprising. Several studies deal with molybdate and tungstate ion complexation by polysaccharides [34,35], as well as by uronic acids [36]. Thus, Ramos et al., show that molybdenum oxo-ions can be bound by varying mechanisms involving several carbon sites, due to the high coordination number of molybdate ions [36].

3.3. Surface reflectance studies

Cross-linked beads mixed with molybdate solutions of increasing concentrations were analyzed (pH 3, solid/liquid ratio = 170 mg/l). Fig. 5 represents the normalized surface reflectance spectra obtained under such experimental conditions.

These spectra show several common characteristics in the appearance of reflectance peaks: (i) a relative maximum is observed near 500 nm, with a shoulder near 460 nm. These peaks correspond to greenish-blue and bluish-

Table 2
 ^{13}C NMR signal assignments (ppm) for several chitosan forms compared to those of β -D-glucosamine (liquid-state analysis)

	C-1	C-2	C-3	C-4	C-5	C-6
β -D-glucosamine	95.2	59.5	74.5	72.2	78.6	63.1
Chitosan	100.5	58.8	73.1	79.3	77.6	63.0
Chitosan flakes	105.2	57.4	75.8	79–84		61.1
Raw chitosan beads	105.0	56.9	75.8	85.8	81.4	60.6
Cross-linked chitosan beads	104.4		75.5			60.6

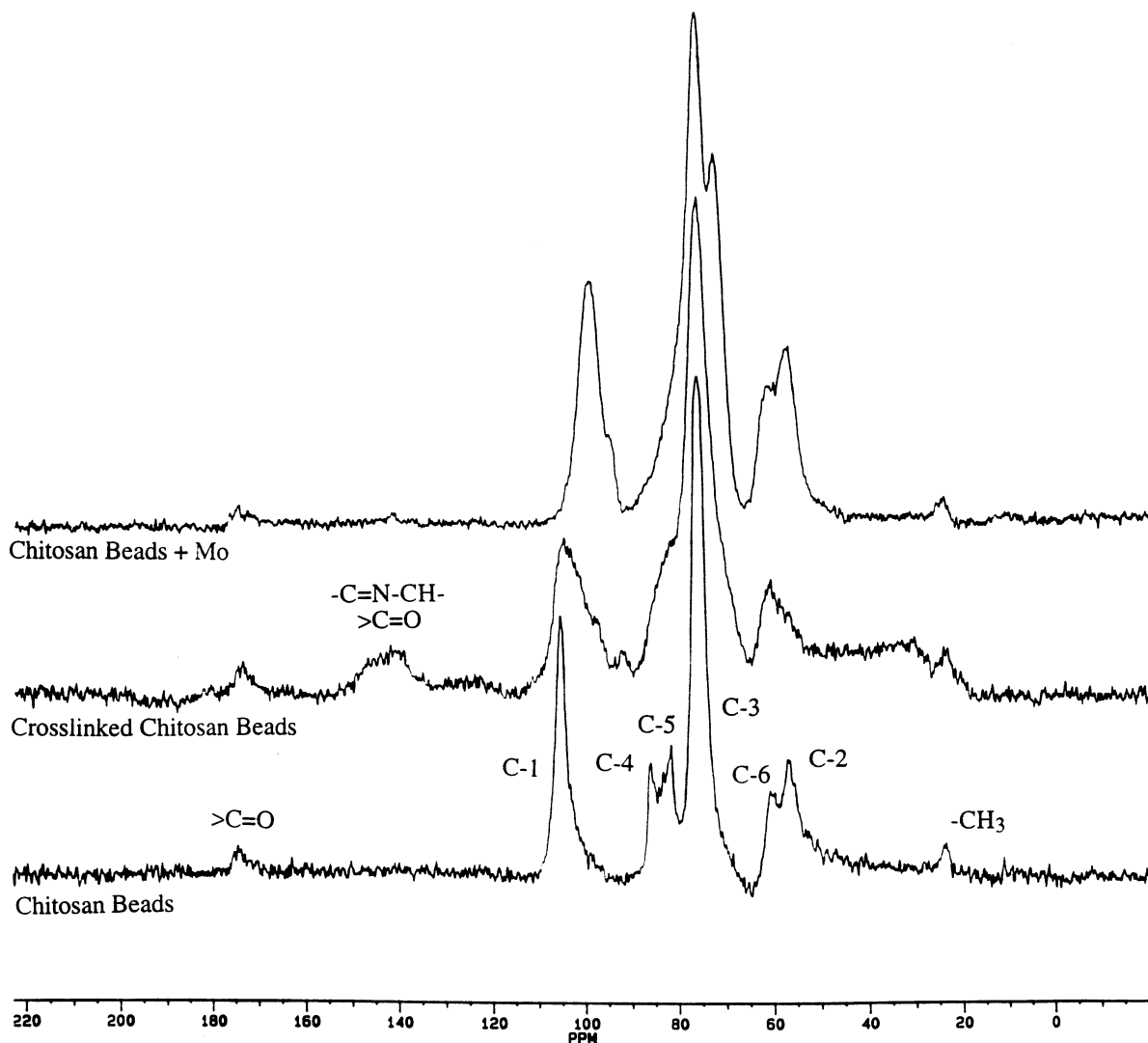


Fig. 4. Solid state ^{13}C NMR spectrum of chitosan beads (on the free amine form), cross-linked chitosan beads (on the free amine form) and raw chitosan beads after molybdate sorption.

purple colours, respectively. (ii) An absolute maximum is reached at varying wavelength between 560 and 580 nm, with a corresponding yellow-greenish to yellow colour. (iii) Significant absorbance is observed above 600 nm (red and infra-red colours).

However, the reflectances vary with the initial concentrations. Increasing the initial concentration leads to an increase in the reflectance at 500 nm and the progressive inversion of the reflectance ratio between the peaks at 500 and 580 nm. Moreover the 580 nm band is progressively shifted to 560 nm. These observations are complemented by the progressive decrease in the reflectance in the 600–700 nm range (red and infra-red regions). These wavelengths can be correlated to the oxidation state of molybdenum (Table 3), which allows molybdate ion sorption to be characterized with regard to the adsorbed molybdenum species. For low initial molybdate ion concentrations, the colour of the cross-linked beads changes from grey to greenish-yellow, while at higher

concentrations, the beads take a greenish-blue appearance (with an increasing intensity correlated to the increasing concentration). The predominance of reduced forms of molybdenum over its oxidized forms could explain the coloured aspect of cross-linked beads when they are mixed with solutions of high molybdate concentration. On the other hand, at low initial concentration, the colourless or light-coloured appearance of cross-linked chitosan beads is attributable to the predominance of molybdenum in a higher oxidation state.

This ranking in colour, correlated to the oxidation state of sorbed molybdenum is consistent with the above observations obtained from infra-red spectrometry. The mild reduction of an acid solution of molybdate gives a strong blue colour, 'molybdenum blue', as the first step in the reduction reaction. While the exact composition and nature of molybdenum blue is disputed, it is commonly accepted that molybdenum blue is a mixture of lower valency hydrous molybdenum oxides [37].

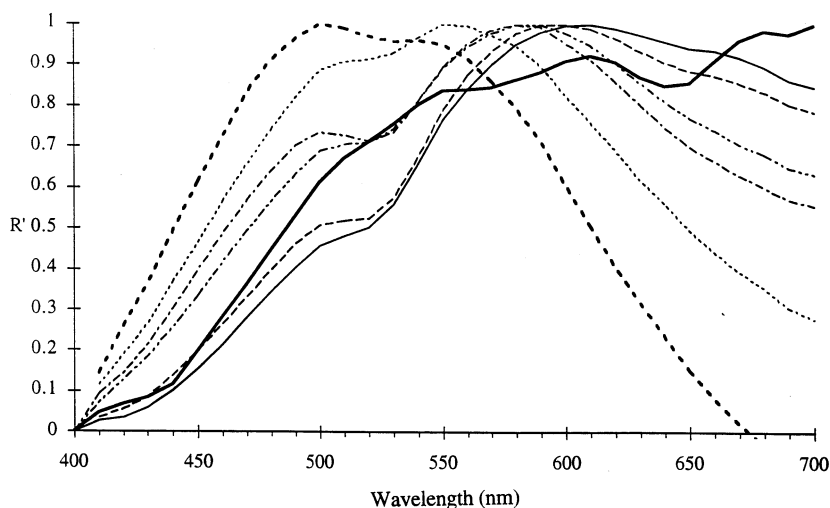


Fig. 5. Normalized reflectance spectra of cross-linked chitosan beads mixed with molybdate solutions of increasing initial concentrations: 20 mg/l (—), 50 mg/l (---), 75 mg/l (- · - ·), 100 mg/l (- · · -), 150 mg/l (---), 200 mg/l (—), and cross-linked beads spectrum as reference (—).

4. Conclusion

This series of experiments involving various spectrophotometric methods shows that the processes of molybdate ion removal are different on raw and cross-linked chitosan beads. It appears that a partial reduction of molybdate occurs on cross-linked sorbents. The cross-linking step is not homogeneous, it occurs mainly near the surface of the biopolymer. Moreover, glutaraldehyde is a bifunctional cross-linking agent which reacts with amino functions according to a mechanism which is not clearly established. Nevertheless, some of the aldehyde groups which do not react with amino groups are freely accessible and partially reduce the metal ions. This preliminary study should be completed by further experiments to identify the molybdenum species present on both raw and cross-linked forms, especially X-ray Photoelectron Spectroscopy. This information could be useful for the prediction of the suitability of these molybdate-impregnated polymers for catalytic oxidation processes [25,38].

Table 3
Correlation between colours, electronic configuration and oxidation state for molybdenum

Oxidation state	Electronic configuration (analogous)	Colour
Mo	4d ⁵ 5s ¹	
Mo I	4d ⁵ (⇌Mn ²⁺)	Blue
Mo II	4d ⁴ (⇌Mn ³⁺)	Yellow, green or blue
Mo III	4d ³ (⇌Cr ³⁺)	Green and purple
Mo IV	4d ² (⇌V ³⁺)	Green to yellow
Mo V	4d ¹ (⇌Ti ³⁺ , V ⁴⁺)	Green
Mo VI	4p ⁶	Colourless

These analytical procedures suggest that amino functions are involved in the sorption mechanisms, together with carbonyl sites (on cross-linked chitosan beads). However, ¹³C NMR on the RCB demonstrates that several carbon sites are affected by molybdate sorption and not solely those neighbouring the amino sites. The comparison with other polysaccharides allows us to suggest that, due to the large size of polynuclear hydrolysed molybdate species, predominant at pH 3, several groups could interact in the sorption mechanism with varying binding energies.

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