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Contribution to the study of the complexation of copper by chitosan and oligomers

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Abstract

The complexation of copper ions by chitosan and its oligomers is investigated using potentiometric and spectrophotometric methods to study the nature of the complexes involved and the role of the degree of polymerisation. Two complexes are demonstrated. Their structure is proposed, the pH range in which they are respectively stable is determined and their stability constants calculated. Finally a degree of polymerisation of 6 appeared as the threshold value for an efficient complexation of copper ions by chitosan oligomers. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Chitosan; Oligomers; Complexation

1. Introduction

Chitin, a homopolymer of β-(1 → 4)-linked *N*-acetyl-D-glucosamine, is one of the most abundant, easily obtained, and renewable natural polymers, second only to cellulose. It is commonly found in the exoskeleton or cuticles of many invertebrates [1] and in the cell walls of most fungi and some algae [2]. Chitosan is produced by thermochemical alkaline deacetylation of chitin. It is a biopolymer with unique properties favourable for a broad variety of industrial and biomedical applications [3–6].

Chitosan presents a large capacity to fix molecules such as pesticides [7,8], proteins [9] and dyes [10]. The free amine function of chitosan gives it a better ability to chelate ions of transition metals [11–13] than other natural compounds such as cellulose derivatives [14,15]. These chelating properties are turned to account for water treatment and particularly to recover metals as traces in sea water [15,16]. Mechanisms seem complex and discussed by different authors who generally do not get on together.

Recovery of metals from metal-bearing effluents may be performed according to different treatment processes [17,18]. Besides mechanical treatments of waste water (sedimentation) or biological ones (activated muds), some chemical treatments are used to eliminate these metals. Most current processes are the precipitation by hydroxides or sulphides, the oxidation–reduction, ion exchange, liquid–solid separation by decanting–flottation and the separation using membranes. But the major drawback of these treatments is the formation of mud, which have to be subject to confining. Hence, alternative processes were developed which are more economical, based on the use of natural polymers, such as the chitosan, to recover metallic ions.

Adsorption on active coal was widely studied. New adsorbents were considered to find more efficient and less expensive materials. Thus, the adsorption by living organisms (bacteria, fungi, seaweeds, etc.) or by chemicals from these organisms was studied. Chitosan is one of these natural chemicals as brown coal or peat for which studies were carried out.

The ability of chitosan to form complexes with metallic ions gave rise to the interest from researchers [19–22]. The comparison between different studies is difficult due to the large variability of experimental conditions. It was shown that the chelation process and the stability of the metal–chitosan complex may be influenced by the stirring (mechanical or ultrasound) [23]. The chelation may depend on the physical state of the chitosan (powder, gel, fibre or

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film, etc.) [23–25]. But the major parameter in the complexation process seems to be the degree of acetylation (DA). Yaku et al. [26] have found that a film of chitin obtained by *N*-acetylation of chitosan does not form complexes with metallic ions such as Cu(II), Co(II) or Ni(II). This was confirmed by an interesting work from Micera et al. [27] on monomers from which it was concluded that *N*-acetyl-D-glucosamine units do not interact with Cu(II). Moreover Kurita [28] has confirmed that complexation was easier with large degree of deacetylation chitosans.

Different models were proposed to elucidate the mechanism of coordination implied in the formation of complexes [29]. The first one is called 'bridge model'. It is supposed that the metallic ion is bound with several nitrogen atoms from the same chain or from different chains [30]. The second one, called 'pendant model' considers that the metallic ion is bound to amino group as a pendant [29,31,32]. This model was reinforced by Domard [33]. Using potentiometric and dichroic measurements it was concluded that totally deacetylated chitosan may form with the cupric ions, in a limited pH range ($pH < 6.1$), only one complex which structure was supposed to be $[CuNH_2(OH)_2]$. The fourth site could be occupied by an H_2O molecule or by the OH group on C-3. Nevertheless, the problem of the interaction of the metallic ions is not yet solved due to the variety of the mechanisms of chelation and the possibility of ion exchange. Recently Monteiro and Airoldi [34] have proposed a structure for the Cu–chitosan complex in which the metal ion Cu is likely to bond to three oxygen and one nitrogen ligands in a square-planar or a tetrahedral geometry. In this proposed structure, two bonded oxygen atoms and the nitrogen atom are believed to emerge from the monosaccharide group and two such groups are involved in forming the metal coordination sphere. In the case of Fe ion from the amino groups content of chitosan, the amount of water, and Fe present in the complex it was concluded that for each of Fe(III) ion there are two moles of amino groups and four moles of oxygen. Bhatia and Ravi [35], from Mossbauer experiments, suggest that ligation around Fe is either penta or hexacoordinate with N/O ligands including two amino groups and three or four oxygen atoms respectively. From X-ray photoelectron spectroscopy Dambies et al. [36] demonstrate that, with copper, the sorption is not followed by reduction of the metal by contrast with Mo(VI) and Cr(VI). Finally density functional theory (DFT) results indicate that transition metal (Cu(II) and Ni(II)) coordination to the chitosan biopolymer takes place in the vicinity of the glycosidic oxygen and includes interactions with nitrogen and hydroxyl oxygen atoms [37] but gas phase mass spectroscopy experiments carried out on chitosan di-, tri- and tetrasaccharides [38] are not in agreement with these calculations.

In a previous work [39] the selectivity of chitosan for metallic ions was studied according their ionic charge. Copper was found as one of the most interacting metals with chitosan. Moreover metal recovery tests were carried out on real effluents confirming the interest in using chitosan as depolluting agent.

In this work the interaction of chitosan and its oligomers with copper is investigated. Potentiometric and spectroscopic methods are used to determine the formed complexes and the role of the degree of polymerisation on the ability of these chemicals to complex Cu(II) ions.

2. Experimental section

2.1. Chemicals

The chitosan is obtained by deacetylation of α-chitin from squilla according to previous procedure carried out in our laboratory [40]. Its viscometric average molecular weight was 84,000 from viscometric experiments [41] and its degree of acetylation determined from NMR experiments was 4% [42]. The chitosan was dissolved in hydrochloric acid under stoichiometric conditions.

The metallic ions were used under their chloride form from Merck samples. The chloride form was preferred due to the fact that most of sulphate salts were not soluble and nitrates may act as oxidants and moreover they absorb in the ultraviolet region which may interfere in the determination of our results.

The oligomers are prepared by partial acid hydrolysis with 12N hydrochloric acid [43]. They were used as series of oligomers obtained for different times of hydrolysis without further fractionation. The average number degree of polymerisation is determined from ^1H NMR using the area of the signals of the anomeric protons and the H-1 atom of the glucosamine unit included in the chain.

2.2. Potentiometry

The potentiometry at variable pH is considered as a fundamental technique to study on the one hand the acid–base reactions and on the other hand the metal–ligand interactions [44,45]. A Minisis 6000 pHmeter from Radiometer (France) is used.

Chitosan is added with hydrochloric acid under stoichiometric conditions to get the water soluble chlorhydrate and then titrated with sodium hydroxide.

The complex formation may be described according to the Lewis acid–base theory: the acid is acceptor of a pair of electrons given by the base. The base is currently called ligand (L), here chitosan, and may form covalent bonds with the metallic ions (M) considered as acid entities. Considering the interaction of chitosan with metals the reaction may be described by:

$$\text{Chit–NH}_2 + \text{H}_3\text{O}^+ \rightleftharpoons \text{chit–NH}_3^+ + \text{H}_2\text{O}$$

$$\text{Chit–NH}_2 + \text{M}^{n+} \rightarrow (\text{Complex})^{n+}$$

$$\text{Chit–NH}_3^+ + \text{M}^{n+} + \text{H}_2\text{O} \rightarrow (\text{Complex})^{n+} + \text{H}_3\text{O}^+$$

The formation of the complex may be accompanied by the release of a proton. Hence the pH will decrease during the addition. The larger the decrease, the larger the fixation of the metallic ion by the chitosan. The study of the pH variation allows to investigate this mechanism.

The chitosan has only one complexation site by monomeric unit, the amino group considered as to initiate a coordination bond with the metallic ions. These bonds are between the free electron pairs of the coordinate (amino group) and the void orbitals of the metal. The importance of the complexation of the metallic ion depends not only on the stability constant of the metal–ligand complex but also on the dissociation constant of the ligand [46]. It was obtained from the reaction:

$$\text{–NH}_3^+ + \text{H}_2\text{O} \rightleftharpoons \text{–NH}_2 + \text{H}_3\text{O}^+$$

$$K_a = [\text{–NH}_2][\text{H}_3\text{O}^+]/[\text{–NH}_3^+]$$

The pK_a of the polyelectrolyte varies with the net charge. The variation of the apparent pK_a as a function of the titration results was calculated using the Katchalsky equation [47]:

$$pK_a = \text{pH} + \log[x/(1-x)]$$

where x is the neutralisation degree of amino groups. The apparent pK_a increases when the net charge decreases and tends towards 6 (intrinsic pK_a, pK_0) when the charge fraction is lower than 0.6 in agreement with previous results [48].

The stability constants of the complex are determined using the Bjerrum method modified by Irving and Rossotti [49]. The principle of this method is based on the determination, from the potentiometric titration, of the different species formed in solution. The chemical equation is as follows:

$$\text{ML}_{n-1} + \text{L} \rightleftharpoons \text{ML}_n$$

with

$$K_n = [\text{ML}_n]/[\text{ML}_{n-1}][\text{L}]$$

then

$$\log K_n = -\log[\text{L}] + \log([\text{ML}_n]/[\text{ML}_{n-1}])$$

Considering $\text{pL} = -\log[\text{L}]$, it may be written:

$$\text{pL} = \log K_n + \log([\text{ML}_{n-1}]/[\text{ML}_n])$$

It is possible to determine, n, the average number of coordinate molecules fixed per one metal atom [50] defined by the relation:

$$n = ([\text{L}]_t - [\text{L}] - [\text{LH}^+])/[\text{M}]t$$

with $[\text{L}]_t$: the total molar concentration of the basic form of the ligand, [L] and $[\text{LH}^+]$ represent respectively the concentrations of free ligand and its protonated form, $[\text{M}]_t$: the total concentration of metal.

In the analysis of the results it will be assumed that in the zone of the formation of the complex, the apparent pK_a is constant and equal to 6.

2.3. Ultra violet spectroscopy

A DU-640 spectrophotometer from Beckman was used, covering the wavelength range from 200 to 800 nm, with quartz cells (with a thickness of 0.2 cm).

For the highly deacetylated chitosan the characteristic bands of the chromophore are around 208 nm. This chromophore does not absorb in the visible wavelength range.

2.4. Circular dichroism

The circular dichroism is a spectroscopic technique usually used to study the interactions of ligands prepared with metallic ions such as copper. The circular dichroism is produced around the wavelength of the maximal absorption of the chromophore in the UV-visible region [51].

A Jobin Yvon Dichograph V (France) was used covering the wavelength range from 200 to 800 nm.

The complexation of copper ions by chitosan was carried out outside the chitosan precipitation pH range. In these conditions, the system is homogeneous allowing the study of the complex formation by spectroscopy. The conditions for which the system is soluble would favour the monochain fixation of metallic ions.

3. Results and discussion

3.1. System Cu^{2+}–chitosan

3.1.1. Potentiometric study

In Fig. 1 the curves (V_{NaOH}, pH) are presented during the neutralisation of the chitosan by sodium hydroxide in presence of copper ions for different ratios R (defined as the ratio $[\text{Cu}^{2+}]/[\text{chitosan}]$ expressed in ion mol of metal/monomol of chitosan).

In acidic medium ($\text{pH} < 5.30$) the curve follows the neutralisation curve of the chitosan chlorhydrate alone and is not affected by the presence of Cu(II). The absence of

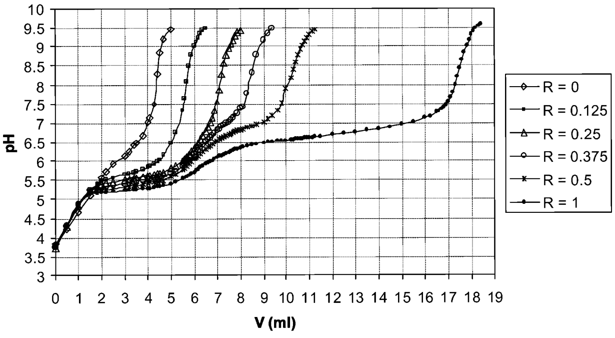


Fig. 1. Influence of R ratio on the neutralisation curves of chitosan solutions by NaOH 0.01N in presence of Cu(II) ions.

difference between the curves demonstrates that in this pH range no complex is formed. The very small complexation of these ions by the polymer may be explained by the fact that it is mainly under protonated form (NH_3^+).

For higher pH values, large modifications appear indicating the presence of complexed species bringing into play the coordination of the amine function of the chitosan with copper ions. Due to the interaction the pH remains lower, during titration, than for chitosan alone. Over a critical degree of neutralisation, the pH increase is related with a change in the colour from pale blue to dark purple in basic pH regions. This shows the implication of nitrogen atom of the amine function of the polymer in the coordination sphere of cupric ions and subsequently the formation of 'chitosan–Cu(II)' complexes.

For pH values near 9, it was observed that the solution is more turbid leading to difficult studies in the high pH ranges. This may be due to the precipitation of copper hydroxides, or to the precipitation of chitosan itself by ionic crosslinking. The precipitation threshold of the chitosan alone is around 6.5, but when the complex (which is ionic) is formed, the precipitation is displaced towards basic media, for pH values around 7.7. It is known that Cu(II) is quite stable in neutral and mild acidic media in the absence of a strong reducing agent.

The shift in the equivalence point between the curve of chitosan neutralisation alone and the one in presence of copper ions varies in close relation with the *R* ratio and consequently on the metallic ion concentration, knowing that the polymer concentration remains constant in these experiments. The excess amount of sodium hydroxide needed for neutralisation of chitosan in the presence of copper corresponds to a molar ratio $[OH^-]/[Cu^{2+}]$ around 2.

When the *R* ratio is larger than 0.25, a second plateau is observed in presence of copper ions. It exists for pH values between 6.2 and 6.7. This plateau is more and more pronounced when the metal concentration is increased. By contrast, no supplementary plateau was observed for *R* values of 0.125 or 0.25. It may be concluded that the ratio *R* is a predominant parameter of the stoichiometry of the complexes.

The research on the Cu^{2+}–chitosan complexes was lead using the Bjerrum method [49]. In the case of our system, *n* (average number of coordinate molecules fixed per one metal atom) may be expressed by the relation:

$$n = ([-NH_2]_t - [-NH_2] - [-NH_3^+])/[Cu^{2+}]_t$$

with $[-NH_2]_t$: total concentration of basic functions, $[-NH_2]$: concentration of free basic functions, $[-NH_3^+]$: concentration of conjugated acidic function and $[Cu^{2+}]_t$: total concentration of cupric ions.

Fig. 2 represents the curve (pL, *n*) obtained from the titration data of the polymer by sodium hydroxide, in presence of Cu^{2+} ($R = 0.25$). It was observed that *n* tends towards 2, demonstrating the presence of essentially two types of complex:

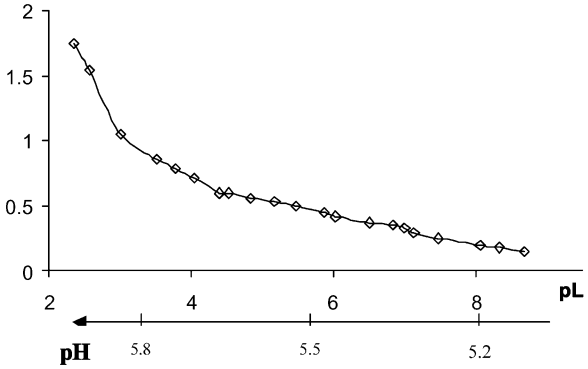


Fig. 2. Formation curve of the complexes from the neutralisation curves of chitosan by sodium hydroxide in presence of copper ($R = 0.25$).

- $[Cu(-NH_2)]^{2+}$ whose formation begins at a pH value of 5.3,
- $[Cu(-NH_2)_2]^{2+}$ which is formed as soon as the pH is larger than 5.8.

The stability constants of these complexes were determined graphically using the method described previously. These values are given in Table 1. The complex implying only one amino group has the larger formation constant, meaning a lower dissociation and hence a larger stability. The constants we have found are in good agreement with those determined by Monteiro and Airoldi [34] from calorimetric experiments. These interactions between the chitosan and the copper ions may be considered as a competition between the following equilibria:

$$Cu^2 + -NH_3^+ + H_2O \rightleftharpoons [Cu(-NH_2)]^{2+} + H_3O^+$$

$$Cu^2 + -NH_2 \rightleftharpoons [Cu(-NH_2)]^{2+}$$

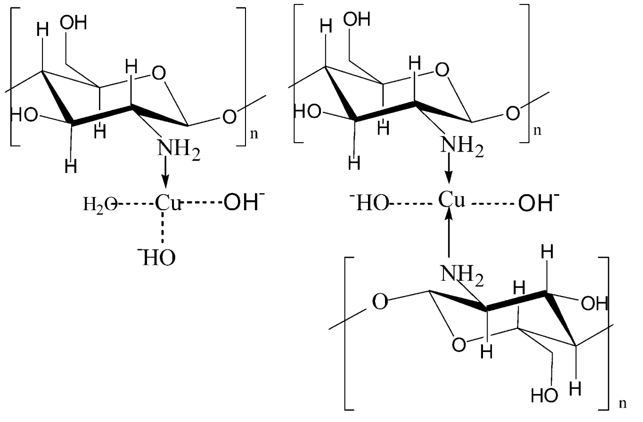
$$[Cu(-NH_2)]^{2+} + -NH_3^+ + H_2O \rightleftharpoons [Cu(-NH_2)_2]^{2+} + H_3O^+$$

$$[Cu(-NH_2)]^{2+} + -NH_2 \rightleftharpoons [Cu(-NH_2)_2]^{2+}$$

For *R* values smaller than 0.25, the pH is smaller than the one observed for the neutralisation of the chitosan chlorhydrate. This may be explained by the release of proton ions by formation of the complex, or the fixation of two OH^- ions for each complexed Cu^{2+} ion, corresponding to a displacement of the equivalence point. Then from all the

Table 1
Formation constants of complexes in the Cu^{2+}–chitosan system; ($R = 0.25$)

Structure of the complex	log K_n
$\{[Cu(-NH_2)]^{2+}, 2OH^-, H_2O\}$	5.47
$\{[Cu(-NH_2)_2]^{2+}, 2OH^-\}$	2.62



Type I
$[Cu(\text{-}NH_2)^{2+}, 2OH^-, H_2O]$

Type II
$[Cu(\text{-}NH_2)_2^{2+}, 2OH^-]$

Fig. 3. Proposed structures for Cu–chitosan complexes.

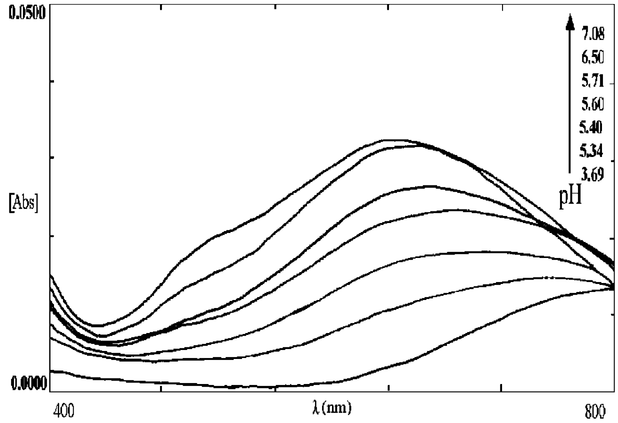


Fig. 4. Evolution of the visible absorption spectra of Cu(II)–chitosan system as a function of pH ($R = 0.25$).

results it may be considered that the first plateau of the neutralisation curves of the polymer in presence of copper ions (Fig. 1) corresponds to the formation of the complex $\{[Cu(-NH_2)]^{2+}, 2\ OH^-\}$ (complex I, Fig. 3). As soon as the metallic ion concentration increases ($R > 0.25$), the pH is increasing illustrated by a transition zone between the two plateaus (Fig. 1). The zone in which pH is increasing corresponds to the neutralisation of a supplementary fraction of NH_3^+: a second type of complex may occur in which an amino group is incorporated in the first complex leading to the $\{[Cu(-NH_2)_2]^{2+}, 2\ OH^-\}$ complex (complex II, Fig. 3).

The number of hydroxyl ions able to bind to the complexed copper determined at the neutralisation point was found to be equal to two. When R is increasing, the number of fixed OH^- ions is increasing with the formation of copper hydroxides. The proposed structures of the complexes are given in Fig. 3. These complexes will be studied using spectroscopic methods.

3.1.2. Spectroscopic methods

The influence of different parameters such as the pH and the R ratio on the absorption (ultraviolet-visible) and dichroic (CD) spectra was studied.

Role of the pH. In Fig. 4 the spectra of the chitosan solutions in presence of copper ions (in a ratio $R = 0.25$) in the 400–800 nm region are registered as a function of the pH. When pH is increased, the difference between registered spectra and the original spectrum of the cupric ion alone (not shown but nearly superimposable with that obtained for pH = 3.6) increases. The pH increase is accompanied with a displacement of the wide band at high wavelength towards smaller values of the wavelength. This band may be considered as the principal characteristic of the different spectra in the visible region. It is due to the d–d electronic transition [52]. The d orbitals of the free cupric ion have the same energy. The presence of the electronic doublets around the metallic ion lift the degenerescence. This leads to transitions which absorb in the visible region and which are responsible for the specific colour of studied solutions. During the pH increase the colour turns from pale blue to purple in the basic media. This is interpreted by the fact that the amino chromophore groups of the chitosan enter the coordination sphere of copper leading to a variation of the energy of the 3d orbitals of Cu^{2+} ions, hence a displacement of the d–d transition towards the smaller wavelengths. This is illustrated in Fig. 5. All these modifications illustrate the strong interaction between the metal and the polymer.

On another side we have observed in the pH range higher than 5.7 the apparition of a shoulder around the wavelength of 510 nm in Fig. 4. It was demonstrated that, generally, the importance of the electronic transition, and subsequently, the intensity of the bands depend upon the position of the ligands around the metallic ion and hence the stability of complexes [53]. Studies on the complexes for which the metallic ion is Cu(II) have shown that the adsorption wavelength depends upon the nature of atoms around the metal

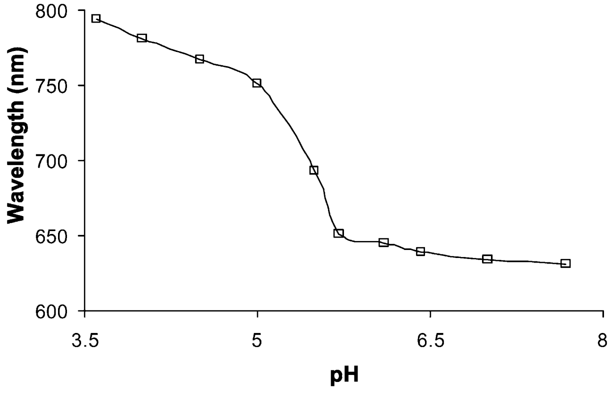


Fig. 5. Influence of pH on the variation of the wavelength at maximal absorption of Cu d–d band of Cu(II)–chitosan system ($R = 0.25$).

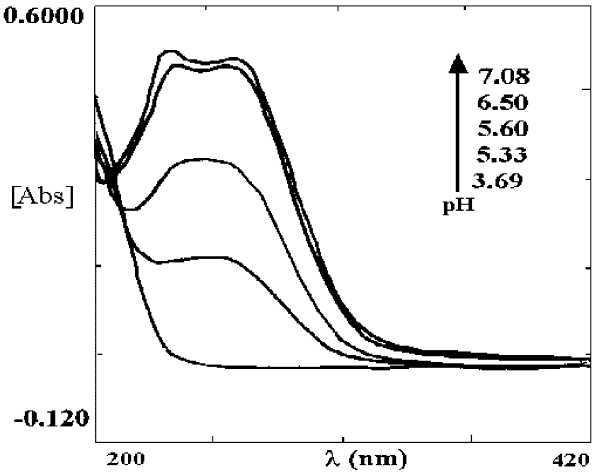


Fig. 6. Influence of the pH on the evolution of absorption spectra of Cu(II)–chitosan system in the ultraviolet region ($R = 0.25$).

[54]. From these considerations and in relation with the potentiometric results the spectroscopic data show that as soon as pH is larger than 5, Cu^{2+} ions are complexed under the most stable form $\{[Cu(-NH_2)]^{2+}, 2OH^-\}$ and for pH values larger than 5.8 the absorbance observed at 510 nm comes from the contribution of the complex $\{[Cu(-NH_2)_2]^{2+}, 2\ OH^-\}$.

In the 200–400 nm range the influence of the pH on the spectra is also demonstrated in Fig. 6. In acidic media ($pH < 5$) no absorbance band was observed, confirming the negligible fixation of Cu^{2+} ions by chitosan. The dissolution of chitosan in an acidic solution favours the formation of the NH_3^+ group. This latter mobilises the free electronic doublet of nitrogen atom, and hence the absence of absorption in the wavelength range. For pH between 5 and 5.8 the apparition of only one wide band at a wavelength of about 257 nm was observed. It is interpreted as a charge transfer transition (CT) [55] between the electronic system of the polymer and the centre of the Cu^{2+} ion. It is on this CT band from the ligand towards the metal that supports the d–d transition. The observed CT band comes from the contribution of the $[Cu(-NH_2)]^{2+}$ species present in solution in this pH range. For pH values larger than 5.8 two maxima were observed, one at a wavelength of 246 nm, the other one at a value of 270 nm. This may be explained by the modification due to the implication of another N atom of an amino group of the polymer in the coordination sphere of Cu^{2+} ions. The apparition of this absorption band with two peaks may come from the contribution of the $[Cu(-NH_2)_2]^{2+}$ form.

As the chitosan is a stereoregular polymer and due to the presence of chromophore amino groups the circular dichroism (CD) may be used to study the complexation of chitosan with metallic ions. The spectra obtained for the same R ratio (equal to 0.25) and different pH values are given in Fig. 7. For pH values smaller than 5 the spectra of the chitosan solution in presence of copper ion are similar to the spectrum of the chitosan alone. This observation confirms that the fixation of the cupric ions on the chitosan is negligible as the amino group is under the protonated form which does not present any dichroic signal [56]. In the pH region between 5 and 5.8 a dichroic signal for the metallic complex is observed with a positive band at 244 nm and a negative one at 287 nm. It is generally attributed to the charge transfer transition from the σ orbital of the ligand to the 3d orbital of the metal [57]. It may be due to the complex $\{[Cu(-NH_2)]^{2+}, 2\ OH^-\}$. For pH values larger than 5.8 the negative band was displaced towards smaller wavelength values (277 nm). This is explained by the implication of a second nitrogen atom in the coordination. The species responsible for this displacement seems to be $[Cu(-NH_2)_2]^{2+}$, 2 OH^-, especially if it is considered that cupric ions may induce the deprotonation of another NH_3^+ group and interact with a second NH_2 group (causing crosslinking).

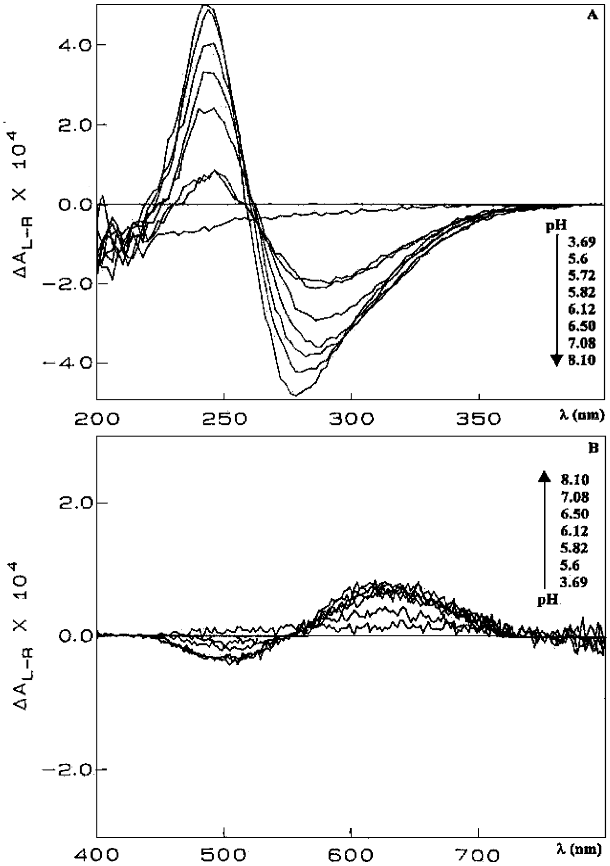


Fig. 7. Influence of pH on the evolution of the dichroic spectra of Cu(II)–chitosan system ($R = 0.25$). A: 200–400 nm region, B: 400–800 nm region.

The pH variation modifies the intensity of the dichroic bands. Indeed, it was observed that the amplitude of these bands increased when the pH was increased. This pH modification was accompanied by the appearance of a large number of non protonated amino groups favouring the complexation of Cu(II) ions by the N atoms, hence an

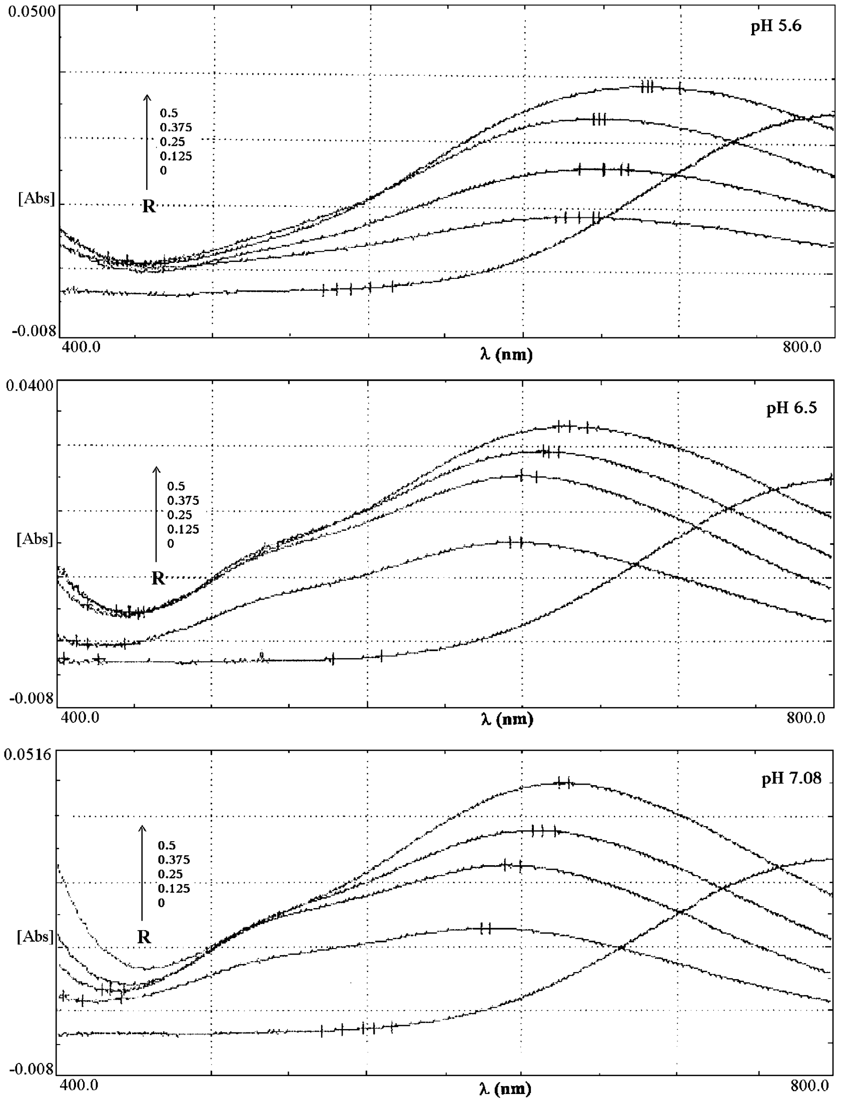


Fig. 8. Evolution of the absorption spectra of Cu(II)–chitosan system in the visible region as a function of the ratio *R* for different pH values.

increase of the amplitude of the peaks related with the number of complexed nitrogen atoms.

The same conclusions can be drawn from the observation of spectra in the 400–800 nm range (Fig. 7B).

Role of the R ratio. In the 400–800 nm range absorption spectra were recorded as a function of the *R* ratio (Fig. 8) at constant pH. Considering a same value of pH, the variation of the *R* ratio does influence only the intensity of the bands without any variation of the wavelength. This demonstrates that the same complexes are formed, the increase of the amplitude being related to the quantity of Cu(II) which is added and complexed. For the pH value of 5.6 only one peak was observed for which the intensity increases with the value of *R*. On another side for pH values of 6.5 and 7.08 a shoulder appears for wavelength values around 530 nm. It is more pronounced when *R* is larger than 0.25. From all these observations, it may be concluded that the increase of the metal concentration favours the deprotonation of ammonium groups and the implication of nitrogen atoms in the coordination with the metal. The same conclusions may be deduced from the observation of the spectra in the 200–400 nm range (Fig. 9). Similar observations were also obtained from the CD spectra.

To illustrate all these results we have represented the variation of the absorbance in UV-visible and CD as a function of *R* for different pH values (Fig. 10). The different curves at 655 nm demonstrate that Cu^{2+} is gradually fixed up to a *R* value of 0.5 with a small influence of pH. From CD, the positive band at 245 nm increases when pH is increased due to an increase of the fixation yield of amines in complex II (Fig. 11). From these curves, it comes that a maximum of 0.5 Cu^{2+} per $-NH_2$ is fixed whatever the pH but that the number of $-NH_2$ per Cu^{2+} is doubled over pH 5.6 corresponding to the complex II.

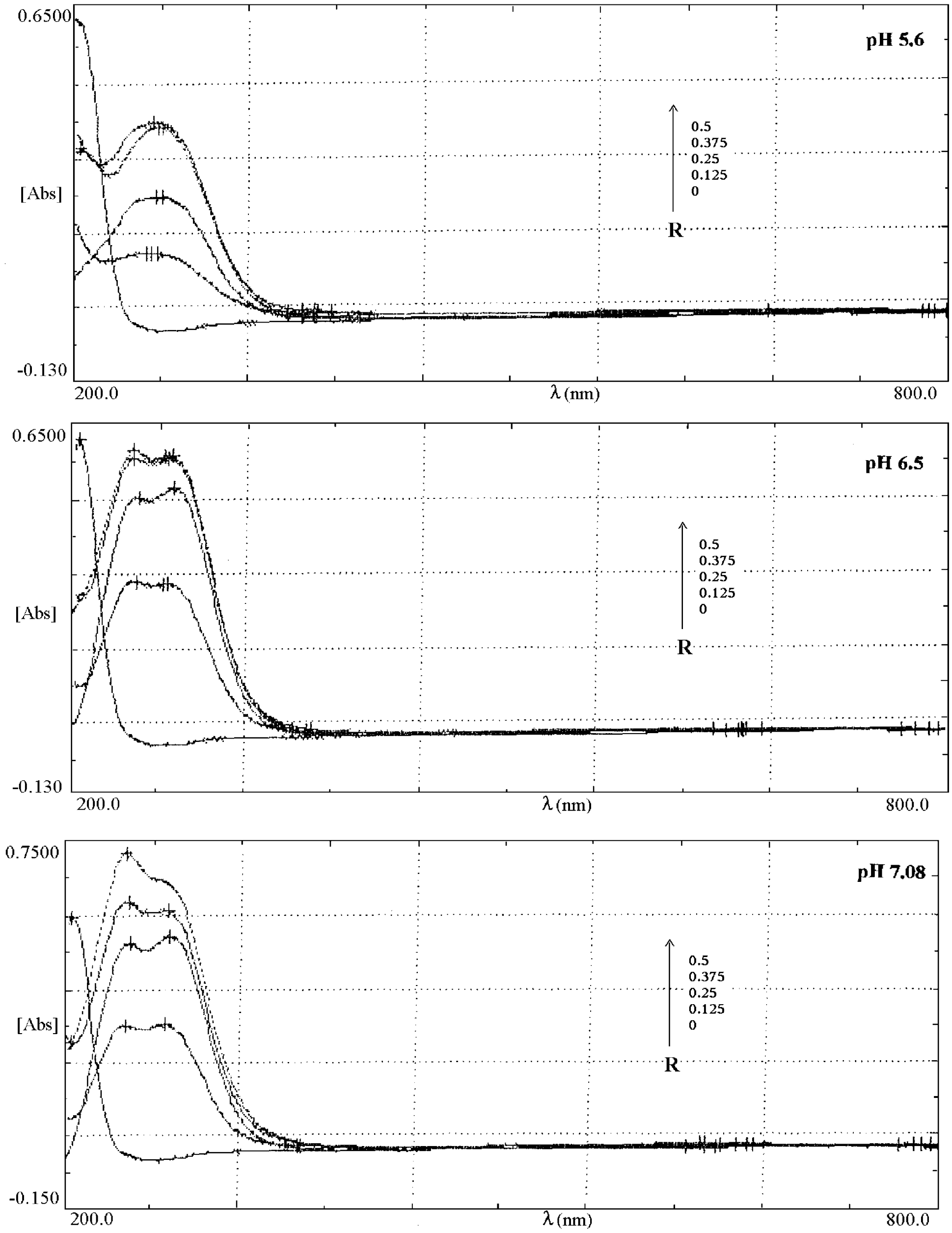


Fig. 9. Evolution of the absorption spectra of Cu(II)–chitosan system in the ultraviolet region as a function of the ratio *R* for different pH values.

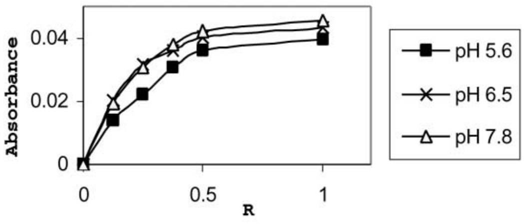


Fig. 10. Variation of the absorbance at 655 nm of Cu(II)–chitosan system as a function of the ratio *R* and the pH.

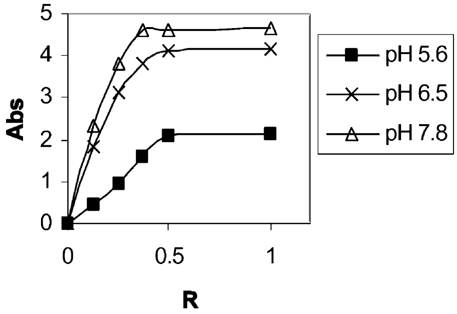


Fig. 11. Variation of the dichroic absorbance at 245 nm of Cu(II)–chitosan system as a function of the ratio *R* and the pH.

Finally it was demonstrated that neither the morphology of the initial chitin (hence the nature of the source), nor the nature of the acidic medium (nature of the acid used), nor the ionic strength have any influence on ability of the chitosan to complex copper ions and on the nature of the complexes.

3.2. System copper–chitosan oligomers.

In all the experiments we have carried out the R ratio value was fixed to 0.25.

In Fig. 12 the neutralisation curves of the different oligomers in presence or in absence of copper ions are presented. When the average degree of polymerisation is 3.5 a very small difference between the different curves was observed indicating a slight complexation of cupric ions. By contrast, for DP values larger than 6.5 the deviations are larger. The capacity of the chitosan oligomers to complex depend upon the degree of polymerisation.

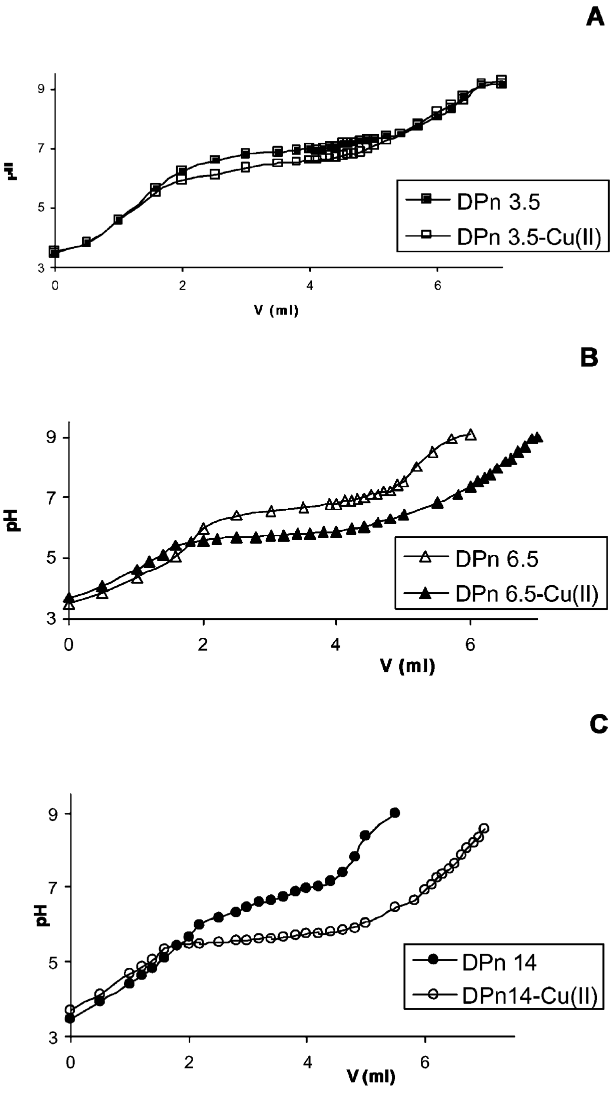


Fig. 12. Neutralisation curves of chitosan oligomers, by NaOH 0.01N, in presence or in absence of Cu(II) ions. A: $DP_n = 3.5$; B: $DP_n = 6.5$; C: $DP_n = 14$.

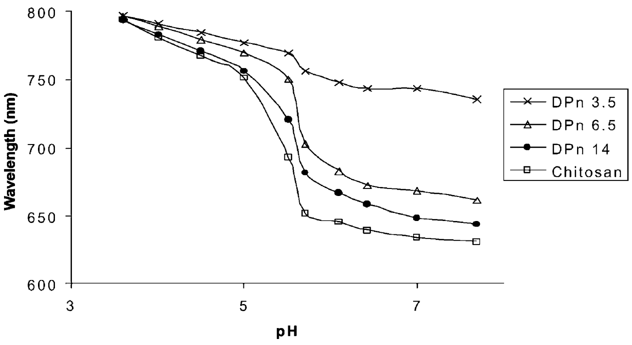


Fig. 13. Variation of the wavelength at maximal absorbance of Cu d–d band for Cu(II)–chitosan and Cu(II)–chitosan oligomer systems as a function of pH ($R = 0.25$).

From the spectroscopic experiments (UV visible and CD) similar observations were done. From UV spectra, as for chitosan, the presence of Cu(II) leads to a displacement of the d–d band towards smaller wavelength values when pH was increased. But the decrease of the wavelength value was more and more important when the number average DP was increased (Fig. 13). For the average value of 3.5 this displacement was very small due to a small amount of oligomers of higher DP able to complex the copper ions. The behaviour of oligomers with average DP_n 6.5 and 14 are very similar to that of the polymer. Considering the intensity of the CT band (Fig. 14) and assuming the extinction coefficient was the same for chitosan complexes than for the oligomer ones, it was determined that the ratio of the degree of complexation of metallic ions by the oligomers, compared with polymer (determined for $R > 0.5$), was around 0.73 for DP_n 6.5 and 0.85 for DP_n 14. The difference may be due to the presence of oligomers (with DP values of 1 or 2 as DP_n characterises the distribution in degree of polymerisation) which are not able to complex the copper ions. The same conclusions were provided from the analysis of CD spectra.

It may be mentioned that in the ultraviolet region of the UV spectra the split of the band was observed for DP_n 14 only but this band is smaller than for the polymer. The two

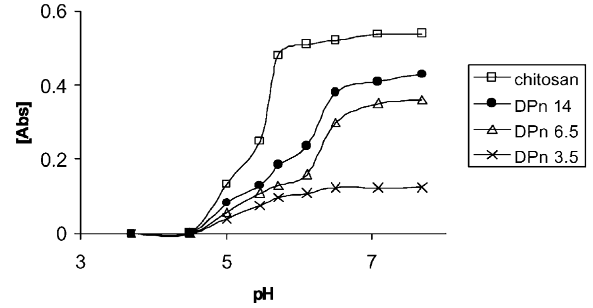


Fig. 14. Variation of the absorbance of the charge transfer band at 240 nm for Cu(II)–chitosan and Cu(II)–chitosan oligomer systems as a function of pH ($R = 0.25$).

complexes are present for this type of oligomer. The ability of the oligomers to complex copper ions depend upon the degree of polymerisation (related with a threshold value around 6) but it may be also related to a minimal amount of macromolecular chain to initiate this complexation. In this case, a minimal concentration would be necessary which is inversely proportional to the degree of polymerisation. Moreover, the structure of the oligomers may be more or less favourable to the spatial organisation of the ligands (oligomers) around to the metallic ion. This may explain that the more stable complex (type I) is also formed in the case of oligomers.

4. Conclusions

Chitosan has a strong affinity for copper ions. The optimal pH for the fixation of cupric ions by chitosan is in the range 5–7. From potentiometric and spectrophotometric studies and taking into account the repartition of the species in solution, it is suggested that the complexes chitosan–Cu(II) have structures as $\{[Cu(-NH_2)]^{2+}, 2OH^-, H_2O\}$ and $\{[Cu(-NH_2)_2]^{2+}, 2OH^-\}$. In the 5–5.8 pH range the first complex is the more stable and for pH values larger than 5.8 the second structure is predominant. The formation constants were determined form potentiometric experiments and found to be in the same order of magnitude that data calculated from calorimetric tests. The maximum Cu fixed corresponds to $[Cu]/[NH_2]$ around 0.5.

The degree of polymerisation plays an important role in the interaction between oligomers and Cu(II) ions; the number average DP value of 6.5 is the threshold for which complexation is efficient.

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