Interaction of Microcrystalline Chitosan with Ni(II) and Mn(II) Ions in Aqueous Solution

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Received 20 December 2004; accepted 4 March 2005
DOI 10.1002/app.22437
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: The protonation constant of the NH_2 function was determined by the method of Katchalsky and Spitnik and by the SUPERQUAD fitting procedure. Samples with higher concentrations of chitosan indicated aggregations of polymer chains, which led to a loss in the effective concentration of the ligand (L). It followed, as a result of potentiometric titrations, that an excess of L of microcrystalline chitosan (MCCh) with a deacetylation degree of 0.90 was a complexing agent toward the metal (M), which was Ni(II) or Mn(II). Species ML and ML_2 were accepted by SUPERQUAD for both of the M's, where coordination occurred via the amino nitrogen. For Ni(II), however, the hydroxyl oxygen may also have been an electron-pair donor at lower excesses of MCCh and, by that, made possible the formation of five-membered chelate rings in the hydroxyl deprotonated MLH_{-1} species. The evaluated formation constants were compared with the values known until now for monomeric D-glucosamine. Additional confirmation of the M–L interaction was determined by the spectrophotometric titration of a Ni(II)–MCCh solution. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 98: 2572–2577, 2005

Key words: biodegradable; chitosan; computer modeling; metal-polymer complexes; UV-vis spectroscopy

INTRODUCTION

Chitosan is a polysaccharide derived from the chitin of crustaceans, with crab and shrimp shell wastes as its principal sources. The properties of a cationic polysaccharide, low toxicity and good biocompatibility, make it interesting for study as a drug excipient.[1] Chitosan has become well known as multifunctional biopolymer with a wide range of applications both in the medical and pharmaceutical areas. New research has indicated that chitosan is an excellent candidate for drug-release formulations for most physiological drug-delivery pathways.[2]

Chitosan is chemically defined as a copolymer consisting of two residues: 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-glucopyranose. The proportion of glucosamine is higher than *N*-acetylglucosamine and yields much better solubility in aqueous solutions of organic acids and numerous inorganic acids.[3] Microcrystalline chitosan (MCCh) is a special multifunctional polymeric material prepared by the aggregation of glucosamine macromolecules from an aqueous solution of an organic acid.[4] It is the only form of polyaminosaccharide characterized by the presence of free unbounded amine groups that can be present in a liquid dispersion form with direct film-forming behavior.

In the available literature, there are few reports on the applications of MCCh in medicine, pharmacy, and other areas.[5] The enhanced sorption behavior of MCCh permits one to incorporate bioactive compounds into the chitosan structure and to prepare suitable controlled release forms, which is an important function in medical and pharmaceutical applications. Different forms of controlled release agents have shown specific release efficiencies depending on the conditions applied during production.[6] MCCh is a safe and effective biopolymer for the achievement of hemostasis at puncture sites.[7] The results of recent studies indicate that MCCh in matrix granules could offer advantages surpassing nonmodified chitosan because the gels form more easily in acidic environments, for example, in the stomach, and drug release is more retarded.[1,8] MCCh might be particularly useful for the preparation of stomach-specific, slow-release dosage forms.

Quantitative studies have confirmed the superior properties of MCCh as a hydrogel compared to its nonmodified form, that is, flakes.[9] MCCh in the form of hydrogels at neutral pH has been used in studies on drug carriers. The preliminary results show that MCCh is useful for the preparation of semisolid and solid formulations.[10,11] The more stable hydrogel systems containing drugs were produced with MCCh

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Contract grant sponsor: Medical University of Łódź; contract grant number: 503-314-2.

hydrogels of higher polymer content. As reported for nonmodified chitosan, its microcrystalline form is a suitable carrier for drugs that are poorly soluble in water.[12]

The ability to form stable, chelated complexes is a unique property of both chitin and chitosan.[13–16] This feature is not exhibited by other known membrane-forming natural polymers. The complexing abilities of chitosan result from the presence of amino and hydroxyl groups in the particular constitutional units. MCCh in the form of a gelatinous water dispersion with a low polymer content of 1.5 wt % and a low weight-average molecular weight ($\bar{M}_w$) of 0.27×10^5 Da has been used until now in our studies on protonation and complexation equilibria with Co (II), Zn (II), and Cu (II) ions.[17] The purpose of this investigation was to evaluate the complexing abilities against Mn(II) and Ni(II) of MCCh in a hydrogel form of higher polymer content, higher average molecular weight, and higher water retention value.

EXPERIMENTAL

Materials and methods

MCCh in the form of a hydrogel with a polymer content of 3 wt %, a water retention value of 1030%, a M_w of 2×10^5 Da, and a deacetylation degree value of 90% was prepared with the previously published unconventional method[4] of the Institute of Chemical Fibers (Łódź, Poland).

Protonation and complexation equilibria for Ni(II)–MCCh and Mn(II)–MCCh systems were carried out by the potentiometric method with an automatic titration kit (Molspin, Ltd., Newcastle upon Tyne, England) with suitable software (MOLSPIN.EXE). The experimental results were then transferred to SUPERQUAD (Protonic Software, Leeds, UK) input files. Titrations in the absence of metal (M) were also used in the calculations of protonation constants based on the method of Katchalsky and Spitnik.[18,19] The coefficients of correlation (R's) and the regression coefficients were tested at $p < 0.001$ by means of the regression tool of Microsoft Excel 2003.

The equilibria in solutions containing chitosan were investigated in a double-walled, 9-cm^3 vessel thermostated at 25 ± 0.1°C; the vessel was equipped with a combined OSH-10-10 electrode (Metron, Czekanów, Poland). Carbonate-free sodium hydroxide (0.1M) used as titrant was purchased from Baker J. T. (Deventer, Holland) (titer = 0.997–0.1 mol/dm^3). Potassium nitrate and nitric acid were analytically pure (ppa) (POCh, Gliwice, Poland). According to IUPAC recommendations, the electrode was standardized with two buffer solutions (pH_{25} = 9.100; 0.01 mol/dm^3 disodium tetraborate; ionic strength = 0.1 KNO_3; pH_{25} = 3.926; 0.05 mol/dm^3 potassium hydrogen phthalate in 0.05 mol/dm^3 of KNO_3)[20] and then calibrated on the electromotive force = function (f)($-\log[H^+]$) scale by titration of a 0.01M HNO_3 (ionic strength (I) = 0.1, KNO_3) with 0.1M NaOH. The total volume (500 μL) of a Hamilton microsyringe in the autoburette was divided into 100 titration points. The volume increments amounted then to 0.0050 mL.

The protonation constants of MCCh were determined at various chitosan concentrations (C_L's) within the range 8.75×10^{-3} to 1.31×10^{-2} mol/dm^3. Each titration was repeated three times. The complexation equilibria were studied in the presence of manganese (II) and nickel (II) nitrate salts, ppa (Fluka AG, Buchs, Switzerland). The stock solutions (0.1–0.29 mol/dm^3) were standardized complexometrically with disodium salt of ethylenediaminetetraacetic acid. The metal concentrations (C_M's) in the titrated samples ranged from 1.31 to 2.62×10^{-3} mol/dm^3. The ligand (L)–M concentration ratios amounted to 5 : 1, 8 : 1, and 10 : 1. For each L–M complex, the titration was repeated five times at various C_M's.

The ultraviolet–visible (UV–vis) absorption spectra of Ni(II) solutions were taken at 25°C on a Cary 50 Bio (Varian, Mulgrave, Australia) spectrophotometer equipped with a cell compartment and thermostated by a Peltier single-cell holder. An external-type spectrophotometric titration was applied; definite pH values were set for the solutions in a number of separate measuring flasks of with volumes of 25 cm^3.

RESULTS AND DISCUSSION

Protonation constant

Evaluation of the amino group protonation was necessary for further studies on the M–MCCh interactions. Chitosan in acidic medium behaves as a polycation due to the positive charge of the amino groups.[21] The method of Katchalsky and Spitnik, suitable for polymeric L's with strong Coulombic repulsion of adjacent constitutional units, brought us to the observation that in our case, with MCCh with a deacetylation degree of 0.90, an increase in C_L led to a decrease in the values of log protonation constant (K_H) and, at the same time, an increase in the values of n parameter (Table I).

This effect was observed despite the limiting of pH to a narrow range (5–6.5), where the linear dependence described by the Katchalsky–Spitnik equation was fulfilled with a determination coefficient (R^2) close to 1 ($p < 0.001$). The calculated standard deviations of log K_H and n were lower at higher C_L values because of the larger numbers of experimental points in the straight line within the low pH range, but the variability in the values of log K_H and n was statistically significant (the values of p were in every case much lower than 0.001). Hence, we presumed that at

TABLE I
Protonation Constant of MCCh with a Degree of Deacetylation of 0.90 and Value of the *n* Parameter Calculated from the Katchalsky–Spitnik Equation[a]

C_L ($\times 10^{-3}$ mol/dm^3)[b]	pH range	log K_H	n	R^2
8.75	4.867–5.995	6.355(15)	1.520(26)	0.9933
9.50	5.006–6.490	6.294(7)	1.543(26)	0.9930
13.1	4.922–6.521	6.011(1)	1.649(5)	0.9996

Temperature 25°C; $I = 0.1$ (KNO_3). Number of replicates = 3. Standard deviations are in parentheses.
[a] $pH = \log K_H - n \log [a/(1 - a)]$, where a is the degree of neutralization of amino groups and n is an empirical parameter related to change in free energy due to electrostatic repulsion.
[b] Total concentration chitosan.

higher amounts of MCCh, with the enhanced mutual interaction of polymer chains, alleviated by the neutralization of —NH_3^+ during titration, the effective C_L was lower than the total C_L.

Confirmation came from the SUPERQUAD calculations. The refinements were successful for a wider pH range (even up to 7.0, where precipitation started to become visible). By treating the number of millimoles of L and the number of millimoles of protons as refined parameters, we could see that at higher C_L's, the millimoles per liter values after refinement were distinctly lower than the total values (Table II). In turn, the number of millimoles of H did not differ essentially before and after refinements; they were only somewhat enhanced at lower contents of MCCh (because the initial excess of acid was referred to fully deacetylated MCCh) and were somewhat lowered at higher C_L values. As a result of the refinements, the tendency of decreasing log β_{011} constants with increasing C_L values was also with the Katchalsky–Spitnik method.

Finally, the mean value of the repeatable protonation constants (log $\beta_{011} = 6.46$) evaluated by SUPERQUAD at lower C_L values seemed the most justified one and was independent of mutual interactions between the polymer chains. Moreover, this value was very close to our previous result (6.5 ± 0.2) for MCCh with a deacetylation degree of 0.87 and to the intrinsic value of 6.5 reported by Domard for various degrees of deacetylation.[16,17] However, the calculated protonation constant of MCCh with a deacetylation degree of 0.90 was also much lower than the value 7.70 of monomeric D-glucosamine (D-Glc), as reported in the literature.[22,23]

Complexing with Mn(II) and Ni(II): Potentiometric titrations

The evaluation of chitosan–M complexation was carried out in approximately the same range of ligand neutralization coefficient ($a \cong 0.1–0.7$) as used for the titrations without the M (Figs. 1 and 2); that is, the

TABLE II
Refinement Results of the Protonation Constants of MCCh with a Degree of Deacetylation of 0.90 and with Simultaneous Refinement of the Millimoles of L and Protons

C_L ($\times 10^{-3}$ mol/dm^3)	mmol of L mmol of H (total)	pH range	log β_{011}	mmol of L mmol of H (after refinement)
8.75	0.0350	5.122–7.034	6.45(2)	0.03424
	0.0400			0.04299
8.75	0.0394	5.107–7.004	6.44(2)	0.03641
	0.0450			0.04710
9.50	0.0380	5.005–7.033	6.48(2)	0.03762
	0.0400			0.04413
9.50	0.0475	5.430–7.006	6.29(2)	0.04518
	0.0500			0.05381
13.1	0.0525	5.027–7.000	6.18(2)	0.04015
	0.0560			0.05380
13.1	0.0525	5.039–6.999	6.18(2)	0.04014
	0.0560			0.05369
13.1	0.0525	5.006–6.952	6.17(2)	0.04229
	0.0560			0.05433
13.1	0.0525	5.008–6.937	6.16(2)	0.04245
	0.0560			0.05443

$\beta_{011} = [LH]/[L][H]$. Standard deviations are in in parentheses. Temperature = 25°C; $I = 0.1$ (KNO_3), number of replicates = 3. SUPERQUAD was the program used.

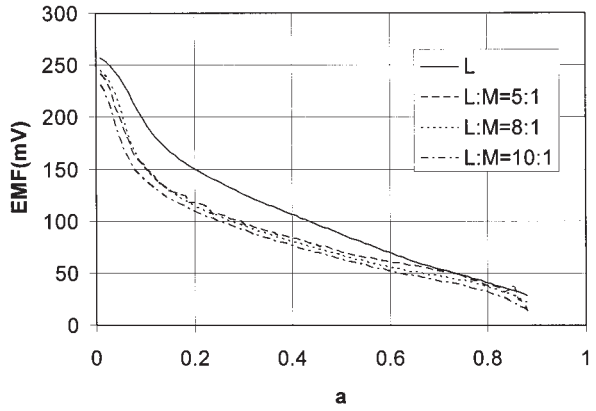

Figure 1 Potentiometric titration of MCCh with a deacetylation degree of 0.90 in the absence and presence of Ni(II). Molar a = mmol of NaOH/mmol of MCCh; C_{MCCh} = 1.31 $\times 10^{-2}$ mol/dm^3; temperature = 25°C; I = 0.1 (KNO_3).

evaluations were started at the neutralization of acid in excess and ended at the first notable measurement disturbances (related to precipitation). The lower a limit corresponded to pH values of 5.0–5.2, whereas the upper limit corresponded to pH values of 6.5–6.7.

L/M ratios were 5 : 1, 8 : 1, and 10 : 1, as an excess of L was necessary to diminish the role of M hydrolysis. At lower excesses of MCCh, the investigation of coordination modes was, as in the previous experiments with MCCh with a deacetylation degree of 0.87, distorted by the formation of sparingly soluble hydroxocomplexes.[17] The formation constant $M_{aq}OH$, known from the literature, was included in every equilibrium model.[20] The corresponding constant ($\beta_{mlh} = \beta_{10\text{-}1} = [MOH][H][M]^{-1}$) under the same (or close) ionic strength and temperature was set as −10.6 in the logarithm for Ni(II) and as −9.87 for Mn(II).

Of all of the potential coordination modes regarding electron-pair donation by the amino and hydroxyl groups (Fig. 3), the calculations confirmed first of all the formation of ML complexes via $—NH_3^+$ proton exchange (Table III). At the highest L excess (10:1), the second accepted species for both the M's was the ML_2 complex. In turn, only for Ni(II) at L/M = 5 : 1, the refinements confirmed chelation to five-membered rings thanks to a $O^- \rightarrow M$ donation from the deprotonated hydroxyl group.

The mean value of log β_{110} = 3.86 for Ni(II) was scarcely higher than for Mn(II) (3.745), but both of them were of the same order as the previously obtained mean log β_{110} constants of Co(II) (4.22) and Zn(II) (3.71) at comparable L/M and C_L values.[17] The comparison with those M's led to a lowering series: Co(II) > Ni(II) > Mn(II) ≥ Zn(II).

On the other hand, these results denied the possibility of the formation of a ML_2H_{-1} species, reported until now for Co(II) at high MCCh excess [but not for Zn(II)] and for Co(II) and Ni(II) with D-Glc.[17,23,24] The only comparison of these results with the data of monomeric D-Glc that could be made for Ni(II) was log β_{120} = 7.01 with MCCh with a deacetylation degree of 0.90 and log β_{120} = 6.73 at 25°C [I = 0.15 ($NaNO_3$)] with D-Glc.[24]

UV–vis absorption measurements with Ni(II)

Because of the d^5 configuration of the high-spin Mn(II), the very low molar absorption coefficients of Mn(II) ions implied an enhanced C_M and, by that, an enhanced C_L. The latter, however, was unfeasible because the solubility of MCCh was limited even in acidic media, and then, it becomes lower with increasing pH. Therefore, the only possible spectrophotometric measurements were carried out with Ni(II).

From the three spin allowed transitions of Ni(II), the most suitable in practice was the energetically highest $^3T_{1g}(P) \leftarrow {}^3A_{2g}$ ligand field transition with a maximum at about 400 nm. Starting from the $Ni(H_2O)_6^{2+}$ ion, we scanned consecutive spectra for solutions also containing initially acidified MCCh and appropriate amounts of sodium hydroxide (to attain the required pH; Fig. 4).

As a result of the relatively weak crystal field in d^8 Ni(II) and the known limited solubility of our MCCh, the concentration of Ni(II) had to be increased and the C_L correspondingly lowered. The optimum overall concentration of Ni(II) and chitosan amounted to 0.02625 and 0.0525 mol/dm^3, respectively. Thereby, the L/M ratio of 2 : 1 ratio was distinctly lower than in the potentiometric titrations, and the L excess could not been used up as a feature permitting a gain of higher pH and displacement of the equilibrium toward a higher degree of complexation. Nevertheless, as shown in Figure 5, up to pH 4 (3.99), the maximum absorbance increased with a concomitant hypsochromic shift in the maximum absorbance wavelength (λ_{max}) in relation to the $Ni(H_2O)_6^{2+}$ aqua ion (Table IV); this indicated an enhanced ligand field splitting in the central ion.

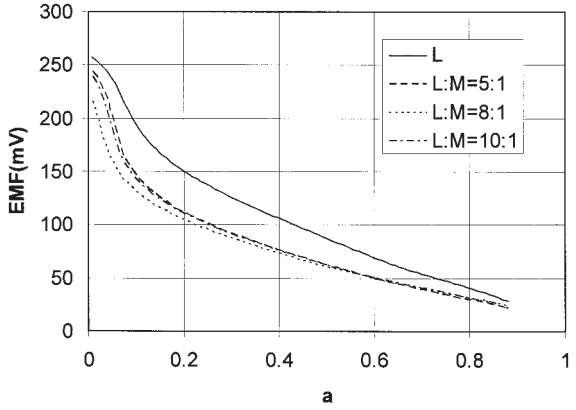

Figure 2 Potentiometric titration of MCCh with a deacetylation degree of 0.90 in the absence and presence of Mn(II). Molar a = mmol of NaOH/mmol of MCCh; C_{MCCh} = 1.31 $\times 10^{-2}$ mol/dm^3; temperature 25°C; I = 0.1 (KNO_3).

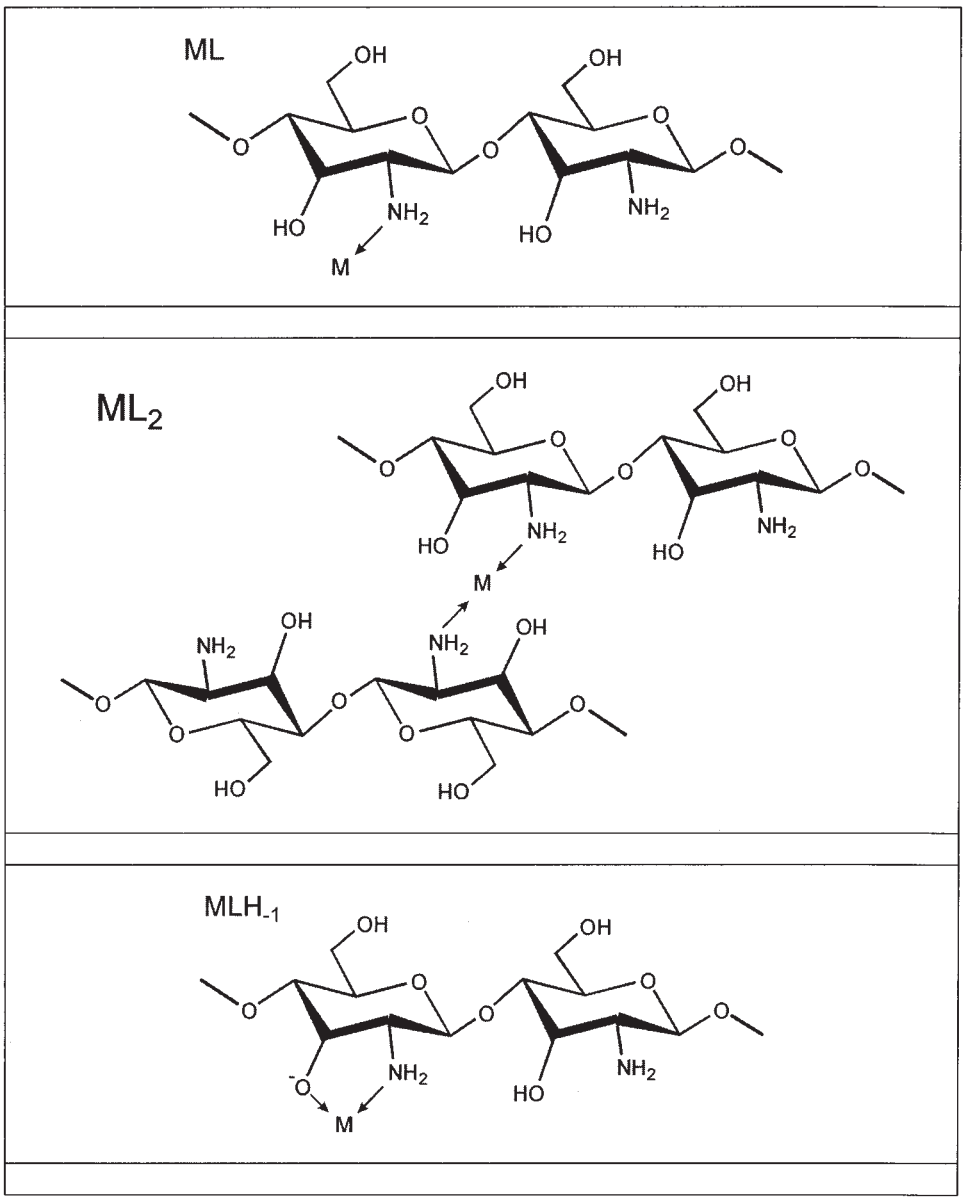

Figure 3 Dative modes found in the Mn(II)–MCCh and Ni(II)–MCCh systems (MCCh had a deacetylation degree of 0.90) in aqueous solutions.

Above pH values of 5.01, the precipitation (probably due mainly to hydrolysis of the M because the precipitation of MCCh alone was observed scarcely at below pH 7) made further spectrophotometric measurements useless; the absorbance fell off as a result of intensive light scattering.

CONCLUSIONS

MCCh deacetylated 90% may be regarded as a complexing agent toward Ni(II) and Mn(II) with features comparable with those reported previously for MCCh deacetylated 87% toward Co(II) and Zn(II). It may be assumed also that for this microcrystalline polymer

TABLE III
Refinement Results of Complex Formation Constants of Mn(II) and Ni(II) with MCCh with a Degree of Deacetylation of 0.90

M	C_L/C_M	log β_{110}	log β_{120}	log $\beta_{11\text{-}1}$
Mn(II)	5 : 1	3.74(2)	—	—
	8 : 1	3.75(4)	—	—
	10 : 1	—	6.91(2)	—
Ni(II)	5 : 1	3.78(3)	—	−1.03(6)
	8 : 1	3.80(4)	—	—
	10 : 1	4.01(3)	7.01(3)	—

β_{110} = [ML]/[M][L]; β_{120} = [ML_2]/[M][L]^2; $\beta_{11\text{-}1}$ = [MLH_{-1}][H]/[M][L]. Temperature = 25°C; I = 0.1 (KNO_3). SUPERQUAD was the program used. For each C_L/C_M, the constants were evaluated by means of comprehensive files consisting of n = 5 titration curves. Standard deviations are in parentheses.

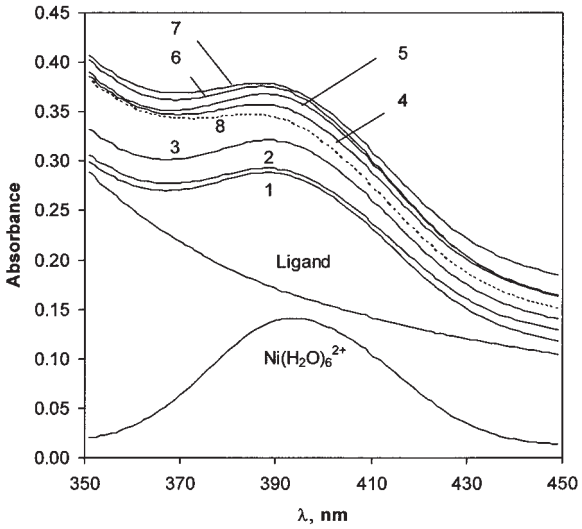

Figure 4 Absorbance changes in the selected $^3T_{1g}(P) \leftarrow {}^3A_{2g}$ band of Ni(II) during the titration of acidified MCCh with a deacetylation degree of 0.90 with 0.1*M* NaOH. pH = (1) 1.98, (2) 3.10, (3) 3.43, (4) 3.65, (5) 3.80, (6) 3.92, (7) 3.99, and (8) 5.01 (precipitation). C_M = 0.02625 mol/dm^3; C_L = 0.0525 mol/dm^3.

form, the mechanism of biodegradation in the living cell could be explained by interactions, among others, with biometals. Furthermore, the knowledge of M–L interaction modes may be involved in the controlled bioactivity of chitosan. In relation to the monomeric D-Glc unit, MCCh with a deacetylation degree of 0.90 created complexes of higher thermodynamic stability; at least, such a conclusion was drawn from the comparison of the ML_2 complex formation constants. Although the participation of one chitosan hydroxyl group was found for Ni(II) at lower excess of L, the rejection of MLH_{-1} (and also the ML_2H_{-1} and ML_2H_{-2} species) in other cases was quite elucidative; the needed higher pH could not be reached due to the

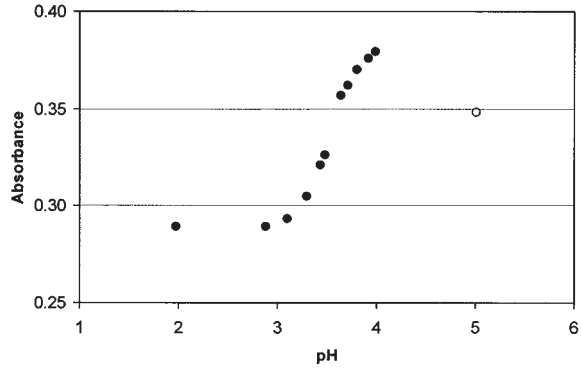

Figure 5 Values of λ_{max} versus pH during the spectrophotometric titration of MCCh with a deacetylation degree of 0.90 in the presence of $Ni(NO_3)_2$. C_M = 0.02625 mol/dm^3; C_L = 0.0525 mol/dm^3. Open circles represent precipitation.

TABLE IV
Changes in the Absorbance During External Spectrophotometric Titration (Selected Samples)

Sample number	pH	λ_{max} (nm)	Absorbance
$Ni(H_2O)_6^{2+}$	—	394.1	0.138
L + M			
1	2.88	389.0	0.289
2	3.10	388.0	0.293
3	3.43	386.9	0.326
4	3.65	384.9	0.357
5	3.80	386.9	0.370
6	3.92	386.9	0.376
7	3.99	386.9	0.379
8[a]	5.01	384.9	0.348

L was MCCh with a degree of deacetylation of 0.90. Number of replicates = 3; path length = 1 cm. The reference was H_2O.
[a] Light scattering due to precipitation.

aggregation of the polymer. It seems highly advisable that some additional studies on other kinds of MCCh's differing in their average molecular weight and/or deacetylation degree should be carried out to evaluate the influence of both of these parameters on their interactions with biologically relevant M's.

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