Protolytic and Complexing Properties of Microcrystalline Chitosan with Co(II), Zn(II), and Cu(II) Ions

KAZIMIERA HENRYKA BODEK* and ALEKSANDER KUFELNICKI

Institute of Chemistry, Faculty of Pharmacy, Medical Academy of Łódź, 90-151 Łódź, Poland

SYNOPSIS

It has been evidenced that microcrystalline chitosan (MCCh) with a determined (by the potentiometric method in nonaqueous medium) degree of deacetylation of 0.87 acts as a polymeric chelating agent with cobalt(II) and zinc(II). The predomination of hydrolytic reactions, in the case of copper(II) ions, has been attributed to a change in crystalline character of the ligand. The protolytic reactions have been studied using the Katchalsky–Spitnik equation. The pH profiles have been shown for Co(II)–MCCh and Zn(II)–MCCh systems and the corresponding equilibrium constants have been determined as well. Both the amino and hydroxyl groups are involved in Co^{2+} complexation, whereas for Zn^{2+}, the complexes are formed only via amino nitrogen. © 1995 John Wiley & Sons, Inc.

INTRODUCTION

Microcrystalline chitosan (MCCh) is a new physical form of the standard chitosan of poly(2-deoxy-2-amino-glucose), the natural polymer derived by deacetylation of chitin–poly(2-deoxy-2-acetyloamino-glucose)[1] (Scheme 1). The new form retains its microcrystalline character above pH > 6.5. In acidic medium, MCCh dissolves and forms a solution of glucosamine macromolecules. Owing to the specific properties, MCCh behaves in a different way during alkalization than does the parent material, Pandalus (Vadsø, Norway). MCCh reveals a high superiority in comparison with standard chitosan,[2] e.g., in respect of the ability for powerful hydrogen-bond formation,[3] water-retention value (500–5000% vs. max. 150%), direct film-forming from a dispersion, as well as high metal chelating ability, controlled bioactivity, and biodegradability.[4] Besides the numerous applications in various branches of the chemical, textile, or food-processing industries and environment protection, chitosan is used as an auxiliary substance in pharmaceutical technology.[5,6] It is a drug carrier of controlled activity, but it also acts in definite directions,[7–9] showing mucoadhesive properties,[10] biostimulatory activity, and capability of tissue reconstruction and is used in hydrocolloids, gels, microspheres, tablets, and other formulations.

Taking into consideration the quite well-known complexing abilities of standard chitosan with first-row transition metals of the living systems, it seemed to be necessary to perform the appropriate studies with the microcrystalline form, not carried out hitherto. Our observations and calculation results were also related to the results known for the corresponding monomeric form of *N*-glucosamine.

EXPERIMENTAL

Materials and Methods

Microcrystalline chitosan (MCCh) in the form of gelatinous water dispersion with a polymer content of 1.5% wt, water retention value (WRV) of 678%, average molecular weight of $M_w = 0.27 \times 10^5$, and deacetylation value of 87% was prepared according to the original method[1] elaborated at the Institute of Chemical Fibres (Łódź, Poland).

The degree of deacetylation (DD, 87%) was determined potentiometrically using an N-5170 pH meter by titration in nonaqueous medium (anhydrous acetic acid and 1,4-dioxane, both from POCh, Gliwice). The glass electrode (kept in an anhydrous acetic acid for 12 h before use) and a calomel elec-

* To whom correspondence should be addressed.

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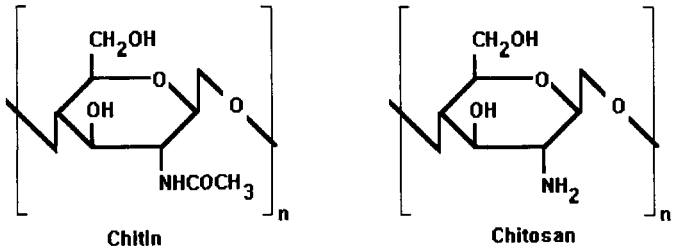


Scheme 1

trode of modified type were joined by a salt-bridge, where a saturated solution of lithium chloride in acetic acid was used instead of potassium chloride. A modified type of calomel electrode was applied to eliminate fluctuation of the diffusion potential. During determinations in nonaqueous media, the aqueous potassium chloride solution in the calomel electrode interacts with the solvent and the potential undergoes changes, due to the evolution of heat.[11] Weighed amounts of chitosan (ca. 1 g) were dried at 30°C to a constant mass of ca. 0.015 g and then dissolved in anhydrous acetic acid with a 1 : 1 addition of 1,4-dioxane. Mixing the neutral solvent of low dielectric constant (1,4-dioxane) with an acidic solvent (acetic acid) increases the sensitivity of the titration endpoints.

The chitosan samples were titrated with perchloric acid (Analar) in 1,4-dioxane (0.1178 mol/dm^3). The distinct inflection (endpoint) in the titration curves of SEM vs. the titrant volume enabled determination of the summary content of amino groups and then the degree of deacetylation (DD) from the formula

$$DD = \frac{[-NH_2]_{determ.}}{[-NH_2]_{theoret.}}$$

The equilibria in solutions containing chitosan were examined in a double-walled vessel thermostatted at 25 ± 0.1°C, under an argon atmosphere. The vessel of 50 cm^3 volume was equipped with a combined SAgP-201W electrode. The used sodium hydroxide, potassium nitrate, and nitric acid were ppa (POCh, Gliwice). The electrode was standardized with two buffer solutions (6.50 ± 0.02 Radiometer and 4.00 ± 0.02, phtalate) and then calibrated on the pH = $f(-\log[H^+])$ scale by the method of Irving.[12]

The protonation constants of MCCh were determined at various concentrations within the range ($2.22 \times 10^{-3} - 2.22 \times 10^{-2}$) mol/dm^3. The com-

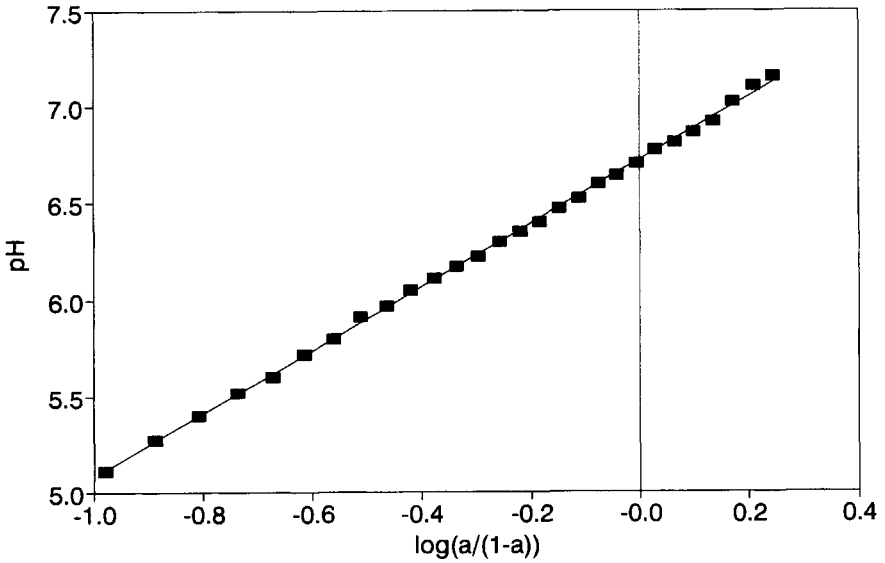


Figure 1 Graphical presentation of the Katchalsky–Spitnik equation for microcrystalline chitosan [0.87]. The sample contained 0.1 mmol of MCCh; initial volume: 45 cm^3; temp 25°C.

Table I Calculated Amino Group Protonation Constant of Microcrystalline Chitosan[a]: Temp 25°C, μ = 0.1(KNO_3)

MCCh (mmol)	log K_H	n[b]
0.1	6.720 (0.004)	1.638 (0.010)
0.2	6.401 (0.006)	1.321 (0.014)
0.4	6.382 (0.011)	1.398 (0.019)
0.5	6.603 (0.013)	1.771 (0.023)
0.6	6.436 (0.015)	1.632 (0.022)

[a] The standard deviations are in parentheses.
[b] Empirical coefficient in eq. (2).

plexation equilibria were studied in the presence of cobalt(II), zinc(II), and copper(II) nitrate salts, ppa (all purchased from POCh, Gliwice). The stock solutions (0.2 mol/dm^3) were standardized with sodium versenate. The metal concentrations in the titrated samples ranged from 2 to 4 $\times$ 10^{-3} mol/dm^3. The ligand-to-metal concentration ratio varied from 1 : 1 to 10 : 1.

The UV/vis absorption spectra were taken on a Specord M-40 (Zeiss) spectrophotometer equipped with a thermostatted cell compartment. Two types of spectrophotometric titrations were applied—an internal titration where the sample was placed directly in the measuring cell and an external one where definite pH values were settled in a number of measuring flasks. The first titration type was used in the case of cobalt due to relatively high concentrations required (2.5–5.0 $\times$ 10^{-2} mol/dm^3), whereas the second type was applied to copper complexes.

The computer programs QuattroPro4.0 and SUPERQUAD were purchased from Borland International, Inc. (U.S.A.) and the Protonic Software (Leeds, U.K.), respectively.

RESULTS AND DISCUSSION

Protolytic Reactions

The protonation constant of the chitosan amino groups, K_H, was determined for the equilibrium

$$-NH_2 + H_3O^+ \overset{K_H}{\rightleftharpoons} -NH_3^+ + H_2O \qquad (1)$$

in the absence of metal, but, also, besides the complexation constants (in the presence of metal), the latter method is discussed in the ensuing part of the present article.

The strong electrostatic interaction exerted by the constitutional units adjacent to given amino groups makes it impossible to apply the typical calculation methods, suitable for monomeric compounds.[13] Hence, a much lower buffer capacity may be observed (appearing itself in a lower slope of the titration curve in the buffer zone).[14]

To determine the amino group protonation constant, a modified Henderson–Hasselbalch equation was applied, in the form known as the equation of Katchalsky–Spitnik.[15] The latter equation may be expressed in relation to the protonation constant as

$$pH = \log K_H - n \log[a/(1 - a)] \qquad (2)$$

Table II Rafination Results of the Protonation Constant and Complex Formation Constants of Microcrystalline Chitosan [0.87] on the Basis of Potentiometric Data: Temp 25°C, μ = 0.1(KNO_3); Program: SUPERQUAD

	mmol L / mmol M	log β_{011}	log β_{110}	log β_{120}	log $\beta_{12\text{-}1}$
Co	0.1/0.1	7.02 (0.06)	3.26 (0.11)	—	—
	0.2/0.1	7.13 (0.03)	3.61 (0.04)	—	—
	0.4/0.1	7.81 (0.02)	4.68 (0.03)	8.29 (0.04)	1.86 (0.06)
	0.5/0.1	7.47 (0.02)	4.48 (0.02)	7.63 (0.08)	1.55 (0.06)
	0.6/0.1	7.30 (0.01)	4.29 (0.02)	7.10 (0.02)	1.12 (0.04)
	0.8/0.1	6.80 (0.01)	4.18 (0.05)	7.10 (0.02)	1.02 (0.08)
	1.0/0.1	6.66 (0.01)	3.94 (0.06)	7.01 (0.05)	0.92 (0.11)
Zn	0.4/0.1	6.82 (0.04)	3.53 (0.03)	—	—
	0.6/0.1	6.31 (0.01)	4.03 (0.03)	—	—
	0.8/0.1	6.32 (0.03)	3.51 (0.08)	5.40	—
	1.0/0.1	6.36 (0.01)	3.77 (0.06)	6.47 (0.08)	—

$\beta_{MLH} = [M_mL_lH_h]/[M]^m[L]^l[H]^h$. Standard deviations in parentheses.

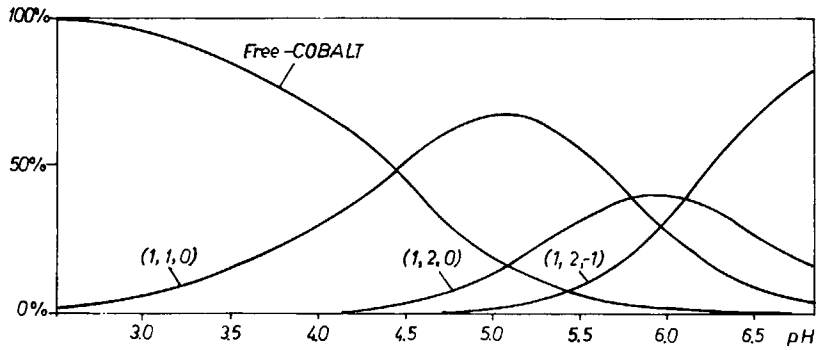


Figure 2 Species distribution diagram in the Co(II) —MCCh [0.87] system. $C_{Co} = 2.22 \times 10^{-3}$ mol/dm^3; $C_L = 1.776 \times 10^{-2}$ mol/dm^3.

where a is the degree of neutralization of the amino groups, and n, an empirical parameter related to the free-energy change during titration.

An exemplary plot of the linear dependence as well as the results of calculations carried out at various concentrations of MCCh [0.87] by means of the QuattroPro worksheet are presented in Figure 1 and Table I. As follows from Table I, the log K_H values did not show any undirectional shift. The average of log $K_H = 6.5 \pm 0.2$ was consistent with the intrinsic constant, independent on the degree of deacetylation, reported by Domard.[16] On the other hand, the n coefficient ranged within 1 and 2, which is typical of polymeric compounds. The log K_H value of 6.5 is of one order of magnitude lower than is the corresponding constant for *N*-glucosamine of 7.47 (Ref. 17) or 7.70 (Ref. 18), being also a result of electrostatic hindrance in the protonation of the $-NH_2$ groups.

The titrations could be continued only up to pH $\approx$ 7, as above this pH, the chitosan solutions indicated precipitation, thus making it impossible to investigate the basic region, where the second, hydroxyl group dissociation constant of the glucosamine unit might be expected.

Complexing Reactions

Besides the above-mentioned classical method of Katchalsky–Spitnik, the determination of the protonation constant was possible also simultaneously with the complexing equilibria. A multidimensional nonlinear regression by means of the least-squares method has been applied in the SUPERQUAD computer program.[19] Apart from equilibrium constants, rafination was related to another regression parameter—the total concentration of hydrogen ions. The calculations indicated a perfect fit of the theoretical model to the experimental data for some titrations with cobalt(II) and zinc(II), in particular, at higher ligand excess (Table II).

In the case of cobalt(II), the results confirmed the formation of complexes of three types: ML, ML_2 with amino nitrogen donation, and $ML(LH_{-1})$ with participation of both amino nitrogen and hydroxyl oxygen (Fig. 2). In turn, for zinc(II), the metal was coordinated only by the amino nitrogen (Fig. 3). In comparison with *N*-glucosamine, the corresponding stability constants with Co(II) are distinctly higher (i.e., for GlcN: log $\beta_{120} = 4.09$, log $\beta_{12-1} = -3.89$; the β_{110} constant has not been reported) (Scheme 2).

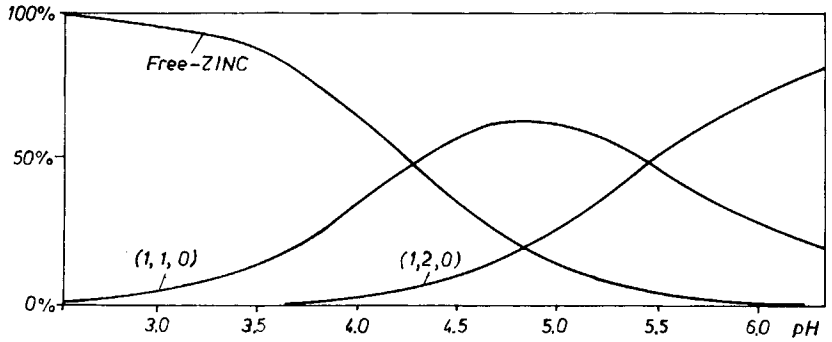
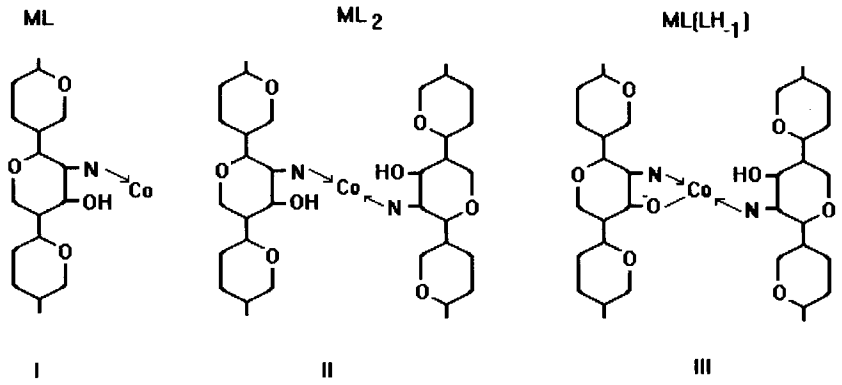


Figure 3 Species distribution diagram in the Zn(II) —MCCh [0.87] system. $C_{Zn} = 2.22 \times 10^{-3}$ mol/dm^3; $C_L = 2.22 \times 10^{-2}$ mol/dm^3.



Scheme 2

As can be seen in Table II, the protonation constants were enhanced in the titrations with moderate ligand excess, reaching the values close to the ones of monomeric *N*-glucosamine. This result is most likely due to the predomination of structures **II** and **III** with a restricted electrostatic repulsion near the metal center.[20]

The titration curves in the case of copper(II) (Fig. 4) were clearly different from the ones of other metals. The plateau at pH $\approx$ 5, following the first increasing part of the curve, corresponded to the increasing turbidity of the solution. The calculations by means of SUPERQUAD confirmed the bis hydroxo-complex of the $Cu(II)(NH_2)(OH)_2$ type described by Domard[16] as the only justified complex species, but the statistical parameters of the goodness-of-fit test were unsatisfactory.

The latter result may be explained based on previous reports[21] in terms of the change in crystallinity of MCCh. Owing to the diminished accessibility of amino groups, following the formation of the macromolecule arrangement of glucosamine, the preponderance of sparingly soluble hydroxo-complexes may take place—for Cu(II), those species are of 2–3 orders of magnitude more stable than for the remaining metals. On the contrary, it is well known, however, that *N*-glucosamine and also the partially *N*-acetylated standard chitosan form complexes with copper(II).[13,18,22,23]

Spectrophotometric Measurements

Owing to the limited solubility of the used polymeric ligand (the solubility decreases with increase in pH), only a quantitative confirmation of complex formation with Co(II) was possible (Fig. 5). The blue-appearing precipitate of cobalt(II) hydroxide vanished as the sample in the cell was alkalized with consecutive portions of NaOH and then the pink color of the sample deepened. At the same time, the observed weak turbidity decreased. The spectral curves were due to the superposition of the ligand field absorption bands and the enhanced absorbance resulting from light scattering on the not entirely transparent sample. Hence, the increase in absorbance at $\sigma \sim 20 \times 10^3$ cm^{-1} [originating mainly from the $^4T_{1g} \rightarrow {}^4T_{1g}(P)$ transition in the quasi-octahedral field with an admixture of forbidden transitions to doublet states] was diminished by a value resulting from the loss of uncomplexed MCCh.

In turn, in the spectrophotometric measurements with copper(II), the increase in pH was accompanied by a constant rise in absorbance within the visible range (13–15 $\times$ 10^3 cm^{-1}) where the characteristic $^2T_{2g} \rightarrow {}^2E_g$ band (in quasi-octahedral symmetry) is observable (Fig. 6). In spite of the increasing turbidity, a slight hypochromic shift indicated an increase in the ligand field strength, which corresponds to the formation of hydroxo-complexes (as the water molecules in the coordination sphere are substituted by hydroxyl ions, which are stronger σ-donors).

CONCLUSIONS

Independently of the transformations in acidic medium, microcrystalline chitosan is an active chelating ligand in relation to cobalt(II) and zinc(II) at a pH corresponding to physiological conditions and a hydrolizing agent to copper(II). Both these features are involved in the mechanisms of biodegradation of the polymeric structure in the living system. Thus, the presented results may confirm the previously established superiority of microcrystalline chitosan, including also biodegradability, in comparison with the standard form of chitosan.

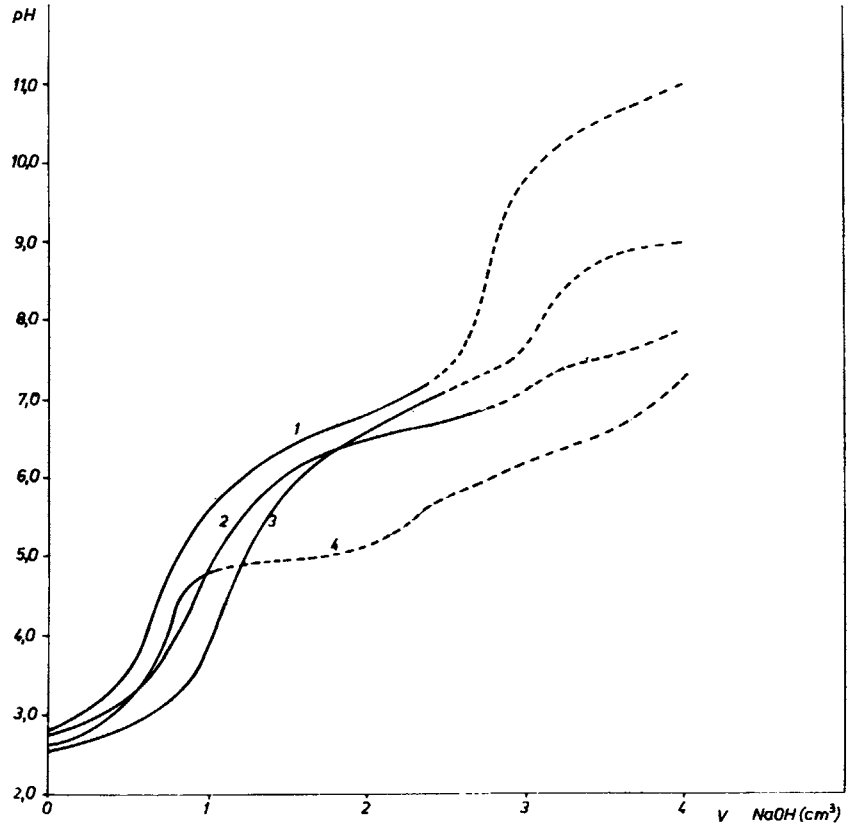


Figure 4 Titration curves of microcrystalline chitosan [0.87] (1) in the absence of metal and in the presence of metal; (2) Zn; (3) Co; (4) Cu. $C_M = 2.22 \times 10^{-3}$ mol/dm^3; $C_L = 4.44 \times 10^{-3}$ mol/dm^3. (-----) precipitation.

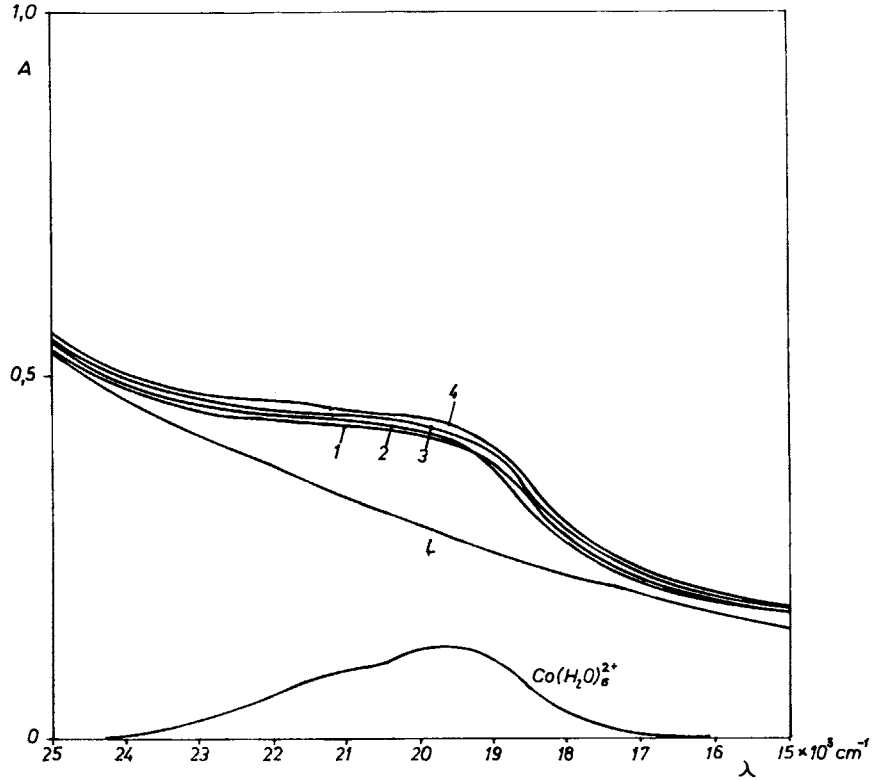


Figure 5 Internal spectrophotometric titration of MCCh [0.87] in the presence of $Co(NO_3)_2$. $C_{Co} = 2.5 \times 10^{-2}$ mol/dm^3; $C_L = 1.0 \times 10^{-1}$ mol/dm^3. Initial volume: 3.0 cm^3. Molar neutralization coefficient (a): (1) 0.000; (2) 0.222; (3) 0.462; (4) 0.884.

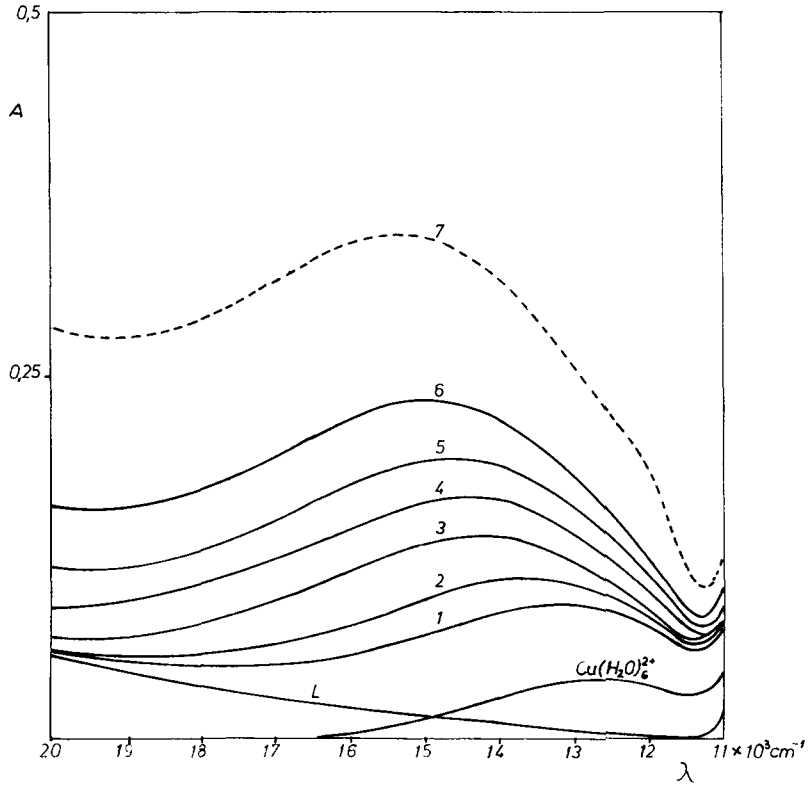


Figure 6 External spectrophotometric titration of MCCh [0.87] in the presence of $Cu(NO_3)_2$. $C_{Cu} = 4.0 \times 10^{-3}$ mol/dm^3; $C_L = 8.0 \times 10^{-3}$ mol/dm^3. Initial volume: 25 cm^3. Curves: (1) pH 2.6; (2) pH 3.8; (3) pH 4.5; (4) pH 4.8; (5) pH 5.1 (opalescence); (6) pH 5.5 (opalescence); (7) pH 5.7 (precipitation).

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