

# Interaction of Microcrystalline Chitosan (MCCh) with Mo(VI) in Aqueous Solution

Aleksander Kufelnicki,<sup>1</sup> Marta E. Lichawska,<sup>1</sup> Kazimiera H. Bodek<sup>2</sup>

<sup>1</sup>Department of Bioorganic and Biocoordination Chemistry, Laboratory of Physical and Biocoordination Chemistry, Faculty of Pharmacy, Medical University of Łódź, Łódź 90-151, Poland

<sup>2</sup>Department of Applied Pharmacy, Faculty of Pharmacy, Medical University of Łódź, Łódź 90-151, Poland

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**ABSTRACT:** The molybdenum(VI) complex formation equilibria of microcrystalline chitosan (MCCh) with three different degrees of deacetylation (DD = 0.750, 0.812, and 0.948) were studied by pH-potentiometric and UV-spectrophotometric methods. The hydrolysis constants of Mo(VI) were evaluated under exactly the same conditions as during the experiments in presence of chitosan. It has been shown that the complexation model depends on DD. Besides the formation of a ligand to metal 2 : 1 MoO<sub>2</sub>B<sub>2</sub><sup>2+</sup> complex ("bridge model") with A = MoO<sub>4</sub><sup>2-</sup>, B = MCCh, H = proton, the 1 : 1 MoO<sub>3</sub>B species ("pendant model")

has been confirmed as well for the two lower values of DD and moderate excess of ligand. At the highest DD, the results indicate only the occurrence of 2 : 1 species. When taking into account the number of protons in reaction as the third index, the corresponding average overall stability constants log  $\beta_{abh}$  of MoO<sub>3</sub>B and MoO<sub>2</sub>B<sub>2</sub><sup>2+</sup> reach log  $\beta_{112}$  = 13.8 and log  $\beta_{124}$  = 27.9, respectively. © 2009 Wiley Periodicals, Inc. *J Appl Polym Sci* 114: 1619–1625, 2009

**Key words:** microcrystalline chitosan; Molybdenum(VI); Metal-polymer complexes; equilibria in aqueous solution

## INTRODUCTION

Chitosan is a well known biopolymer which is composed of 2-acetamido-2-deoxy- $\beta$ -D-glucose (*N*-acetyl-D-glucosamine) and 2-amino-2-deoxy- $\beta$ -D-glucose (D-glucosamine) with various contents of both monosaccharides (Fig. 1). In other words, chitosan is a partially deacetylated derivative of chitin (poly *N*-acetylglucosamine), that naturally occurs in crustaceans. By converting the mass of D-glucosamine and *N*-acetyl-D-glucosamine to moles, we can use the percentage of D-glucosamine in the chitosan sample as an estimate of the so called degree of deacetylation (DD).<sup>1</sup>

Microcrystalline chitosan (MCCh) is a special form of chitosan which is prepared via its physicochemical modification using aqueous hydroxides and their salts.<sup>2</sup> After precipitation, the MCCh is degraded by enzymatic, hydrolytic, and oxidative methods to attain the desired average molecular weight and polydispersity.<sup>3</sup> So far, most pharmaceutical-formulation studies have been carried out using chitosans produced commercially by conventional methods.

Such chitosans, unlike MCCh, are fairly amorphous, as shown by powder X-ray diffraction patterns. MCCh also has favorable functional properties, e.g., a high water-retention value, which reflects the substantial surface area of MCCh. MCCh can retain three to four times as much of water as the parent chitosan, potentially increasing its capacity to form gels. Such a functional property could be of particular value in hydrogel-based slow-release formulations. Therefore, the effect of chitosan crystallinity on drug release seems to require evaluation. The bioadhesive properties of chitosan could make hydrogel-based MCCh formulations valuable as carriers for drugs with their sites of action in the stomach. The use of MCCh could offer advantages over other hydrophilic excipients, because gel formation by other excipients is regarded less efficient in markedly acidic environments such as the stomach. For example, a stable hydroxypropylmethylcellulose (HPMC) gel is formed over the pH range 3–11.<sup>4–6</sup>

The problem of interaction of chitin and chitosan (in both standard and substituted forms) with biologically relevant metal ions has been attracting more and more attention during the last years, parallel to the progress that has been observed in the methods of biocoordination chemistry.<sup>7–11</sup> At the same time, applications of chitosan as a chelating agent to metals and thus removing metal contaminations from water has become of great interest.<sup>12,13</sup> In

Correspondence to: A. Kufelnicki (akuf@ich.pharm.am.lodz.pl).

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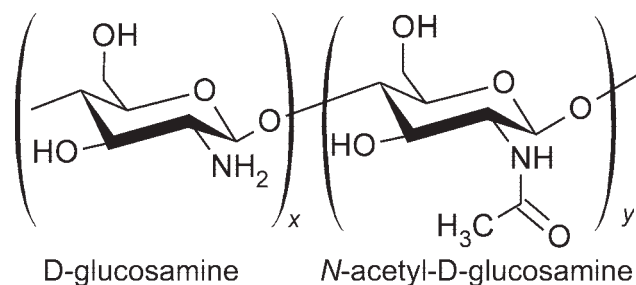


Figure 1 Structure of the two monomers of MCCh.

our previous studies, we applied MCCh gelatinous water dispersions of definite polymer contents and weight-average molecular weight up to  $2 \times 10^5$  Da. The complexing abilities toward such metal cations as Co(II), Zn(II), Cu(II), Mn(II), and Ni(II) have been described as well.<sup>14,15</sup> In the present study, we tried to assess the not described hitherto interactions of MCCh with molybdenum(VI) - one of the metals occurring in solutions as anions and, moreover, we paid special attention to the influence of DD degree on the complexing abilities of chitosan. The influence of DD on some other physicochemical behaviors has been already described.<sup>16,17</sup>

Molybdenum, generally assimilated in nature as Mo(VI) oxoanions ( $\text{MoO}_4^{2-}$ ), is an essential biometal, which functions as part of different enzymes (e.g., nitrogenases)—catalytic agents of numerous, live-important reactions. It is also a well established micronutrient for microorganisms, plants, and animals.<sup>18,19</sup> Both deficiency and excess of molybdenum may lead to definite loss in plant cultivations and to certain diseases in living populations. Thus, for instance, the delivery of molybdenum (VI) via complexes with MCCh, a well known biodegradable excipient, might be treated as one of the appropriate therapeutic possibilities.

## EXPERIMENTAL

### Materials

MCCh (weight-average molecular weight  $\bar{M}_w = 2.10^5$  Da, Institute of Biopolymers and Chemical Fibers, Łódź, Poland) was used in the form of hydrogel of definite polymer content (from 2.3 up to 3.2 wt %) at three different average degrees of DD: 75.0%, 81.2%, and 94.8%. The degree of DD, necessary to estimate the content of  $-\text{NH}_2$  groups in the samples, was determined by the method of first derivative UV-spectrophotometry (1DUVS) according to Khor and coworkers.<sup>1</sup> Molybdenum(VI) stock solution was prepared from  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$  (Riedl-de-Haën), P.A. The metal ion concentration was checked gravimetrically via precipitation of quinolin-8-olate. Carbonate-free  $0.1000 \pm 0.0003$  M

NaOH solution (Mallinckrodt Baker B.V.) was used as titrant. Other reagents, i.e., nitric acid and potassium nitrate were from grade pro analysis (P.A.)

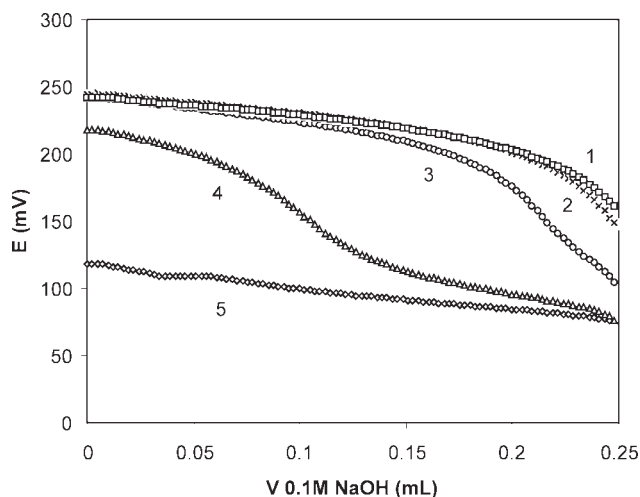
### Potentiometric titrations

A Molspin instrument (Newcastle upon Tyne, England) equipped with an OSH-10-10 combined electrode (METRON, Poland), and autoburette was used for EMF measurements. The total volume of the Hamilton microsyringe in the autoburette amounted to 500  $\mu\text{L}$ . The number of up to 100 titration points was attained by volume increments 5.0  $\mu\text{L}$ . The titration course was controlled by MOLSPIN software. All the measurements were carried out at  $25.0 \pm 0.1^\circ\text{C}$  and ionic strength 0.1 mol  $\text{L}^{-1}$  ( $\text{KNO}_3$ ). The cell was standardized according to IUPAC recommendations with two buffers: pH = 3.926 (potassium hydrogen phthalate 0.05 M + 0.05 M  $\text{KNO}_3$ ) and pH = 9.10 (disodium tetraborate 0.01 M + 0.07 M  $\text{KNO}_3$ ).<sup>20</sup> It follows then, that the buffer solutions were of the same ionic strength, medium and temperature as in the tested solutions. In addition, prior to each titration the electrode system had been calibrated in the  $-\log [\text{H}^+]$  scale by strong acid-strong base titrations, according to the procedure recommended by Irving et al.<sup>21</sup> In particular, 0.005 M  $\text{HNO}_3$  (adjusted to  $I = 0.1$  mol  $\text{L}^{-1}$  by adding  $\text{KNO}_3$ ) was neutralized with carbonate-free 0.1 M NaOH at temperature  $25 \pm 0.1^\circ\text{C}$ . The ionization product of water ( $pK_w$ ) under these conditions was 13.77 in our study and was in accordance with the references.<sup>22</sup> The values of standard electromotive force ( $E_0$ ), which also comprises the liquid junction potential, and slope ( $s$ ) from the equation  $E = E_0 - s \cdot 59.16(-\log [\text{H}^+])$  were evaluated by SUPERQUAD and HYPERQUAD2003.<sup>23-25</sup> The parameters, that were different from the ones obtained from the two-point cell standardization on pH, were then inserted into the input files of the programs SUPERQUAD and HYPERQUAD2003 to evaluate the overall, concentration formation constants:  $\beta_{abh} = [\text{A}_a\text{B}_b\text{H}_h] / [\text{A}]^a[\text{B}]^b[\text{H}]^h$ , where A =  $\text{MoO}_4^{2-}$ , B = MCCh, H = hydrogen (proton).

Goodness-of-fit was tested by two parameters:  $\sigma$  (connected with the objective function  $U$ ) and  $\chi^2$  statistics (test of randomness). When the observation errors are uncorrelated, the  $U$  function is defined for the number of experimental points  $n$  as:

$$U = \sum_{i=1}^n W_i r_i^2$$

where:  $W_i$  - weight of the  $i$ -th experimental point, calculated as  $1/\sigma_E^2$  when regarding only the estimate of error in emf ( $\sigma_E$ ) and when the estimate of



**Figure 2** Titration curves of the  $H^+$  - Mo(VI) system.  $C_{Mo(VI)}$  ( $mol\ L^{-1}$ ): 1— $6.769 \times 10^{-4}$ , 2— $8.461 \times 10^{-4}$ , 3— $1.354 \times 10^{-3}$ , 4— $3.385 \times 10^{-3}$ , 5— $6.769 \times 10^{-3}$ .

the error in burette reading  $\sigma_V$  is nearly equal to zero,  $r_i - i$ -th residual in emf ( $E_{exp} - E_{theoret}$ ).

The  $\sigma$  parameter is calculated from formula  $\sigma = \sqrt{U/(n - n_p)}$ , where  $n_p$  is the number of refined parameters (i.e., for instant the number of refined equilibrium constants). The critical values were assumed as  $\sigma < 3$ .

The statistical test of randomness  $\chi^2$  is based on the hypothesis that the residuals should follow a normal distribution. With this assumption the results are divided by magnitude into  $k = 8$  groups (classes). A  $\chi^2$  test is then applied to the  $n$  experimental points:

$$\chi^2 = \sum_{i=1}^k \frac{(n_i - np_i)^2}{np_i}$$

where:  $n_i$  - size of the  $i$ -th group,  $p_i$  - theoretical frequency of the  $i$ -th group,  $np_i$  - theoretical size of the  $i$ -th group.

If the distribution is approximately normal the value will be  $\chi^2 \leq 12.60$  at 0.95 confidence level.

### Spectrophotometric measurements

UV-visible absorption spectra were recorded on a Cary 50 Bio spectrophotometer, slit width, 1.5 nm, range, 200–400 nm. Silica cells of path length 1 cm and 0.5 cm were used depending on total metal concentration. A Peltier accessory was used to maintain the temperature constant at  $25 \pm 0.1^\circ C$ . Acidity of the solutions was changed directly in the cell by small aliquots of base (NaOH) or acid ( $HNO_3$ ). The pH was measured by means of a multifunctional microprocessor digital instrument CX-731 (ELME-

TRON, Poland) with a combined Inlab Micro (METTLER TOLEDO) electrode.

## RESULTS AND DISCUSSION

### Hydrolysis of Mo(VI)

As it has been stated previously molybdenum(VI) is able to form polyoxomolybdate ions in acidic medium whereas mononuclear species predominate in solution in the neutral and basic ranges and especially at very low concentrations ( $< 10^{-4} M$ ).<sup>26–31</sup> It has also been proved that most of the molybdenum is found in heptamolybdate ions at higher concentrations but there are also reports of octamolybdates and even higher polyoxomolybdates in the low pH region and in electrolytes of high ionic strength.<sup>30</sup> The protonation and condensation reactions that may occur in presence of the molybdate ion are represented by equation:



However, the constants determined previously referred to other (although sometimes quite similar to ours) experimental conditions. Hence, it was necessary to study the  $H^+$  -  $MoO_4^{2-}$  system under exactly the same conditions as in the measurements with MCCh carried out in the present work. The representative curves, obtained under titration conditions ensuring attainment of equilibrium, are presented in Figure 2.

The best fit between the calculated and measured curves was observed at medium concentrations of Mo(VI):  $2.5\text{--}5.0 \times 10^{-3}$  and  $3.385 \times 10^{-3}\ mol\ L^{-1}$  in particular. The species accepted here (from all the ones confirmed in the studies that had been carried out under similar conditions)<sup>26–28</sup> and their overall stability constants, are presented in Table I.

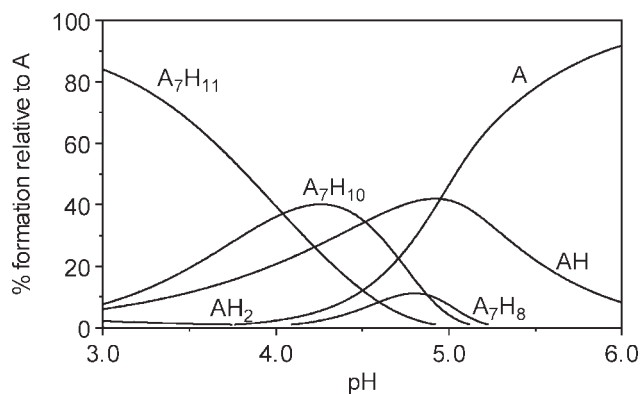
As it appears from the speciation diagram (Fig. 3), obtained by means of HySS2 (Protonic Software 2000)<sup>32</sup> upon the results of HYPERQUAD

**TABLE I**  
Equilibrium Model and Stability Constants ( $\log \beta_{mh}$ )  
of the  $H^+$  - Mo(VI) System

	$\log \beta_{mh}$
$HMoO_4^-$	4.95(2)
$H_2MoO_4$	7.51(10)
$H_8(MoO_4)_7^{6-}$	55.28(9)
$H_9(MoO_4)_7^{5-}$	<sup>a</sup>
$H_{10}(MoO_4)_7^{4-}$	65.03(3)
$H_{11}(MoO_4)_7^{3-}$	69.06(7)

$T = 25^\circ C$ ,  $I = 0.1\ mol\ L^{-1}$  ( $KNO_3$ ). Standard deviations in parentheses.

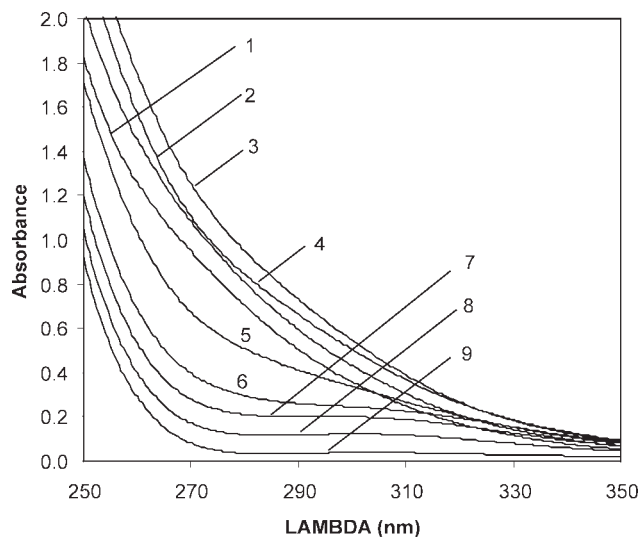
<sup>a</sup> The only one from the predicted species not accepted.



**Figure 3** Species distribution of the hydrolysis products formed in the  $\text{H}^+$  - Mo(VI) system in the pH range 3.0–6.0.  $\text{A} = \text{MoO}_4^{2-}$ .

refinements, the predomination of polyoxomolybdates occurs in a more acidic medium. At the same time, the only essential monomeric hydrolytic product of  $\text{A} = \text{MoO}_4^{2-}$  occurring at pH 5 is  $\text{HMoO}_4^-$  (AH). The  $\text{AH}_2$  product, i.e., the neutral monomeric molybdic acid  $\text{H}_2\text{MoO}_4$ , is found in a scarce amount above pH 3.0.

The UV absorption spectra recorded for this system (Fig. 4) within the wide pH range 1.79–10.73 show an increase in absorbance starting from pH 1.79 up to 3.52 due to the known higher molar absorptivity of the monomeric hydrolytic species ( $\text{HMoO}_4^-$ ).<sup>33</sup> Then at higher pH (>4) the arising share of the  $\text{MoO}_4^{2-}$  ion, absorbing only below 250 nm<sup>34</sup> due to the  $d$  charge transfer transitions, leads to an evident decrease in absorbance within 250–350 nm.

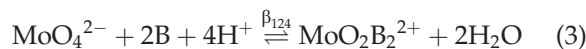
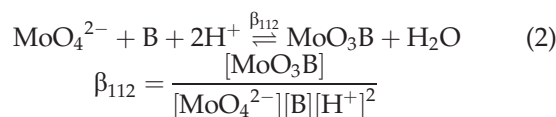


**Figure 4** UV spectra of the  $\text{H}^+$  - Mo(VI) system.  $C_{\text{Mo(VI)}} (\text{mol L}^{-1}) = 1.2 \times 10^{-3}$ . Reference: 0.024 M  $\text{HNO}_3$ . Path length: 0.5 cm. pH: 1–1.79; 2–2.14; 3–3.52; 4–4.21; 5–4.61; 6–5.00; 7–5.58; 8–7.18; 9–10.73.

### Complexation with Mo(VI)

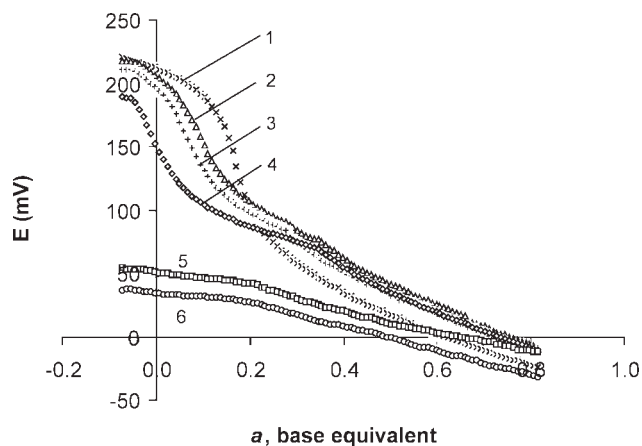
The titrations in the presence of MCCh (for each of the DD) were carried out within the range of ligand neutralization coefficient (base equivalent)  $a = \text{mmol of base/mmol of ligand} = -0.1$  to 0.8. The experiments were started with neutralization of the excessive mineral acid and ended with the occurrence of the first notable disturbances related to precipitation (representative titrations are shown in Fig. 5).

The complexation model under consideration comprised both the possibility of attaching a single amino group of B (i.e., MCCh) by one metal anion (reaction 2) and the possibility of coordinating two amino groups (reaction 3):



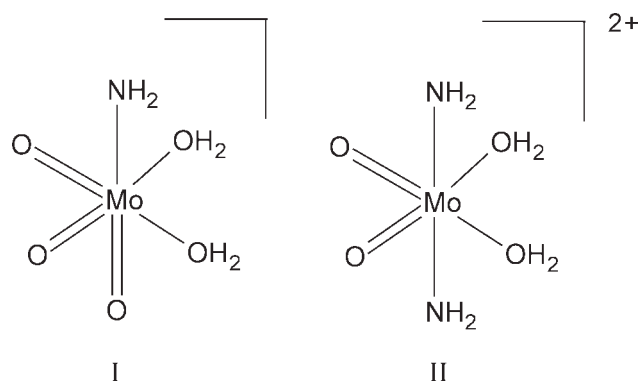
$$\beta_{124} = \frac{[\text{MoO}_2\text{B}_2^{2+}]}{[\text{MoO}_4^{2-}][\text{B}]^2[\text{H}^+]^4}$$

It seems that hydrogen ions present in the solution enable the complexation due to displacement of one or two oxygen atoms of  $\text{MoO}_4^{2-}$  by one or two amino groups. According to the previously presented Figure 1, the two amino groups originate most probably from different polymer chains (Fig. 6,



**Figure 5** Titration of the  $\text{H}^+$  - Mo(VI) - MCCh (DD = 0.812) system.  $C_{\text{MCCh}} (\text{mol L}^{-1}) = 7.0 \times 10^{-3}$ . The value of base equivalent  $a = -0.1$  corresponds to  $\text{HNO}_3$  in excess as related to ligand. Curves: 1 - MCCh in absence of Mo(VI), 2 - MCCh : Mo(VI) = 10 : 1, 3 - MCCh : Mo(VI) = 8 : 1, 4 - MCCh : Mo(VI) = 5 : 1, 5 - MCCh : Mo(VI) = 2 : 1, 6 - MCCh : Mo(VI) = 1 : 1.





**Figure 6** Coordination modes of Mo(VI) with amino groups of MCCh.

mode II), as otherwise the complexation would require formation of highly stressed poly-membered rings.

Apart from the equilibria mentioned above, the calculations for all the degrees of DD involved protonation of MCCh under the same conditions as in the experiments with the metal (i.e.,  $\log \beta_{011} = 6.50$ , which is in accordance with our previous data and also with reports from the literature).<sup>14–16</sup> All the hydrolysis reactions of Mo(VI) were taken into account as it was mentioned in the previous section. The calculations were made for titrations with chitosan excess (5 : 1, 8 : 1, and even 10 : 1) because hydrolytic reactions overwhelmed the complexation equilibria at lower ligand to metal ratios. As a result, the pH-metric titration curves could be fitted very well if the species  $\text{MoO}_3\text{B}$  (Fig. 6, mode I) and  $\text{MoO}_2\text{B}_2$  (Fig. 6, mode II) were considered besides the protonated form of MCCh and the hydrolytic products of Mo(VI). The refined overall constants are presented in Table II. As it can be seen, both the complexes were accepted at lower DD (0.750 and 0.812) but not at the highest excess of chitosan. At such a high excess of ligand the total concentration of molybde-

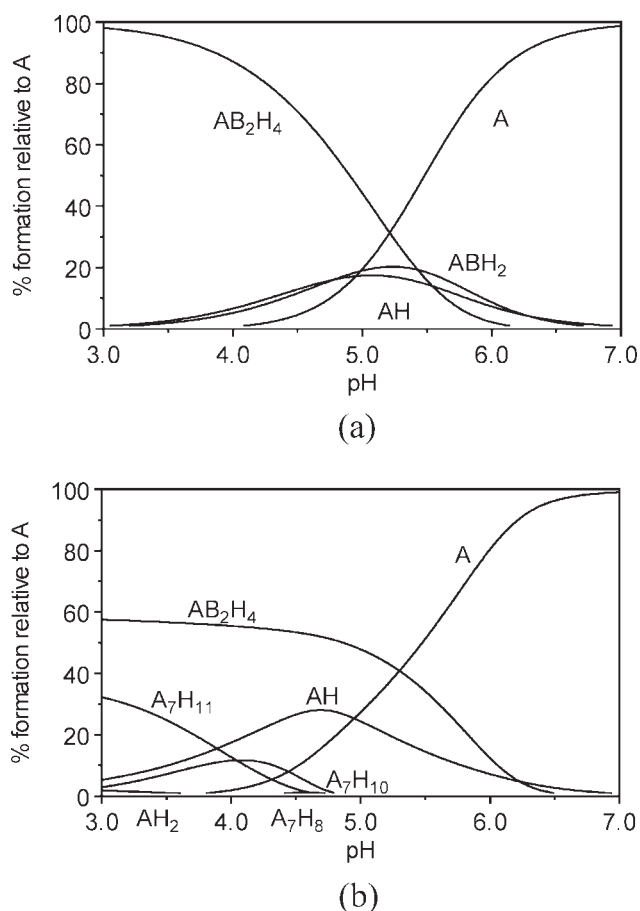
Degree of DD	$C_L/C_M^a$	$\log \beta_{112}$	$\log \beta_{124}$	pH
0.750	5 : 1	14.19(6)	28.16(9)	3.42–7.20
	8 : 1	13.85(11)	27.55(10)	3.03–7.02
	10 : 1	–	–	3.00–7.31
0.812	5 : 1	13.83(5)	28.35(2)	3.65–6.85
	8 : 1	13.45(10)	27.78(3)	3.14–6.97
	10 : 1	–	27.69(3)	3.02–6.93
0.948	5 : 1	–	28.55(8)	3.87–6.55
	8 : 1	–	27.63(5)	3.53–6.93
	10 : 1	–	27.78(16)	3.22–6.60

$T = 25^\circ\text{C}$ ,  $I = 0.1 \text{ mol L}^{-1}$  ( $\text{KNO}_3$ ). Standard deviations in parentheses.

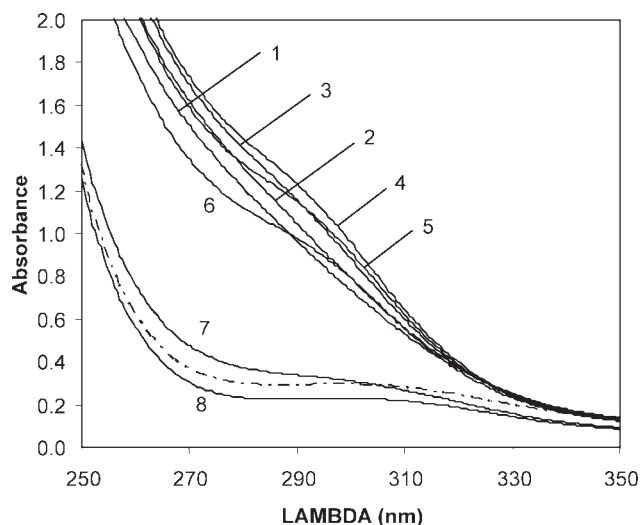
<sup>a</sup>  $C_L$  = total concentration of ligand;  $C_M$  = total concentration of metal.

num was the lowest, hence owing to low accessibility of amino groups at DD 0.750 the complexation has not been confirmed. In case of the highest DD (0.948), the refinements supported only the II type complex, probably due to higher accessibility of the  $-\text{NH}_2$  groups. Thus, the “bridge model” with the metal coordinating to amine groups of two neighboring polymer chains seems to be the most attractive one. However, the “pendant model” with a single metal ion attached as pendant to one amine group of chitosan is justified for mode I.

The concentration distribution curves obtained by means of HySS2 indicate unequivocally that the formation of polyoxomolybdates is completely hindered at the excess of chitosan [Fig. 7(a)]. The ligand to metal 2 : 1 complex ( $\text{MoO}_2\text{B}_2 = \text{AB}_2\text{H}_4$ , according to reaction (3)) is formed in acidic solution already at the beginning of titration. Then, at higher pH (>5) an essential share of the 1 : 1 species ( $\text{MoO}_3\text{B} = \text{ABH}_2$ , according to reaction (2)) can be observed besides the only one hydrolytic product confirmed AH (i.e.,  $\text{HMoO}_4^-$ ). On the contrary, the competition between metal complex formation and protonation



**Figure 7** Species distribution of the complexes formed in the  $\text{H}^+$  - Mo(VI) - MCCh (DD = 0.812) system at ligand to metal ratios: (a) 5 : 1 and (b) 2 : 1. A =  $\text{MoO}_4^{2-}$ . B = MCCh.  $C_{\text{MCCh}}$  ( $\text{mol L}^{-1}$ ) =  $7.0 \times 10^{-3}$ .



**Figure 8** UV spectra of the  $\text{H}^+$  - Mo(VI) - MCCh (DD = 0.812) system at ligand to metal ratio 5 : 1.  $C_{\text{Mo(VI)}} (\text{mol L}^{-1}) = 1.2 \times 10^{-3}$ . Reference: 0.024 M  $\text{HNO}_3$ . Path length 0.5 cm. pH: 1—1.76; 2—2.12; 3—3.06; 4—3.66; 5—4.57; 6—4.95; 7—5.43; 8—6.13. Dashed line corresponds to precipitation (pH = 6.58).

reactions is distinctly visible at a lower excess of MCCh [Fig. 7(b)]. In this case, however, the  $\text{ABH}_2$  complex ( $\text{MoO}_3\text{B}$ ) could not be evidenced due to its relatively low concentration.

Although, except for coordination via  $-\text{NH}_2$ , we took into account the possibility of a metal promoted deprotonation of the chitosan  $-\text{OH}$  group and interaction of Mo(VI) with  $\text{O}^-$  (similarly as in some other metals),<sup>10,11,14,15</sup> the fitting procedures rejected the corresponding equilibria.

Unlike the spectra in the absence of the ligand, the UV absorption spectra (Fig. 8) were recorded up to pH  $\sim 6.5$  because the precipitation of chitosan notably disturbed the measurements at higher pH. Starting from pH 1.76, we observed an increase in the absorbance and then a distinct inflection occurred at ca 290 nm. From pH 3.06 up to 4.95, and most of all at 3.66 the inflection was best visible (apparently, as compared with Figure 7(a), due to arising share of the 1 : 1  $\text{ABH}_2$  complex with MCCh and the monomeric hydrolytic species AH). At the same time, however, the absorbance decreased with the rise in pH for the same reasons as in the absence of chitosan (i.e., the arising share of  $\text{MoO}_4^{2-}$ , absorbing only below 250 nm). Above pH  $> 5$ , the absorbance decreased distinctly and at pH  $> 6$ , we observed a notable precipitation resulting in measurement disturbance. It is worth noticing that the course of spectral changes is similar but the precipitation is observable at lower pH (already 5.5) for MCCh of highest DD (0.948), thus explaining the previous potentiometric results (lack of the 1 : 1  $\text{MoO}_3\text{B}$  complex, just mainly occurring in this region).

## CONCLUSIONS

Our results indicate that MCCh interacts with molybdenum(VI) in acidic aqueous solution. In excess of the ligand (total concentration ratio 5 : 1 and 8 : 1) two types of metal complexes are confirmed:  $\text{MoO}_3(\text{MCCh})$  - that may be assigned as "pendant model" and  $\text{MoO}_2(\text{MCCh})_2^{2+}$  - apparently the "bridge model" (Fig. 6). The species decompose at pH ca 6.0. However, the molybdenum binding modes depend on the DD of the polymer. At lower DD (0.750 and 0.812), the equilibrium system comprises both species with predomination of  $\text{MoO}_2(\text{MCCh})_2^{2+}$  whereas at DD 0.948, the  $\text{MoO}_2(\text{MCCh})_2^{2+}$  complex is, in practice, the only one present in the solution. Such a result could be explained by the accessibility of amine groups in the highly deacetylated polymer which favors the coordination to two neighboring polymer chains. An additional observation results from the highest total ligand to metal concentration ratio 10 : 1. At such a significant deficiency of the metal it seems quite understandable that the "bridge model" concerning complexes of higher thermodynamic stability, is more preferred.

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