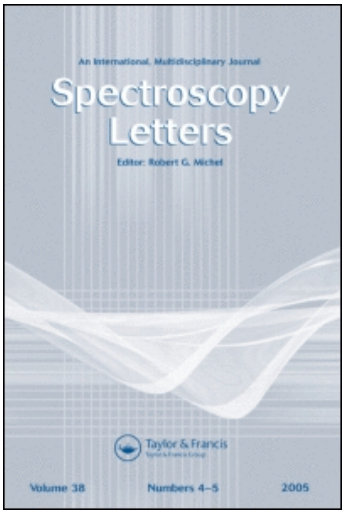

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Spectrophotometric and 27-Al NMR Characterization of Aluminum(III) Complexes with l-Histidine

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Abstract: The complex formation between L-histidine (HHis) and aluminum(III) ion in water solutions was studied by UV spectrophotometric and 27-Al NMR measurements at 298 K. UV spectra were measured on solutions in which the total concentration of histidine was from 15.0 to 50.0 mmol/dm^3 and the concentration ratio of histidine to aluminum was varied from 3 : 1 to 10 : 1 in the pH range between 4.2 and 6.0. The spectra were taken in the wavelength interval 240–340 nm. Nonlinear least-squares treatment of the spectrophotometric data indicates the formation of the complexes $Al(HHis)^{3+}$, $Al(His)^{2+}$, $Al(HHis)His^{2+}$, and $Al_2(OH)His^{4+}$ with the overall formation constants $\beta_{p,q,r}$: log $\beta_{1,1,1} = 11.90 \pm 0.04$, log $\beta_{1,1,0} = 7.25 \pm 0.08$, log $\beta_{1,2,1} = 20.1 \pm 0.1$, and log $\beta_{2,1,1} = 5.92 \pm 0.12$ (p, q, r are stoichiometric indices for metal, ligand, and proton, respectively). ^{27}Al-NMR spectra were taken on solutions with the concentration of aluminum 50 mmol/dm^3 and that of histidine 250 mmol/dm^3.

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In the pH interval 5.0–6.1, two resonances at 9.5 ppm and 12.0 ppm were assigned to $Al(HHis)^{2+}$ and $Al(HHis)(His)^{2+}$ (or $Al(OH)(HHis)_2^{2+}$), respectively.

Keywords: Aluminum, 27-Al NMR, complex formation, L-histidine, spectrophotometry

INTRODUCTION

Aluminum exerts harmful and toxic effects on the human organism. It can enter the human body from the environment, the diet, or from medication and passes into systemic circulation from the gastrointestinal tract, the lungs, or by a parenteral route (hemodialysis or parenteral nutrition).[1–3]

Because Al^{3+} is the hardest metal ion, it forms complexes of enhanced stability with ligands containing hard donor groups. The most effective are the ligands possessing strongly basic, negatively charged oxygen atoms (phenolates, alcoxides, carboxylates, phosphonates, etc.) and nitrogen atoms, which are appropriately arranged inside the ligand molecule to form five- or six-membered metalacycles.[4–6]

The amino acids possess amino and carboxylate groups able to bind metal ions. Though amino acids generally do not form strong complexes with aluminum ion, those with effective side-chain donors (glutamic, aspartic), can form chelates of appreciable stability with Al^{3+} ion as a result of favorable steric arrangements of donor groups.[7–11]

Among the amino acids, L-histidine is a unique case. It has an imidazole group that may bind to a metal ion, and this amino acid itself is a component of protein and enzyme active sites. The histidine possesses four potential coordination sites, the carboxyl group, the amino group, and the pyridinic and pyrollic nitrogens from the imidazole residue. The imidazole pyridinic nitrogen is among the strongest electron pair donors to metal ions, and its Lewis basicity depends on the protonation state of the rest of the molecule and in proteins, on the vicinity of carboxylate groups. Thus, imidazolic nitrogen is fairly basic (pK_a in the range 6–7) and can serve as a site for aluminum binding.

Histidine is involved in a large number of biochemical processes such as biosynthesis of histamine, secretion of prolactin and antidiuretic hormone, production of red and white blood cells, and so further. Histidine possesses vasodilating and hypotensive actions and may boost the activity of soothing alpha waves in the brain. Histidine is now used in the treatment of anemia, allergies, rheumatoid arthritis, and other inflammatory reactions. Solution chemistry of aluminum ion and histidine may be of importance in elucidating the interactions between protein hydrolizates used in parenteral nutrition and aluminum, which is unavoidably present in such solutions. Also, histidine taken as food supplement may interact with concomitantly administered aluminum-based antacids. In human organism, likely sites of

aluminum–histidine interaction may involve GI tract, blood, kidney tissue, cell cytosol, and lysosomes. The histidine taken either for therapeutic purposes or released from ingested food or drinks may affect the solubilization of aluminum and enhance its bioavailability. Hence, understanding of the nature of aluminum–L-histidine interactions would help in knowledge of the toxicity problems of aluminum.[2,3]

The objective of the current work was to study the complex formation between trivalent aluminum ion and L-histidine with respect to speciation (composition and stability of the formed complexes) and binding mode of histidine to aluminum. Review of available literature data shows that no unambiguous description of aluminum complexation with L-histidine exists. Duc et al.[12] studied the complexation of aluminum with α-amino acids that constitute the collagen by potentiometric measurements in 0.5 mol/dm^3 $NaClO_4$ ionic medium at 298 K. With histidine, the mononuclear mixed hydroxo complex was found. Berthon et al.[7] studied the complexation of aluminum ion with glycine, serine, threonine, and histidine in 0.15 mol/dm^3 NaCl medium at 37°C by potentiometry and ^{1}H and ^{13}C-NMR spectroscopy. The complex $Al_2(His)H_{-2}$ was identified with the overall stability constant $\log \beta_{2,1,-2} = 1.16 \pm 0.12$. In our previous work,[13] we identified the complexes Al(HHis) ($\log \beta_{1,1,1} = 12.21 \pm 0.08$), Al(His) ($\log \beta_{1,1,0} = 7.25 \pm 0.08$), and Al(HHis)(His) ($\log \beta_{1,2,1} = 20.3 \pm 0.1$) by potentiometric measurements in 0.1 mol/dm^3 LiCl ionic medium at 298 K.

In the current work, we studied the complex formation between aluminum ion and L-histidine by UV spectrophotometry and 27-Al NMR spectroscopy.

EXPERIMENTAL

Reagents and Analysis

The aluminum chloride stock solution was obtained by dissolving $AlCl_3 \cdot 6H_2O$, p.a. Merck (Darmstadt, Germany), in doubly distilled water. The appropriate amount of 0.1 mol/dm^3 of HCl was added to prevent initial hydrolysis of aluminum. The aluminum content in solution was determined gravimetrically by precipitation with ammonia or 8-hydroxyquinoline. Both methods gave the same results within 0.3%. The concentration of the free acid was determined potentiometrically with standard NaOH using the Gran plot. The metal and proton content in the solution was periodically checked before each series of experiments. L-histidine, p.a. Merck, was dissolved in doubly distilled water and assayed potentiometrically. The sodium hydroxide solution was prepared from a concentrated volumetric solution, p.a. Merck, by diluting with freshly boiled doubly distilled water, cooled under constant flow of purified nitrogen. The alkali concentration was checked by titration against potassium hydrogen phthalate. The hydrochloric

acid solutions were prepared from HCl, "suprapure", Merck, and standardized against tris(hydroximethyl) aminomethane.

Instruments and Procedure

All pH measurements were made by using Tacussel model Isis 20000 digital pH/mV meter (resolution, ± 0.001 pH units) equipped with the Radiometer combined electrode. UV spectra were obtained using a Beckman model UV 5260 double-beam spectrophotometer. Operational parameters were scan speed, 2 nm/s, slit width, 0.3 nm, photometric sensitivity, 0.2 abs. units. Matching pair of 1-cm quartz cells was used for measuring the spectra. The metal-free ligand solutions with the same total ligand concentration and pH as in test solutions were used as the reagent blanks. The species formed in the systems were characterized by the general equilibrium:

$$p\mathrm{Al} + q\mathrm{His} + r\mathrm{H} = \mathrm{Al}_p(\mathrm{His})_q\mathrm{H}_r$$

and the corresponding constants are given by:

$$\beta_{p,q,r} = \frac{[\mathrm{Al}_p(\mathrm{His})_q\mathrm{H}_r]}{[\mathrm{Al}]^p[\mathrm{His}]^q[\mathrm{H}]^r}$$

where His is the nonprotonated molecule of the ligand. Fully protonated histidine is denoted as H_3His^{2+}. The concentration stability constants of complexes, $\beta_{p,q,r}$, were calculated with the aid of the computer program Squad.[14] To derive the reliable complexation model, correct data on pure hydrolysis of aluminum are needed. Hydrated aluminum(III) ion is subject to extensive hydrolysis in water solutions. The extent of hydrolysis, the identity and stability of hydrolytic species formed in solution, depend upon many factors such as the type and concentration of supporting ionic medium, the kind and concentration of the base used to force the hydrolysis, aging time, and the presence of other substances that may interact with either aluminum ion or water molecules or both. Pronounced hydrolysis of aluminum ion could considerably obscure weak complexation in solution. Furthermore, polynuclear hydrolytic species, whose rate of formation is quite slow so that they may persist in solution for long period of time as metastable species even if the actual pH of the solution corresponds to the conditions where they are no longer the most stable species, make the identification of complexes very difficult. The solution could also become supersaturated with respect to one or more polynuclears or solid hydrated oxide of aluminum, which further complicates the complexation. To be confident that the measured pH effects are due to complexation and not to pure hydrolysis of aluminum, very reliable data on aluminum hydrolysis are necessary, relatively high concentration ratios of ligand to aluminum should be used, and several experimental techniques must be combined. The diversity of factors that influence the hydrolysis creates the situation where there is no unique model for aluminum ion

hydrolysis. In the milimolar range of the total aluminum concentration in solution and the pH interval from ca 3 to 5, the common consensus is that $Al(OH)^{2+}$, the oligomer, and $Al_{13}(OH)_{32}^{7+}$ are the main constituents of the solution.[15] Essentially, the same model was determined in our previous works, in 0.1 mol/dm^3 LiCl ionic medium at 298 K. Thus, based on the previously reported hydrolytic models[1] for the analysis of the titration curves, the hydroxo species AlH_{-1} (−5.27), Al_3H_{-4} (−13.81), AlH_{-4} (−23.1), $Al_{13}H_{-32}$ (−109.23), and AlH_{-3}(aq) (−14.68) were taken into account.[13]

The ^{27}Al-NMR spectra were recorded a Bruker MSL 400 spectrometer at 104.26 MHz. Samples were recorded in 10-mm tubes, with $AlCl_3$ in 6 mol/dm^3 HCl, as an external standard. D_2O was added in each sample for a lock. The FT-NMR measurement conditions were as follows: pulse width 7 μs, flip angle 45°, acquisition time, 98.3 ms, spectral width, 20,833 Hz, number of transients, 200–500, pulse repetition time, 1 s, number of data points, 4k, digital resolution, 10.17 Hz/point.

RESULTS AND DISCUSSION

The histidine possesses absorption in far UV with a maximum at 211 nm arising from $\pi \rightarrow \pi^*$ transition in imidazole residue. The band is symmetrical and approaches zero at wavelengths higher than 240 nm. Upon complexation, however, the tail extending into wavelengths higher than 240 nm is observed. Though in the presence of aluminum, absorbance does not exceed 0.1 absorbance unit, it is clearly distinctive from that of pure histidine which is virtually zero (Fig. 1). This property can be used to evaluate the extent of complexation

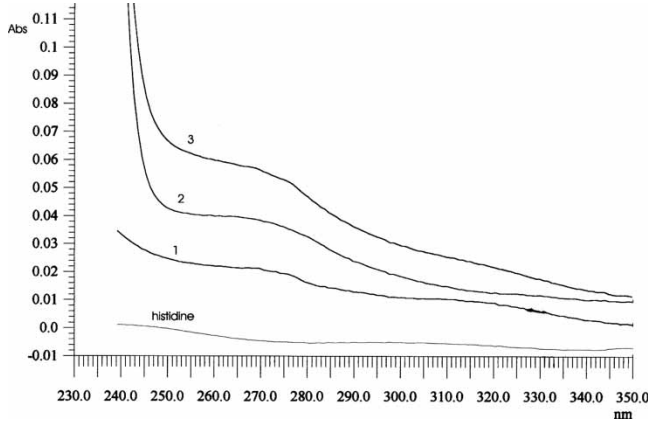

Figure 1. UV spectra of histidine and histidine + Al^{3+} solutions. $C_{His} = 30$ mmol/dm^3, $C_{Al} = 5$ mmol/dm^3. (1) pH = (1) 5.0; (2) 5.5; (3) 6.0. For the spectra 1–3, the corresponding metal-free solutions were used as reagent blank.

in aluminum–histidine solutions. The UV spectra of the solutions in which the total histidine concentration was 15.0, 30.0, or 50.0 mmol/dm^3 and that of aluminum was 3.0, 5.0, 10.0, 20, or 25.0 mmol/dm^3 in the pH interval 4.20 to 6.20, were taken in the wavelength range 240–340 nm (10 solutions in each series). No lower pH values were used, since in these cases absorbencies are too small to be reliably measured. The pH of the test solutions was measured with a combined glass electrode calibrated as hydrogen ion concentration probe with a series of standard solutions of hydrochloric acid in 0.1 mol/dm^3 LiCl medium. All test solutions were left to stand 3 days before measurements were made. The initial opacity, observed in some solutions, completely disappeared during this time interval. The pH of solutions was then recorded every 30 min. The stable, reproducible values within ± 0.01 pH units were obtained after couple of hours and remained constant for the next hour. Slightly higher equilibration time was required for the solutions with pH higher than 4.5. Hence, spectrophotometric measurements were made in nearly true equilibrium conditions. The obtained spectra, part of which is shown in Fig. 2, show monotonous decrease in absorbance with increasing the wavelength. The plateau appears between 250 and 275 nm. Its position is pH dependent. The histidine itself does not show any appreciable absorption in the chosen wavelength range.

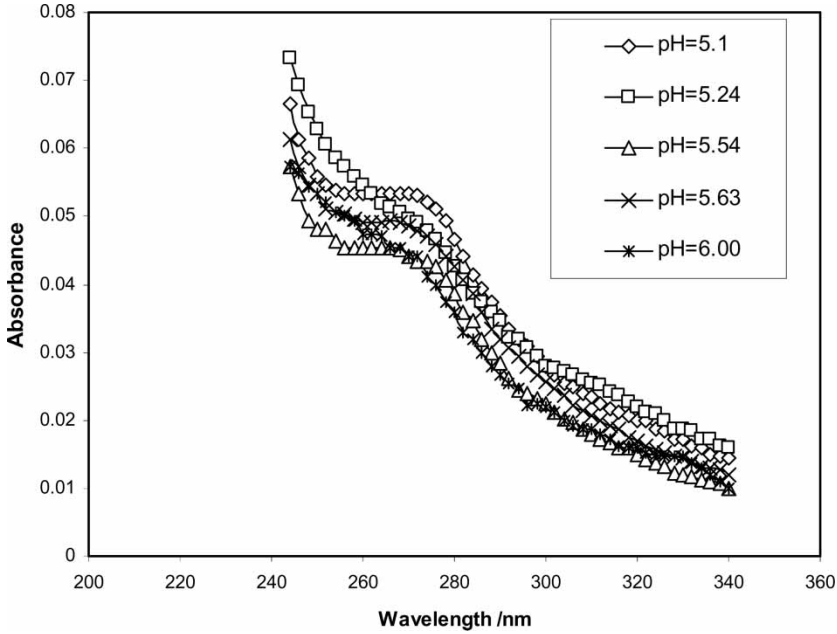

Figure 2. Spectra of L-histidine + Al^{3+} solutions: 30.0 mmol/dm^3 L-histidine, 3.0 mmol/dm^3 Al^{3+}.

The dependence of the intensity and position of the spectral bands on pH indicates the presence of several absorbing complexes in solution. In order to evaluate spectrophotometric data, the Squad[14] program was used. The program calculates overall stability constants, $\beta_{p,q,r}$, and molar absorptivities, $\varepsilon_{p,q,r}$, of the complexes formed by minimizing the sum, S, defined as:

$$S = \sum w_i (A_{obs} - A_{calc})^2$$

where the calculated absorbance, A_{calc}, is given by:

$$A_{calc} = \sum \beta_{p,q,r} [Al]^p [H]^q [His]^r \varepsilon_{p,q,r}$$

His represents the anion of histidine. In Squad calculations, the protonation constants of histidine and stability constants of aluminum hydrolytic complexes were taken from our previous work[13] and were not optimized, while these of Al^{3+} with histidine and their molar absorptivities were varied until minimum value of the sum, S, and standard deviations of the fit, SD, were obtained. During the calculations, various models were tested. The acceptance criteria for each particular model tested were (a) the standard deviation of the fit of each spectrum not higher than 5.0×10^{-3}, (b) the standard deviation in calculated stability constants not higher than 0.1 log units, and (c) the lowest value of the sum, S. The models tested comprised a set of trial complexes together with an estimate of their stability constants. In the first calculation cycle, the following complexes, AlL, AlL_2, $AlLH_{-1}$, AlL_2H_{-1}, AlLH, $AlLH_2$, AlL(LH), Al_2LH, Al_2LH_{-1}, $Al_2L_2H_{-2}$, $Al_2L_2H_{-1}$ (L = His), were tested. The refinement operations for each total histidine to aluminum concentration ratio (L/M) resulted in different and often acceptable models. Different strategies were employed in the refinement operations: (i) fixing selected constants to simplify optimization procedure, (ii) reducing the number of spectra included in calculations, (iii) increasing the number of iterations. In a preliminary calculations, multiple regression option of the program was used. When speciation model was established, the nonlinear regression mode was used. Several models appeared as acceptable (Table 1). It is seen from the data in Table 1 that the species M_2LH_{-1}, MLH, and M(HL)L are always calculated with low standard deviation and are accepted in most tested models. Therefore, these species were accepted as the basic set of fitting the spectra. Their stability constants were fixed in further calculations. Subsequently, to this model we added various dinuclear and polynuclear species, but all were rejected or unacceptably high values of standard deviations were obtained. Addition of AL complex did not change the fit appreciably, and bearing in mind that in some models this species appeared with low standard deviation, we decided to keep it in the accepted mode. To examine the effect of aluminum hydrolysis on the complexation, we performed sensitivity analysis of the calculated stability constants, i.e., we co-varied stability constants of accepted aluminum–histidine complexes with the constant of Al_{13}-mer. Such analysis was necessary since hydrolytic

Table 1. Optimization procedure for spectrophotometric equilibrium data treatment.

Complex	log $\beta_{p,q,r} \pm \sigma$		
	Model 1	Model 2	Model 3
L/M = 10 (M_T = 3.0, 5.0 mM)			
M(HL)L	20.1 ± 0.1	20.3 ± 0.1	20.60 ± 0.07
MHL	12.20 ± 0.07	11.87 ± 0.07	11.87 (fixed)
ML	7.40 ± 0.09	—	—
MLH_{-1}	—	2.40 ± 0.12	—
M_2LH_{-1}	—	—	—
S	1.5×10^{-2}	8.2×10^{-3}	4.1×10^{-3}
L/M = 5 (M_T = 5.0, 10.0 mM)			
M(HL)L	—	20.41 ± 0.05	—
MHL	11.98 ± 0.15	11.90 (fixed)	11.93 ± 0.06
ML	7.35 ± 0.08	—	—
M_2LH_{-1}	5.95 ± 0.04	6.10 ± 0.1	5.95 (fixed)
S	6.0×10^{-3}	2.0×10^{-2}	8.0×10^{-3}
L/M = 3 (M_T = 20 mM)			
MHL	11.90 ± 0.15	11.90 (fixed)	12.10 ± 0.05
ML	7.40 (fixed)	—	—
M_2LH_{-1}	5.90 ± 0.07	6.31 ± 0.06	5.95 (fixed)
S	3.1×10^{-2}	4.0×10^{-3}	2.1×10^{-3}
L/M = 2 (M_T = 25 mM)			
MHL	—	11.90 (fixed)	11.89 ± 0.03
M_2LH_{-1}	6.40 ± 0.02	5.92 ± 0.07	5.92 (fixed)
S	1.0×10^{-3}	3.1×10^{-3}	3.8×10^{-3}

L/M denotes total ligand to total metal concentration ratio. *S* represents total residual sum of squares of the absorbance data (should be less than 1×10^{-2}).

tridecamer considerably interferes with the complex formation. Co-variation of (13,−32) and (1,2,1) complexes leads to decrease of the stability constant of the (13,−32) complex, from −109.23 to −111.50 and the stability constant of (1,2,1) complex was decreased from accepted value, 20.41 to 20.10, with fairly good standard deviation (0.03) and improved statistical parameters of the fit. When, however, (1,1,1) or (1,2,−1) complexes were co-varied with (13,−32) complex, the stability constant of tridecamer increased to −106.80 and stabilities of Al-His complexes decrease to 11.90 and 5.85, respectively. Co-variation of (1,1,0) complex with (13,−32) complex resulted in rejection of (1,1,0) complex. Inclusion of (13,−32) complex as refinable species into the calculation substantially improved standard deviation of calculated stability constants as well as the standard deviation of the fit of the individual spectra. We decided to keep the complex (1,1,0) in the model as nonrefinable species, since its inclusion did not change overall fit. In the final calculation cycle, we selected the species from the various models that appear with the lowest values of standard

deviation and (13, −32) complex as refinable species and looked for the best set of statistical parameters of the overall fit. We finally adopted those values of stability constants that appeared in the recalculated models with the best statistical parameters. The selected model with the most reliable constants is given in Table 2. It is in good agreement with our previous data with two important differences. Since relatively high concentrations of aluminum were used, the hydrolytic polymerization was favored and the complex $Al_2(OH)His^{4+}$ was detected. This complex was not found in our previous work[13] because of very low total aluminum concentrations we used in potentiometric titrations (0.5–2.0 mmol/dm^3). Second, important difference concerns the slight discrepancy between the present and previously reported values of the stability constants of aluminum–histidine complexes. It may be attributed to variation of the activity coefficients of aluminum at higher aluminum concentrations used in current work.

The calculated spectra of the main complexes, Al(HHis) and Al(HHis) His are shown in Fig. 3. Both spectra show poorly defined maximum of absorption at 275 nm with rather low molar absorptivities (less than 20 L mol^{-1}cm^{-1}).

Reliability of the Speciation Model

In Table 3, some selected literature data on amino acids–aluminum ion complexation are presented. By inspection of the data (Table 2), it is seen that our values for the stability constants of the complexes formed in Al/His solutions are considerably higher than those reported for other aluminum–amino acid complexes. The enhanced stability of the Al(HHis) complex may be explained by the formation of five-membered chelate where histidine acts as a bidentate ligand with carboxyl oxygen and ammino nitrogen as donors. The electrostatic repulsion between ammonium group and central aluminum ion is weakened

Table 2. Calculated stability constants of Al^{3+}–His complexes in 0.1 mol/dm^3 LiCl ionic medium at 298 K (data for protonation constants of histidine are from Ref. 13

Species	$\log \beta_{p,q,r} \pm \sigma$
H_3His^{2+}	17.01
H_2His^{+}	15.29
HHis	9.17
$Al(HHis)(His)^{2+}$	20.1 ± 0.1
$Al(His)^{2+}$	7.25 ± 0.08
$Al(HHis)^{3+}$	11.90 ± 0.04
$Al_2(OH)His^{4+}$	5.92 ± 0.12
Statistical parameters of the fit	$S = 3 \times 10^{-3}$, $SD = 1.0 \times 10^{-3}$

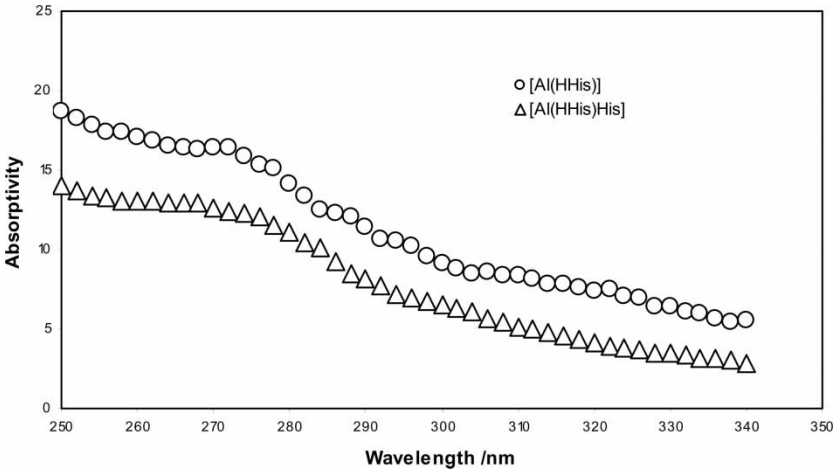

Figure 3. Calculated UV spectra of the Al–histidine complexes.

by the possibility of the proton transfer from ammonium to pyridinic nitrogen in histidine molecule and by ion pairing with the chloride ion. The maximum formation of Al(HHis) complex is around pH ~4, which is very close to the protonation constant of the imidazolic nucleus in histidine and should thus facilitate proton transfer.

To examine the effect of pH and histidine to aluminum concentration ratio on the formation of Al_{13}-mer hydrolytic complex and ternary complex formation in solution, factor analysis using response surface approach was performed. The statistical analysis was performed with the aid of the program Statistica v. 6.[18] The result of the analysis is given in Figs. 4a and Figs. 4b.

In Fig. 4a, the effect of pH and L/M on the formation of Al_{13}-mer complex is shown. It can be seen that at lower pH values and higher L/M values, the response surface flattens indicating insensitivity of tridecamer formation on these factors. Also, the interaction between factors in this region is negligible. In contrast, the formation of Al(HHis)His (Fig. 4b) shows that response surface at lower pH values and low L/M values is relatively insensitive to change of factors. From Figs. 4a and 4b is seen that hydrolytic tridecamer is dominantly formed at lower His to Al concentration ratio and pH values higher than 4.0. In contrast, the optimum formation of the mixed complex is at higher His to Al concentration ratios and at pH values beyond 4.2. Thus the measured effects at higher His to Al concentration ratios are due to complex formation and not to the formation of hydrolytic tridecamer. However, the presence of histidine cannot suppress the tridecamer formation so that the hydrolysis parallels the complexation.

The distribution diagram of the Al^{3+}–L-histidine complexes in solution is shown in Fig. 5. For the calculation of relative concentration of the species the

Table 3. Selected literature data on aluminum complexation with amino acids (potentiometric measurements)

	log $\beta \pm \sigma$					
Species	Gly	Ser	Thr	His	Asp	Glu
MLH_2					14.48 ± 0.04^b	14.74 ± 0.04^b 15.025 ± 0.025^c
MLH		11.18 ± 0.1^f		12.21 ± 0.08^d	11.76 ± 0.06^a 11.24 ± 0.03^b	10.88 ± 0.22^a 11.07 ± 0.06^b 10.68 ± 0.02^e 12.02 ± 0.04^f
ML	5.91 ± 0.1^a 6.23 ± 0.1^b	5.66 ± 0.11^a 5.97 ± 0.05^b 5.71 ± 0.02^f	5.51 ± 0.12^a 5.71 ± 0.08^b	7.08 ± 0.20^b 7.25 ± 0.08^d	7.87 ± 0.04^a 7.77 ± 0.02^b	7.29 ± 0.04^a 7.69 ± 0.03^b 7.42 ± 0.03^f 7.86 ± 0.01^e
MLH_{-1}			0.94 ± 0.15^a		3.30 ± 0.03	2.56 ± 0.03^f
M_2LH_{-1}	4.35 ± 0.09^a	3.75 ± 0.11^a 4.65 ± 0.03^f				
$K(M + HL \rightarrow MLH)$					2.14^a	1.38^a 1.88^b 1.72^e 2.54^f

[a]Ref. 11. $\mu = 0.2\,mol\,dm^{-3}$ KCl, 25°C.
[b]Ref. 16. $\mu = 0.15\,mol\,dm^{-3}$ NaCl, 37°C.
[c]Ref. 9. $\mu = 0.15\,mol\,dm^{-3}$, 37°C.
[d]Ref. 13. $\mu = 0.1\,mol\,dm^{-3}$ LiCl, 25°C.
[e]Ref. 10. $\mu = 0.1\,mol\,dm^{-3}$ KCl, 25°C.
[f]Ref. 17. $\mu = 0.1\,mol\,dm^{-3}$ LiCl, 25°C.

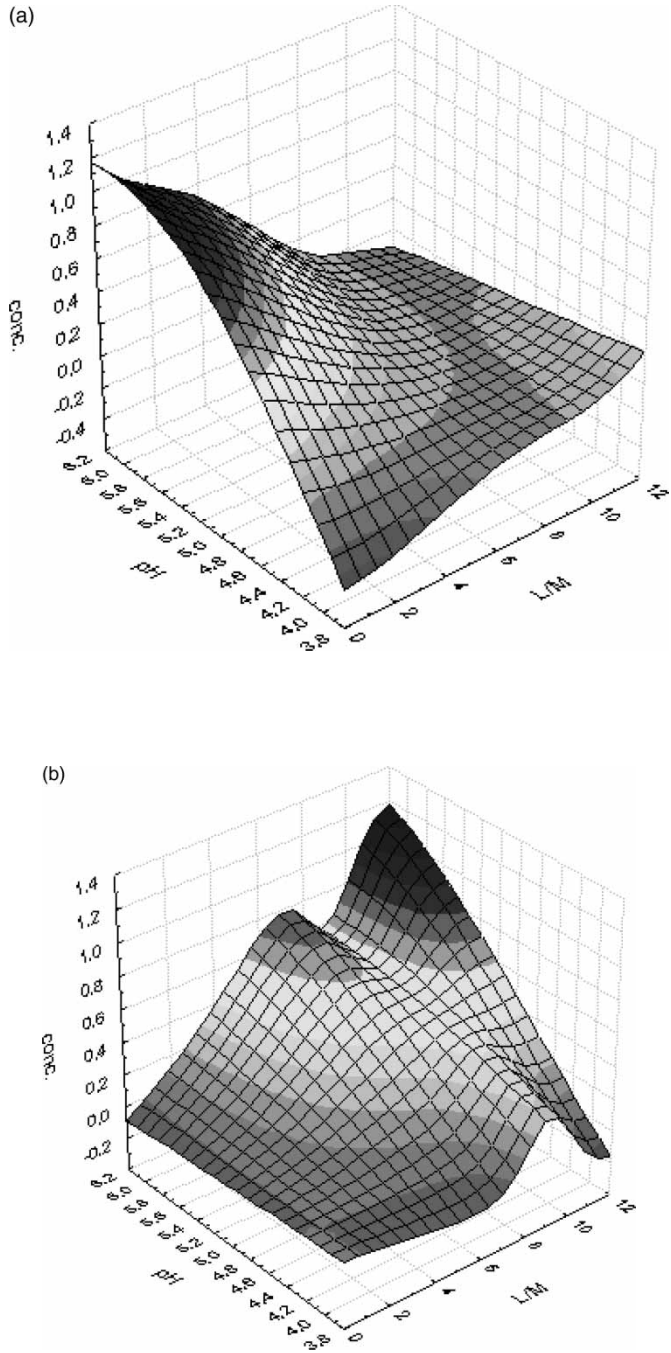

Figure 4. (a) Response surface for the formation of Al_{13}-mer in solution. (b) Response surface for the formation of Al(HHis)His in solution.

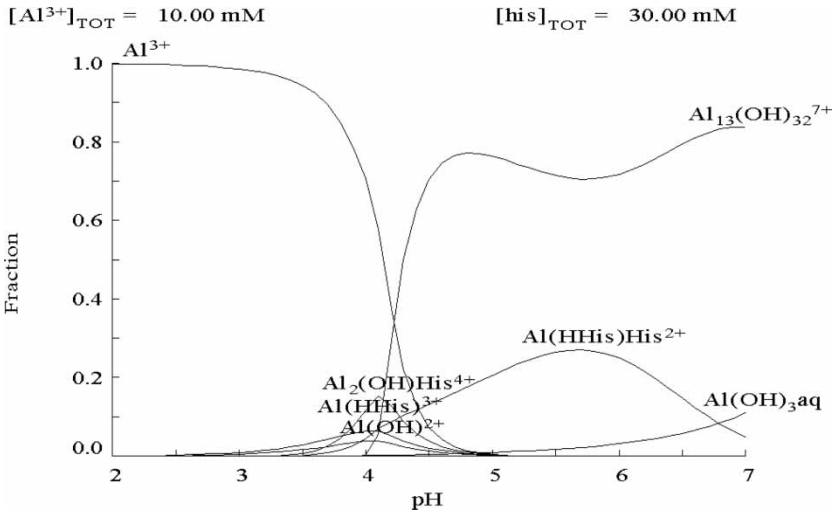

Figure 5. The distribution diagram of histidine–Al^{3+} complexes.

stability constants of the Al–histidine complexes were taken from Table 2, while for the stability constant of (13,−32) hydrolytic complex, the refined value −106.80 was used.

The distribution diagram shows that the complex Al(HHis) starts to form at pH about 2.0 and reaches maximum concentration at pH about 4.0. From this pH value, the mixed binary complex Al(HHis)(His) is formed, and its concentration increases with pH increase reaching the maximum at pH around 6.0. The microcolloidal hydrated $Al(OH)_3$(aq) hydrolytic complex exhibits increase in concentration at pH values higher than 6.0. The formation of tridecamer, Al_{13} starts at pH around 4.5 and slightly falls at pH 5.5 where the complex $Al(HHis)His^{2+}$ reaches high concentration.

The distribution diagram of pure hydrolytic species[13] indicates that in the pH interval 4.0–4.5, the most important reactive species of aluminum is monohydroxo complex $Al(OH)^{2+}$ so that the probable mechanism of the formation of binary ligand complexes should be:

$$Al(OH)^{2+} + H_2(His)^{+} \rightleftharpoons Al(HHis)^{3+} + H_2O$$

At pH values higher than 4.5, formation of the mixed complex Al(HHis)His becomes significant. This complex attains maximum concentration at pH about 5–6 and then its concentration rapidly falls. In the same time the concentration of hydrolytic complexes increases. Hence, it may be assumed that this complex is formed by the reaction of protonated histidine and hydrolytic species of aluminum, probably $Al(OH)_3$(aq). The composition of the complex may be represented as $Al(OH)(HHis)_2$, since it is more probable that at pH < 6 histidine is in protonated form, i.e., aluminum cannot completely

displace all dissociable protons from the histidine. However, the two microforms perhaps coexist in dynamical equilibrium. The dimer, $Al_2(OH)His^{4+}$, forms in the approximately same pH region as $Al(HHis)^{3+}$ and $Al(OH)^{2+}$ complexes. Its concentration increases with increasing the total concentration of aluminum and with decreasing the histidine to aluminum concentration ratio. Dimerization may proceed according to reaction:

$$Al(OH)^{2+} + Al(His)^{2+} \rightleftharpoons Al_2(OH)His^{4+}$$

which may explain why the binary complex, Al(His) is only minor species at total aluminum concentration higher than 10 mmol/dm^3.

27-Al NMR Measurements

To evaluate the complexation in histidine + Al^{3+} solutions, number of solutions were prepared. The total concentration of histidine was 250.0 mmol/dm^3 while that of aluminum was 50.0 mmol/dm^3 in the pH range 1.0–6.0. The pH of all solutions was adjusted by the addition of either standard 1.0 mol/dm^3 HCl or 1.0 mol/dm^3 NaOH solution and was finally checked by pH metric measurements. The prepared solutions were left to stand 72 hr before measurements were made. Final check of the pH was done 30 min before recording the spectrum. It should be noted that the solutions whose pH was in the interval 4.0 to 6.0 showed initial opacity, but after 48 hr they became totally clear with no apparent precipitate. The turbidity of solutions, measured with the aid of turbidimetric attachment to UV spectrophotometer, showed low values so that it may be assumed that the NMR measurements were performed on clear solutions in the state close to thermodynamic equilibrium. Some of the spectra of solutions, recorded in the pH interval 1.80 to 6.0, are shown in Fig. 6. At pH values less than 3.00, the observed pattern closely resembles that seen in pure hydrolysis. At pH = 3.0, small, rather broad peak at $\delta \sim 4.3$ ppm appears and the resonance at $\delta \sim 0$ ppm widens as the pH increases. Small sharp peak at $\delta \sim 63$ ppm also appears (not shown in Fig. 6).

The resonance at 4.3 ppm arises from hydrolytic oligomers, broadening of the zero resonance is assigned to the formation of mononuclear hydrolytic products, while the resonance at 63 ppm denotes beginning of the Al_{13}-mer formation. At pH 4.04, the peak at 4.3 ppm shifts to 4.69 ppm and in the same time new broad peak at $\delta \sim 9.54$ ppm appears. The resonance at 9.54 ppm is near to expected value of $\delta \sim 10$ ppm when the amino acid acts as bidentate ligand[19] and may be thus assigned to the formation of binary complex between aluminum ion and histidine. In this pH region, the dominating complex is $Al(HHis)^{3+}$, and therefore protonated histidine acts as a bidentate ligand in fairly symmetrical environment. Bearing in mind that pK value of pyridinic nitrogen in imidazole residue is about 6.5, it appears that the proton resides on this donor. Thus the complex is formed by

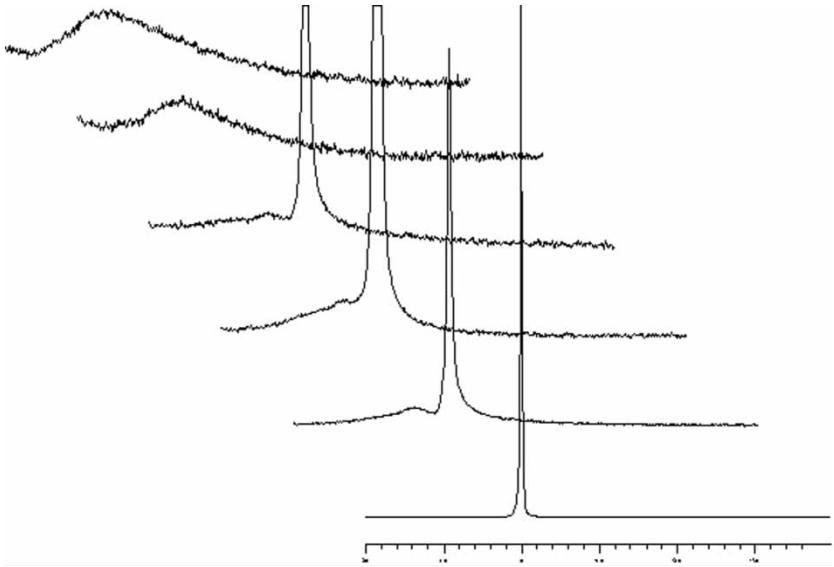

Figure 6. 27-Al NMR spectra of Al^{3+} + L-histidine solutions (expanded scale). $C_{Al} = 50\,mmol/dm^3$, $C_{His} = 250\,mmol/dm^3$. The pH values from bottom to top: 1.80, 3.00, 4.04, 4.55, 5.00, 6.08.

binding carboxylate oxygen and amino group of histidine to aluminum ion. Considering the equilibrium:

$$Al(H_2O)_6^{3+} + H_2His^+ \rightleftharpoons Al(H_2O)_4(HHis) + H^+ + 2H_2O$$

for which the equilibrium constant is given by the expression $\log K_{eq} = \log \beta_{1,1,1} - \log \beta_{0,2,1} = -3.39$, we see that it is less probable than the equilibrium:

$$Al(H_2O)_6^{3} + HHis \rightleftharpoons Al(H_2O)_4(HHis) + 2H_2O$$

for which the equilibrium constant is $\log K_{eq} = \log \beta_{1,1,1} - \log \beta_{0,1,1} = 2.73$, in line with other bidentate amino acids. Since the above reaction is a dynamical chemical exchange, it causes broadening of resonances at 0 ppm and 9.54 ppm. HHis represents the protonated histidine molecule in which the proton from the ammonium group, in the presence of aluminum, was transferred to pyridinic nitrogen of imidazole residue thus enabling the closure of five-membered glycine-like ring. Further increase of pH to 4.55–5.54, leads to the shifting and merging of the broad band at 4.3 ppm into the tail of the resonance at $\delta \sim 9.54$ ppm. The poorely resolved new resonance appears at 12.08 ppm and probably indicates formation of new complex. In the same time, the sharp resonance at 63.01 ppm decreases in intensity (Fig. 7). The resonance at $\delta \sim 12$ ppm may be attributed to mixed binary complex

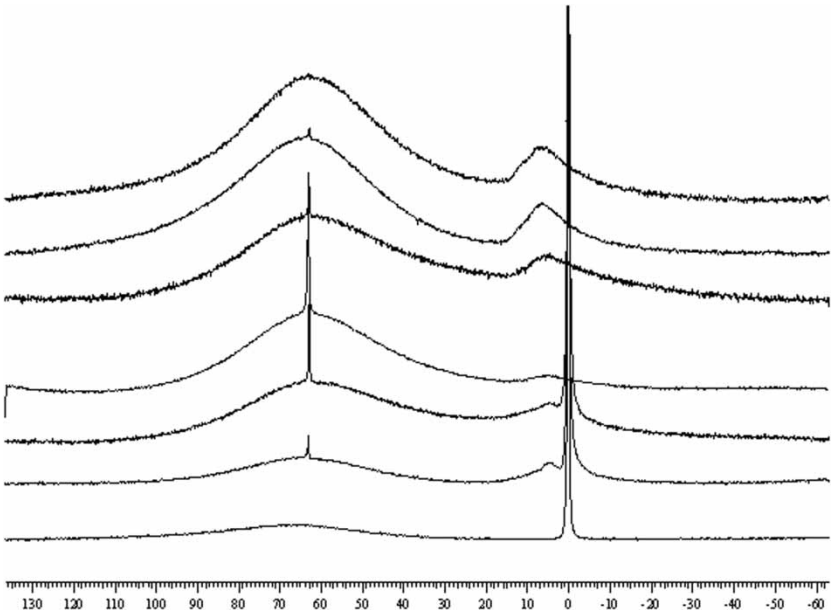

Figure 7. 27-Al NMR spectra of aluminum–histidine solutions. $C_{Al} = 25\,mmol/dm^3$, $C_{His} = 250\,mmol/dm^3$. The pH values from bottom to top: 1.80, 3.85, 4.18, 4.40, 4.65, 4.92, 5.40.

$Al(HHis)His^{2+}$ in which the coordination sphere around aluminum is less symmetric than in $Al(HHis)^{3+}$.

CONCLUSIONS

Histidine and aluminum ion may form mononuclear and dinuclear binary and mixed complexes in which histidine is bound to aluminum in bidentate fashion using ($-COO^-$, $-NH_2$) or ($-COO^-$, imidazole pyridinic-N) couples as donors. The later one can be used at pH values where deprotonation of imidazolium is appreciable (pH $>$ 5).

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