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Binding of ions to chitosan—selectivity studies

Inger M.N. Vold[a], Kjell M. Vårum[a,*], Eric Guibal[b], Olav Smidsrød[a]

[a]*Department of Biotechnology, Norwegian Biopolymer Laboratory (NOBIPOL), Norwegian University of Science and Technology, Sem Sælands vei 6/8, N-7491 Trondheim, Norway*
[b]*Ecole des Mines d'Alès, Laboratoire Génie de l'Environnement Industriel-6, avenue de Clavières, F-30319 Ales cedex, France*

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Abstract

Selectivity coefficients for binding of negative and positive ions to chitosans of different chemical composition have been determined by equilibrium dialysis. Chitosans with different fraction of acetylated units (F_A of 0.01 and 0.49) generally behaved similarly in their selectivity towards both negative and positive ions. No selectivity was found in the binding of chloride and nitrate ions, while chitosan showed a strong selectivity towards molybdate polyoxyanions, with selectivity coefficients around 100. Chitosan showed a strong selectivity towards copper (Cu^{2+}) compared to the metal ions zinc (Zn^{2+}), cadmium (Cd^{2+}) and nickel (Ni^{2+}), with selectivity coefficients from 10 to 1000, while little or no selectivity could be detected with the other metal ions. Ionic strength and pH did not influence the selectivity coefficients of the chitosans towards the metal ions.

Keywords: Chitosan; Metal ion; Selectivity coefficient

1. Introduction

Chitosan may be considered as a family of linear binary copolymers of (1 → 4)-linked 2-acetamido-2-deoxy-β-D-glucopyranose (GlcNAc) and 2-amino-2-deoxy-β-D-glucopyranose (GlcN). It is produced from chitin, one of the most abundant biopolymers in nature. Chitosans have a very diverse range of established and potential applications. The great potential of chitosans is more or less related to its poly-cationic properties, which are unique among abundant polysaccharides and natural polymers in general.

It is well known that chitosans may complex with certain metal ions (Muzzarelli, 1977). Possible applications of the metal binding property are waste water treatment for heavy metal and radio isotope removal with valuable metal recovery, and potable water purification for reduction of unwanted metals (Onsøyen & Skaugrud, 1990). Another well-documented application of chitosan is as a cholesterol-lowering agent (Ormrod, Holmes, & Miller, 1998), and the much more controversial use as a weight reducing agent (Ernst & Pittler, 1998). It is important to investigate the competitive binding of essential metal ions to chitosan in order to judge if chitosan could be harmful to humans consuming chitosan.

The binding mechanism of metal ions to chitosan is not yet fully understood. Various processes such as adsorption, ion exchange, and chelation are discussed as the mechanisms responsible for complex formation between chitosan and metal ions. The type of interaction depends on the metal ion, its chemistry and the pH of the solution (Guibal, Milot, & Roussy, 2000; Inoue, Baba, & Yoshizuka, 1993). Metal anions (e.g. molybdate) can be bound to chitosan by electrostatic attraction. It is likely that the chitosan–metal cation complex formation occurs primarily through the amine groups functioning as ligands (Roberts, 1992). The competitive binding of two ions, e.g. Cu^{2+} and Ni^{2+}, at pH-values around the pK_a-value of chitosan may formally be expressed by the equations:

$$-NH_3^+ + Cu^{2+} \leftrightarrow -NH_2Cu^{2+} + H^+ \tag{1}$$

$$-NH_3^+ + Ni^{2+} \leftrightarrow -NH_2Ni^{2+} + H^+ \tag{2}$$

(1) − (2) gives:

$$Cu^{2+} + -NH_2Ni^{2+} \leftrightarrow Ni^{2+} + -NH_2Cu^{2+} \tag{3}$$

It is seen that any selectively binding of metal cations is independent of pH according to Eq. (3) in the reaction

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[*] Corresponding author. Tel.: +47-73-59-33-24; fax: +47-73-59-12-83.
E-mail address: kjell.morten.vaarum@biotech.ntnu.no (K.M. Vårum).

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scheme above. The reaction schemes indicate that the formation of a metal complex at pH-values around the pK_a-value of chitosan must be accompanied by the release of protons, as also pointed out earlier (Domard, 1987; Koshijima, Tanaka, Muraki, Yamada, & Yaku, 1973; Piron & Domard, 1998; Tsezos, 1983).

Different theories exist concerning the structure of the chitosan–metal ion complex. Some experiments support the theory that two or more amino groups from one chain bind to the same metal ion; the bridge model (Blair & Ho, 1980; Focher, Massoli, Torri, Gervasine, & Morazzoni, 1986). Other studies indicate that only one amino group is involved in the binding, and that the metal ion is bound to the amino group as a pendant; the pendant model (Domard, 1987; Piron & Domard, 1998). Domard (1987) found that only one type of complex was made, and suggested the structure to be $[CuNH_2(OH)_2]$. It seems that the most probable structure is the one where the metal ion is bound to the amino group in the chitosan as a pendant (Roberts, 1992). Nevertheless, the variety of the mechanisms of chelation and the possibility of ion exchange makes the situation even more complex. Recently Rhazi et al. (2002a) suggested that two different complexes exist, dependent on pH. $([Cu(-NH_2)]^{2+}, 2OH^-, H_2O)$ is the more stable for pH-values between 5 and 5.8, and $([Cu(-NH_2)_2]^{2+}, 2OH^-)$ is more stable at pH-values larger than 5.8.

Many parameters will affect chitosans ability to complex metal ions and the stability of the metal–chitosan complex. One of the major parameter in the complexation seems to be the fraction of acetyled units, F_A (Guibal, Dambies, Milot, & Roussy, 1999; Kurita, Sannan, & Iwakura, 1979). The chain length is also an important parameter; Rhazi et al. (2002a) have demonstrated that a degree of polymerisation of six appears as the threshold value for an efficient complexation of copper ions by chitosan oligomers. Various ways of mixing and the physical state of the chitosan will also influence the metal ion capacity of chitosan (Rhazi et al., 2002a). In addition external factors as pH and ionic strength will affect the complexation of metal ions to chitosan.

The aim of most of the studies in this field has been to determine whether or not chitosan will complex with a given ion, determine the amount of ion that can be bound, or to understand the process involved. Not many studies have involved determination of the selectivity of binding of different metal ions to chitosan, however, this aspect has recently been studied. It is of great importance to determine the selectivity of chitosans towards metal ions. Rhazi et al. (2002b) determined the following order in the selectivity from mixtures of ions; $Cu(II) \geq Hg(II) > Zn(II) > Cd(II) > Ni(II) > Co(II), Ca(II)$, using potentiometric and spectrophotometric methods, and showed that the selectivity was independent of the physical form of chitosan.

The aim of this study has been to develop methods to quantify the binding of different ions to chitosan through the determination of selectivity coefficients, and to determine the influence of pH, ionic strength and F_A on the selectivity coefficients.

2. Experimental

The following chitosan samples were used in this study: $F_A = 0.01$, $[\eta] = 800$ ml/g ($M_n = 250,000$), $F_A = 0.49$, $[\eta] = 1270$ ml/g ($M_n = 290,000$). The chitosan with $F_A = 0.01$ was prepared by further deacetylation of a commercial chitosan provided by Pronova Biopolymer (Drammen, Norway), while the chitosan with $F_A = 0.49$ was prepared by homogeneous deacetylation. The fraction of acetylated units (F_A) was determined by ^1H NMR spectroscopy (Vårum, Anthonsen, Grasdalen, & Smidsrød, 1991) and intrinsic viscosities were measured according to Dragnet, Vårum, moen, Gynnild, & Smidsrød (1992a). The number-average molecular weights (M_n) were calculated from the Mark–Houwink–Sakurada (MHS) equation using a calibration against chitosan samples with known M_n (from osmometry) and intrinsic viscosity. The coefficients K and a in the MHS equation used in the calibration were calculated from the relationships given by Anthonsen, Vårum, and Smidsrød (1993).

The selectivity of chitosan in different binary mixtures of ions was determined as described below. The chitosans were dissolved in deionised water of Milli-Q grade (1% (w/v)), and adjusted to the actual pH.

2.1. Chloride/nitrate

Three millilitre (or 6 ml) of the chitosan solution was enclosed in small dialysis-bags (Inf Dia 18/32″–14.3 mm, MWCO 12–14,000 Da), and dialysed towards 3×50 ml^2 solutions of the mixed anions at pH 4.5, using $NaNO_3$ and NaCl in a total concentration of 0.2 M. The ratio of the two competitive anions was varied. The solutions were exchanged three times, to ensure that the ion concentrations at equilibrium in the dialysate were the same as the predetermined ratio. The chitosan solutions were then dialysed exhaustively against deionised water adjusted to pH 4.5 to remove all unbound salts. Then the chitosan solutions were dialysed three times against 0.2 M NaOH to displace the bound ions, and the resultant final three dialysates were combined and analysed. The chloride ions were analysed by complex titration with mercury(II)nitrate using diphenylcarbazone as indicator (Clarke, 1950; Førland, 1989; Meites, 1963). Nitrate was analysed by UV spectroscopy after reduction of nitrate to nitrite followed by a diazotiation reaction (Hellebust & Craigie, 1978; Strickland & Parsons, 1972).

2.2. Molybdate/chloride and molybdate/nitrate

Six millilitre of the chitosan solutions were enclosed in small dialysis-bags, and dialysed towards 3×50 ml^2

solutions of the mixed anions adjusted to pH 5, using MoO_3 and NaCl or $NaNO_3$ in a total concentration of 0.2 M. The experiments were performed as described above. Molybdate ions were analysed by atomic absorption spectroscopy using a Varian SpectrAA 400. Chloride and nitrate ions were analysed as described above.

2.3. *Cu^{2+}/Cd^{2+}, Cu^{2+}/Ni^{2+}, Cu^{2+}/Zn^{2+}, Ni^{2+}/Cd^{2+}, Zn^{2+}/Cd^{2+}, Zn^{2+}/Ni^{2+}*

Three millilitre of the chitosan solutions were enclosed in small dialysis-bags, and dialysed towards 3×50 ml^2 solutions of the mixed metal ions at a total concentration of 0.1 M (or towards 3×100 ml solutions at a total concentration of 0.05 M) adjusted to the actual pH. Metal sulphates, metal chlorides or metal nitrates were used. The ratio of the two competitive ions was varied. The metal ion solutions were kept at a pH lower than 4.7 to avoid copper hydroxide precipitation. The solutions were exchanged three times, to ensure that the ion concentrations at equilibrium in the solution phase were according to the predetermined ratio. The chitosan solutions were then dialysed exhaustively against deionised water to remove all unbound salts. Then the chitosan solutions were dialysed three times against 0.2 M hydrochloric acid to displace the bound ions, and the resultant final three dialysates were combined and analysed. The ionic strength of the metal ion solutions was varied by addition of sodium sulphate. Copper, cadmium, nickel, and zinc were analysed by atomic absorption spectroscopy using a Perkin–Elmer 560 Atomic Absorption Spectrophotometer.

3. Results and discussion

To quantify the binding of anions to chitosan, a selectivity coefficient, k_A^B, was defined in the ion-exchange equilibrium.

$$\text{Chitosan-A} + \text{B} \leftrightarrow \text{Chitosan-B} + \text{A}$$

by the equation:

$$k_A^B = \frac{X_B \cdot C_A}{X_A \cdot C_B}$$

where A and B represent the two anions, X_A and X_B is the mole fraction of ions bound to the chitosan ($X_A + X_B = 1$), and C_A and C_B are the molar concentrations of ions in solution at equilibrium. This definition of the selectivity coefficient has been widely used in ion binding studies on alginate (Haug & Smidsrød, 1970; Smidsrød & Haug, 1968, 1972). In systems concerning metal cations and chitosan, the sorption probably occurs by complexation rather than ion-exchange. The definition of the selectivity coefficient given above will nevertheless be a measure of the selectivity in the system, regardless of the sorption mechanism, and this definition will be used throughout the paper.

3.1. *Selectivity of chitosan for anions*

3.1.1. *Binary mixtures of chloride and nitrate*

It is practical to prepare chitosan salts (e.g. chitosan chloride and chitosan nitrate), as these salts, as opposed to chitosan in its free amine form, are directly soluble in water. Chitosan chlorides are widely used also commercially, and we initially tested the selectivity of chitosan towards chloride and nitrate. The experiments were performed using equilibrium dialysis as previously described to determine selectivity coefficients for alginates (Smidsrød & Haug, 1968). Starting with a 1% chitosan chloride solution in the dialysis bag, the chitosan was dialysed against solutions with varying amounts of chloride to nitrate (sodium salts) to obtain the same concentration of the anions inside the dialysis bag as on the outside. The chitosan was thereafter dialysed extensively against deionised water, and then against 0.2 M NaOH to remove the ions bound to chitosan. The amounts of chloride and nitrate were determined in the final combined dialysates. The selectivity of chitosans towards nitrate and chloride ions was investigated for two different chitosans of different chemical composition ($F_A = 0.01$ and 0.49) in binary salt solutions of constant ionic strength, but varying composition of the two ions. Fig. 1 shows the calculated selectivity coefficients in the exchange reaction between chloride and nitrate at different ionic composition for the two chitosans. The selectivity coefficients are around one, meaning that chitosans show no selectivity towards chloride or nitrate ions. In the experiment the total amount of ions bound to the chitosans was nearly constant at the different ionic composition, with around 0.8 mol ion bound per mol GlcN. The pH in the dialysate increased during the dialysis against the sodium salts. During dialysis with deionised water the pH in the dialysate decreased while the pH inside the dialysis bag increased, probably caused by the Donnan equilibrium.

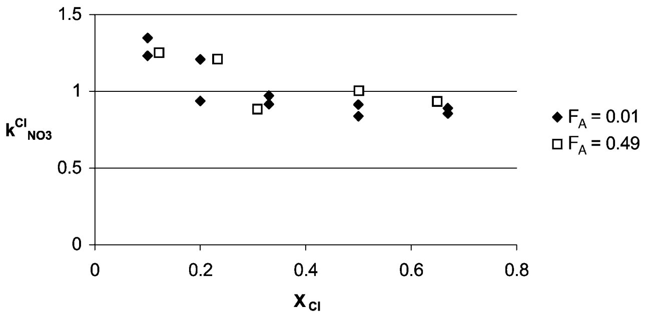

Fig. 1. Selectivity coefficients of chitosans ($F_A = 0.01$ and 0.49) in the exchange reaction between chloride and nitrate as a function of the ionic composition in the bound anion fraction at pH 4.5. The fraction of chloride in the exchange solution varies from 0.1 to 0.7.

3.1.2. Binary mixtures of molybdate/chloride and molybdate/nitrate

In earlier experiments (Draget et al., 1992a; Draget, Vårum, & Smidsrød, 1992b) cross-linking of chitosan with Mo(VI) polyoxyanions has been used to prepare chitosan gels. The selectivity in binary systems of molybdate and nitrate ions, and molybdate and chloride ions was investigated for two chitosans of different chemical composition ($F_A = 0.01$ and 0.49) in salt solutions with constant ionic strength, but different ionic composition in the binary salt solution. Fig. 2 shows the selectivity coefficients for chitosans in the binding of molybdate and chloride, and molybdate and nitrate, and it is clearly seen that both chitosans show strong selectivity for binding of molybdate, with selectivity coefficients around 100. However, the evaluation of the results is complicated by the fact that the molybdate solution is very complex with different composition of polyoxyanions at different pH and molybdate concentrations (Tytko, Baethe, Hirschfeld, Mehmke, & Stellhorn, 1983). In addition the analysis of chloride and nitrate ions at high molybdate concentrations were complicated by the fact that the molybdate interfered with the chloride and nitrate analysis. It is, however, obvious that chitosans can bind molybdate ions in the presence of a large excess of chloride or nitrate ions. The selectivity coefficients increased considerably when the system changed from chitosan in solution to precipitated chitosan (from the point $X_{Mo} \approx 0.5$ to $X_{Mo} = 1$), suggesting some inter-chain binding as in the binding of Ca^{2+} to alginate (Smidsrød & Haug, 1968). The chitosans with F_A of 0.01 and 0.49 show no differences in the value of the selectivity coefficients. This supports the hypothesis that the increased gel strength of chitosan molybdate gels formed with chitosans of higher F_A is caused by interactions between the acetyl groups (Draget, 1996). The molar ratio between amino groups and molybdenum has been determined. At high metal concentration, this ratio exceeds one (between 1.3 and 1.9). This confirms that molybdate is adsorbed in the form of polynuclear species. It is consistent with previous results (Draget et al., 1992a).

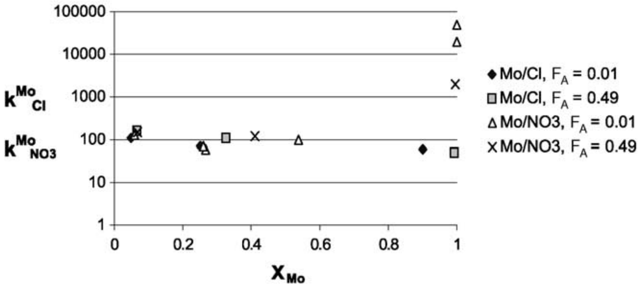

Fig. 2. Selectivity coefficients of chitosans ($F_A = 0.01$ and 0.49) in the exchange reaction between molybdate and chloride, and molybdate and nitrate as a function of the ionic composition in the bound ion fraction at pH 5.0. The fraction of molybdate in the exchange solution varies from 0.001 to 0.1.

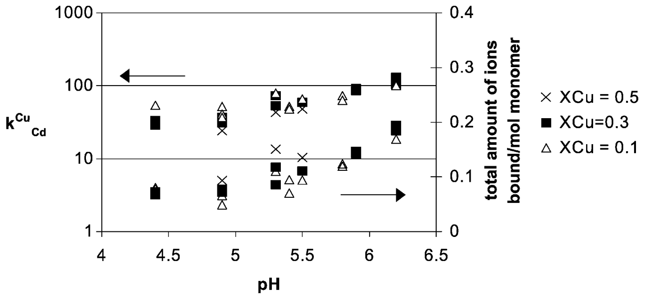

Fig. 3. The influence of pH on the selectivity coefficient in the Cu^{2+}/Cd^{2+} exchange reaction (upper points) and on the total amount Cu^{2+} and Cd^{2+} ions bound to the chitosan per mol GlcN (lower points). The selectivity experiments are carried out at three different ionic compositions of Cu^{2+} and Cd^{2+}.

Guibal et al. (2000) also found that molybdate in the form of heptamolybdate species was sorbed at pH 3, reaching sorption capacities as high as 7–8 mmol Mo/g.

3.2. Selectivity in the binding of metal cations to chitosan

It has previously been shown that pH is important in the binding of metal ions to chitosan, and we have tested the influence of pH and ionic strength on the chitosan selectivity coefficient between Cu^{2+} and Cd^{2+}, k^{Cu}_{Cd}. Also, the total amount of ions bound to the chitosan was determined as a function of pH and ionic strength. When the dialysis bags containing dissolved chitosan chloride were immersed in solutions of Cu/Cd sulphate the interaction of the ion with chitosan was accompanied by the appearance of a blue colour and precipitation of the chitosan. The pH in the dialysis bags was found to decrease during dialysis towards metal solution, indicating that the formation of the complex was accompanied by the release of protons.

Figs. 3 and 4 show the influence of pH and ionic strength, respectively, on the selectivity coefficient of chitosan ($F_A = 0.01$) towards Cu^{2+} and Cd^{2+}, k^{Cu}_{Cd} (upper points)

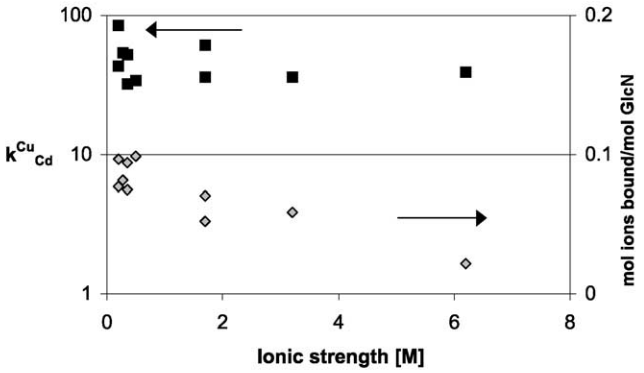

Fig. 4. The influence of ionic strength on the selectivity coefficient in the Cu^{2+}/Cd^{2+} exchange reaction (upper points) and on the total amount Cu^{2+} and Cd^{2+} ions bound to the chitosan per mol GlcN (lower points). The selectivity experiments are carried out at pH 5.3 with a fraction of copper, $X_{Cu} = 0.1$, in the exchange solution.

and on the total amount Cu^{2+} and Cd^{2+} ions bound to the chitosan per mol GlcN (lower points).

Fig. 3 suggests that the selectivity coefficient increases slightly with increasing pH. According to Eq. (3), the selectivity should be independent of pH. The slight increase in the selectivity coefficients could be due to some kind of cooperativity or interchain binding. Results from Juang and Shao (2002) on binary systems of Cu^{2+}/Ni^{2+} and Cu^{2+}/Zn^{2+} using cross-linked chitosan indicate on the other hand that the selectivity coefficient increased up to a pH range maximum and then decreased. The optimum pH-range was determined to 5.1–5.3 for binary mixtures of Cu^{2+} and Ni^{2+}, and 4.5–4.9 for binary mixtures of Cu^{2+} and Zn^{2+}. The discrepancy between their result and the present data is not understood. Fig. 3 also shows that the total amount of metal ions bound to the chitosan increased with increasing pH, in agreement with previous results (Becker, Schlaak, & Strasdeit, 2000; Dzul Erosa, Saucedo Medina, Navarro Mendoza, Avila Rodriguez, & Guibal, 2001; Juang and Shao, 2002; Koshijima et al., 1973). The increase in the capacity of the chitosan at higher pH-values can be explained by the fact that at low pH the metal ions compete with protons for binding sites, as the reaction schemes in the introduction indicate. Moreover, the increased protonation of the amine groups at lower pH-values also induce long-range electrostatic repulsion of metal ions. Both these effects will favour binding of metal ions at high pH-values, far above the pK_a-value of the amino group of chitosan. Conversely binding will be impossible at pH-values far below the pK_a-value of the amino groups.

Fig. 4 suggests that the selectivity coefficient decrease slightly with increasing ionic strength. However, no dramatic changes in the selectivity coefficient or the amount of metal ion bound was found up to 0.5 M ionic strength, and even at an 1.7 M ionic strength the selectivity coefficient remained high. At low ionic strength a small increase in the ionic strength seems to lead to an increasing amount of ions bound to the chitosan. This is consistent with the findings of Mitani, Nakajima, Sungkono, and Ishii (1995), who found that the removal efficiencies of cobalt and nickel by swollen chitosan beads increased with increasing ionic strength from 5 to 25 mM. This could be

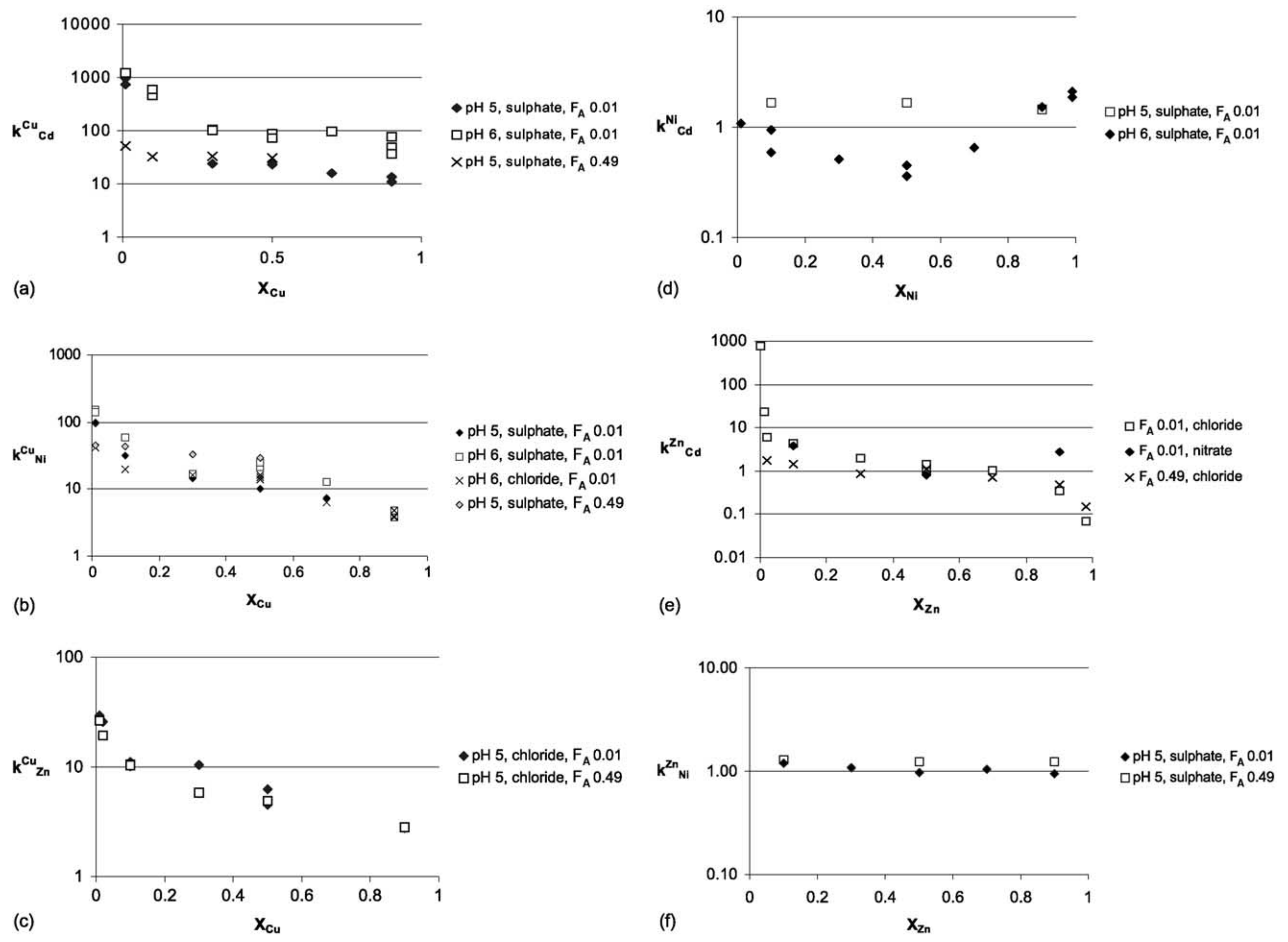

Fig. 5. Selectivity coefficients in the exchange reaction between (a) Cu^{2+} and Cd^{2+} as sulphate salts at pH 5 and pH 6; (b) Cu^{2+} and Ni^{2+} as sulphate and chloride salts at pH 5 and pH 6; (c) Cu^{2+} and Zn^{2+} as chloride salts at pH 5; (d) Ni^{2+} and Cd^{2+} as sulphate salts at pH 5 and pH 6; (e) Zn^{2+} and Cd^{2+} as chloride and nitrate salts at pH 5; (f) Zn^{2+} and Ni^{2+} as sulphate salts at pH 5, as a function of the ionic composition in the exchange solution for chitosans with $F_A = 0.01$ and 0.49.

caused by screening of the long-range electrostatic repulsion between the positively charged metal ions and the positively charged chitosan. Fig. 4 shows that when the ionic strength is further increased, the amount of ions bound to chitosan decreases.

The selectivity coefficients of chitosans ($F_A = 0.01$ and $F_A = 0.49$) towards the metal ions Cu^{2+}, Cd^{2+}, Ni^{2+}, and Zn^{2+} were investigated by studying the selectivity coefficients in binary systems: (a) Cu^{2+} and Cd^{2+}, (b) Cu^{2+} and Ni^{2+}, (c) Cu^{2+} and Zn^{2+}, (d) Ni^{2+} and Cd^{2+}, (e) Zn^{2+} and Cd^{2+}, (f) Zn^{2+} and Ni^{2+} as a function of the ionic composition of the metal solution, as shown in Fig. 5(a)–(f).

Cu^{2+} ions were found to bind selectively to the chitosans in the presence of Ni^{2+}, Zn^{2+}, and Cd^{2+} ions. The highest selectivity coefficients were determined for Cu/Cd at pH 6, with $k_{\mathrm{Cd}}^{\mathrm{Cu}}$− values between 100 and 1000 with $X_{\mathrm{Cu}} < 0.7$. The selectivity of chitosans for Cu^{2+} was confirmed by the fact that the total uptake capacity of chitosan increased when the fraction of Cu(II) in the solution increased. This is shown in Fig. 6(a) for the Cu–Cd system. The increasing uptake capacity was most pronounced in the Cu–Cd and Cu–Ni systems (about five times), and less pronounced for the Cu–Zn system. This is in agreement with other results (Bassi & Prasher, 2000; Juang & Shao, 2002; Rhazi et al., 2002b), and it is clear that chitosan selectively adsorb Cu^{2+} from such multi-component solutions.

Fig. 5(d)–(f) indicate that chitosans have no selectivity in the binding of ions in the binary systems $\mathrm{Ni}^{2+}/\mathrm{Cd}^{2+}$, $\mathrm{Zn}^{2+}/\mathrm{Cd}^{2+}$, and $\mathrm{Zn}^{2+}/\mathrm{Ni}^{2+}$. This is in accordance with results from Becker et al. (2000) who investigated the selectivities between chlorides, nitrates and sulphate salts of Cd^{2+}, Ni^{2+} and Zn^{2+}, and found that the metal ion selectivities of chitosan derivatives (including a chitosan derivative made by about 20% cross-linking with glutardialdehyde and subsequent reduction) were low. Our results indicate that in the Zn/Cd and Zn/Ni system the total uptake of ions are independent of the ionic composition in the solution (shown in Fig. 6(b) for the Zn–Ni system), whereas in the Ni/Cd system nickel has a depreciating effect on the sorption capacity.

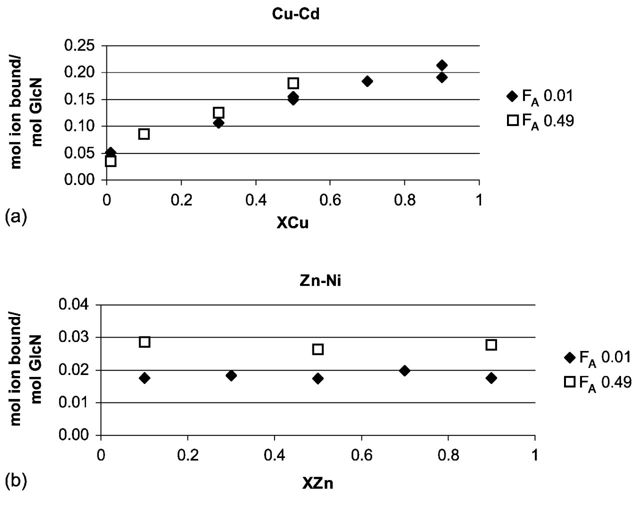

Fig. 6. The total amount of ions bound to the chitosans per mol GlcN in the binary systems (a) $\mathrm{CuSO_4/CdSO_4}$, pH 5 and (b) $\mathrm{ZnSO_4/NiSO_4}$, pH 5, as a function of the ionic composition in the exchange solution for chitosans with $F_A = 0.01$ and 0.49.

It seems that neither the anion of the metal salt (Fig. 5(b)) nor the chemical composition of the chitosan, F_A, (Fig. 5(a–c,e, f)) influence the selectivity coefficient. The anion in the metal salt does, however, influence the total amount of ions bound to chitosan. Results from experiments with Cu/Cd at pH 4.3 and 5.3 (data not shown) show that sulphate salts bind to a greater extent to chitosan than chloride salts. This result is in agreement with results from other research groups (Becker et al., 2000; McKay, Blair, & Grant, 1987; Mitani, Fukumuro, Yoshimoto, & Ishii, 1991; Mitani et al., 1995). In most cases the total amount of ions bound pr mol GlcN was slightly higher in experiments with chitosan of the high degree of acetylation, $F_A = 0.49$, as compared to chitosan with a very low degree of acetylation, $F_A = 0.01$ (shown for the systems Cu–Cd and Zn–Ni in Fig. 6).

4. Conclusion

No selectivity in the binding of chloride and nitrate ions to chitosan can be determined. Molybdate anions are selectively bound to chitosans in the presence of excess nitrate or chloride ions, with selectivity coefficients around 100. Cu^{2+} ions are bound selectively to chitosan in the presence of Ni^{2+}, Zn^{2+} or Cd^{2+} ions, with selectivity coefficients in the range 10–1000. Chitosans with low and high F_A do not show any difference in their selectivity towards ions. Binding of metal ions increases with pH indicating that only the deprotonised amino groups can bind the ions.

Acknowledgements

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