Journal of Coordination Chemistry

ISSN: 0095-8972 (Print) 1029-0389 (Online) Journal homepage: http://www.tandfonline.com/loi/gcoo20

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To cite this article: Agnieszka Chylewska, Małgorzata Ogryzek, Lech Chmurzyński & Mariusz Makowski (2015) Spectrophotometric, potentiometric, and conductometric studies of binary complex formation between copper(II) and three forms of vitamin B_6 in aqueous solutions, Journal of Coordination Chemistry, 68:21, 3761-3775, DOI: 10.1080/00958972.2015.1088149

To link to this article: http://dx.doi.org/10.1080/00958972.2015.1088149

Accepted author version posted online: 01 Sep 2015.
Published online: 11 Sep 2015.

Journal of Coordination Chemistry, 2015
Vol. 68, No. 21, 3761–3775, http://dx.doi.org/10.1080/00958972.2015.1088149

Spectrophotometric, potentiometric, and conductometric studies of binary complex formation between copper(II) and three forms of vitamin B_6 in aqueous solutions

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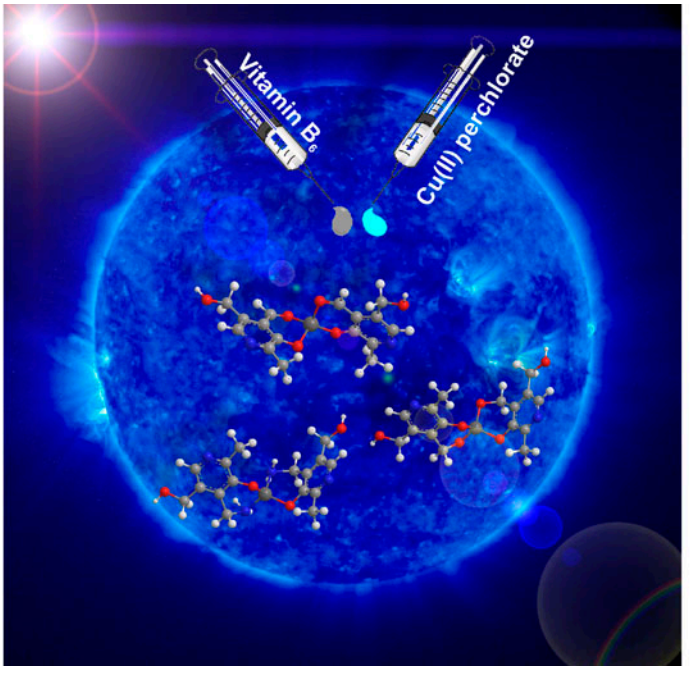
(*Received 19 June 2015; accepted 14 August 2015*)



This article reports the detailed study concerning the mode of binding of three forms of vitamin B_6, pyridoxamine (pm), pyridoxine (pn), and pyridoxal (pl), with Cu(II) in aqueous solutions using three independent methods: potentiometry, conductometry, and UV–vis spectroscopy. The stability constants of complexes formed between copper(II) and vitamin B_6 were investigated by potentiometric titration in 0.1 M KNO_3 ionic medium at 25 °C. While drawing the relations between molar conductance and the ratio of metal to ligand concentrations, different types of lines were obtained indicating the formation of 1 : 1 and 1 : 2 stoichiometric compounds. The stability constants have been

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determined using EQUID and CVEQUID computer programs and the obtained results were in agreement. The relatively high values of stability constants of Cu(II)-vitamin B_6 complexes obtained from three independent methods in comparison to those with other competing cations suggest that the complexes studied are relatively stable in aqueous solutions.

Keywords: Cu(II) complexes; Vitamin B_6; Potentiometry; Spectrophotometry; Conductometry

1. Introduction

Metal ions play key roles in structural organization and activation of certain enzymes, which are involved in the transfer of genetic information from DNA. Metal ions commonly used form low-molecular weight complexes and, therefore, prove to be active against several diseases.

Biologically relevant metal complexes have several requirements in terms of their structure. First, a biologically active metal complex should have a sufficiently high thermodynamic stability to deliver the metal to the active site. The metal–ligand binding should be hydrolytically stable. The compounds of low-molecular weight with a neutral charge and some water solubility are soluble in almost any medium and may slip through biological membranes by passive diffusion.

Research of transition metal complexes has shown significant progress in the use of these compounds as drugs to treat human diseases like carcinoma, infection control, anti-inflammatory and neurological disorders. This activity has started the development of metal-based drugs with promising pharmacological applications and may offer unique therapeutic opportunities.

Transition metal complexes, with their versatile electrochemical and spectroscopic properties, are suitable for designing metal-based therapeutic agents. Among these metal ions, after zinc, copper is the most abundant metal element in the human body and plays significant roles in several biological processes. Copper complexes are extensively examined as copper is available between the +I/+II oxidation state involving oxidative metabolism, ascorbic oxidase and numerous other procedures in cells [1]. Copper complexes can cause oxidative cleavage of DNA in the presence of oxidant or chemical reductant by generating reactive oxygen forms such as O_2^-, $OH^{\cdot}$ or hydrogen peroxide. A number of Cu(II) complexes with strong ability to bind and cleave DNA have been studied. Moreover, many of them exhibit excellent apoptosis-regulating and anticancer activity [2]. Copper(II) coordination compounds, as antitumor agents, cleave DNA not only through a direct oxidation by ROS, but also through intercalation into DNA base pairs. Additionally, copper polypyridine complexes have been studied as potential artificial nucleases [3, 4]. Early work demonstrated that coordination compounds of Cu(II) may facilitate DNA transformations [5]. Furthermore, Cu(II) ion forms complexes with the nonsteroidal anti-inflammatory drug group. These complexes exhibit better chomopreventive and chomosuppressive effects than the uncoordinated drugs [6].

Vitamin B_6 exists in three natural forms, pyridoxal, pyridoxamine, and pyridoxine, and is a necessary water soluble vitamin for a normal growth and development. It functions as a cofactor in numerous reactions of amino acid metabolism including transamination, deamination, and decarboxylation [7–9]. The primary role of vitamin B_6 is to act as a coenzyme to many other enzymes in the body, which are involved in a metabolism. The immune,

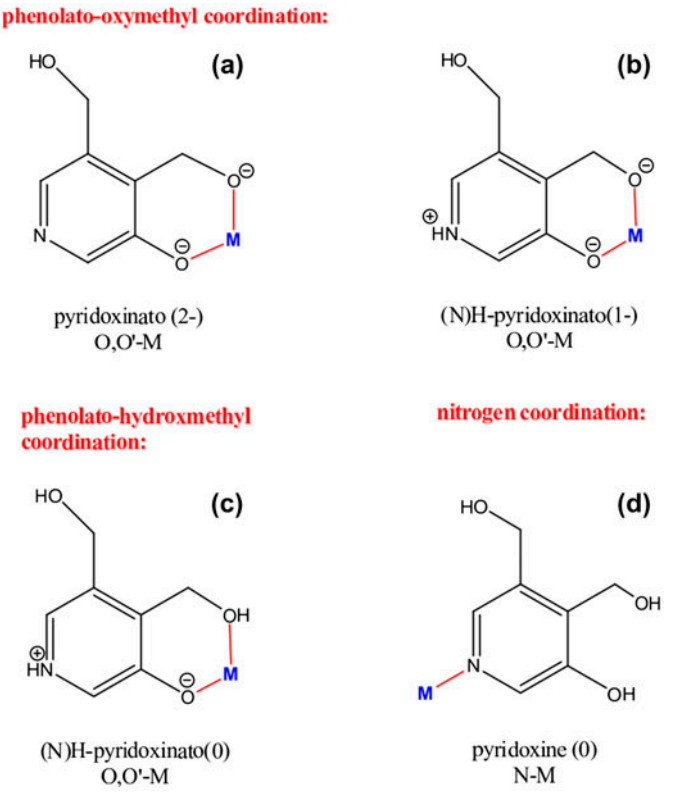

Figure 1. Coordination modes of metal-pyridoxine in mononuclear complexes: (a) pyridoxinato (2-), (b) (N)H-pyridoxinato (1-), (c) (N)H-pyridoxinato (0), and (d) pyridoxine (0).

endocrine, and nervous systems need this vitamin to function successfully (neurotransmitter synthesis) and it is also essential for the synthesis and metabolism of red blood cells [10, 11]. Moreover, complexes of vitamin B_6 with various transition metal ions have been reported to inhibit the growth as well as biosynthesis of RNA, DNA, and protein of gram negative bacteria *Escherichia coli* B-766 [12].

Pyridoxine exhibits different metal ion coordination sites with different charges and "hard"/"soft" character [13]. A chelation through the oxymethyl oxygen and adjacent phenolate is common for pyridoxine metal complexes [14]. Other chelating mode of this form of vitamin B_6 is a simple coordination through the pyridine nitrogen in the palladium complex [15]. In dinuclear and polynuclear complexes, the phenolate-oxymethyl coordination plus bridging through the pyridine nitrogen (towards Cu^{2+}) [16, 17] were observed. Moreover, the chelation plus bonding through the coordinated phenolate or hydroxyl was reported [18, 19] [figure 1(a)–(d)].

Pyridoxamine, in particular, forms quite stable binary complexes in solution and is also involved in many ternary metal complexes [20, 21]. This form of vitamin B_6 acts as a monodentate or bidentate ligand with coordination through phenolate oxygen and an adjacent amino moiety of the aminomethyl common for this ligand.

The structural characteristic of pyridoxal highly influences the possibility to form supramolecular networks. The pyridine nitrogen, the oxygens of aliphatic alcohol and

phenol groups can act as hydrogen bond acceptors. The π electron system of the pyridine ring can participate in π–π stacking interactions. The use of pyridoxal as one of the building blocks of the complex may improve the solubility of the final compounds and may promote an establishment of supramolecular networks [22, 23].

The formation of complexes in aqueous solutions is a matter of importance not only in inorganic, but also in biochemistry, analytical, and other industrial and scientific fields [24, 25]. Metal ions can induce toxicity in humans. Classic examples being heavy metal poisons such as mercury and lead. Even essential metal ions can be toxic when present in excess. Wilson's disease leads to an excessive accumulation of copper in the liver and brain which leads to various neurological abnormalities. The treatment of metal toxicity involves chelation therapy, in which metal-specific chelating agents are administrated as drugs to complex and facilitate the removal of unwanted excess element. The stability constants of metal-chelates are valuable for designing and selecting chelators [26, 27].

To study the formation constant of ligand with metal ions in solution the conductomeric method is widely used because of its various advantages such as low cost, eco-friendly, less time consuming, proper thermodynamic calculation with different parameters and, most important, ease of operation [28–30]. The factors responsible for the stability of complexes are charge, size and type of the metal ion, the counter ion, the nature of the ligand, the temperature, and physical properties of solvents.

In this work, we focus on the important mechanism of chelation of pyridoxal, pyridoxamine and pyridoxine with Cu(II) as a model to understand the biosynthetic role of vitamin B_6 *in vivo* as well as to develop bioactive compounds. Furthermore, our research group studied the copper(II) compound stabilities in solution of three independent titration methods, potentiometry, conductometry, and spectrophotometry.

The stability of vitamin B_6 in aqueous solutions has never been investigated using conductometric technique. Here, we report the results of the first conductometric determination of the gradual equilibrium constants of the three forms of vitamin B_6 with Cu(II) in water. The obtained data clearly demonstrate the formation of 1 : 2 metal to ligand complexes for all of the investigated compounds. The conductometric titration curves have also been used to calculate the gradual formation constants of the complexes produced by the interaction of pm, pn, and pl with Cu(II). The results show that the ionic size of the metal ion and the nature of the interaction between the metal and ligand are the major factors that determine the stability of the complexes. This study is useful to understand the effect of ionic size and the nature of the complexing agents. This article is a continuation of our earlier investigations of the coordination compounds of three forms of vitamin B_6 with nickel(II) in the various stoichiometries [31]. Those complexes were investigated with regard to their spectral, potentiometric, and conductance properties, which provide chemical bases for their biological activity. Our previous studies showed that the structure of the ligand had an important impact on the stability and the ability to inhibit the microbial of compounds used [32, 33].

2. Experimental

2.1. *Materials (chemical reagents)*

All chemicals, of analytical purity grade, were purchased from the commercial source (Sigma-Aldrich): pyridoxamine dihydrochloride (pure ≥98%); pyridoxine hydrochloride

(pure ≥98%); pyridoxal hydrochloride (pure ≥99%); copper(II) nitrate trihydrate (pure p.a. 99–104%); copper(II) perchlorate hexahydrate (pure 98%). All reagents were used without purifications. Sodium hydroxide and hydrochloric acid were supplied by Chempur.

Doubly distilled water (Hydrolab-Reference purified) with the conductivity not exceeding 0.09 μS cm^{-1} was used in the preparation of all the solutions. Ethanol (C_2H_5OH, cz.d.a grade 99.9%) was purchased from StanLab.

Stock solutions of HCl and NaOH were prepared by dissolution of appropriate amounts of the compounds in doubly distilled water. The hydrochloric acid stock solution was standardized by pH titration against Na_2CO_3. Sodium carbonate (from Sigma Aldrich) was calcined at 220 °C before standardization of HCl.

2.2. *Spectrophotometric titrations*

The solutions of the compounds studied and metal Cu(II) cation were prepared in water directly before measurements and were maintained at a constant temperature of 25 °C. The concentrations of ligands measured spectrophotometrically were around 10^{-4} M. The concentration of metal ion was about 15–30 times higher than vitamin B_6.

The spectrophotometric titrations were performed by adding to the solution of appropriate ligand a solution of metal salt dissolved in the ligand. These titrations were performed at the constant addend concentration.

The composition and stability of investigated copper(II) complexes were studied with the spectrophotometric titration method in aqueous solutions. In these measurements Cu(II) perchlorate hexahydrate, $Cu(ClO_4)_2 \cdot 6H_2O$, was used since perchlorates are known for their weak complexing properties. For all coordination compounds, the molar absorption coefficient values were determined.

Stability constants of copper(II)-vitamin B_6 complexes were determined using the EQUID program by a minimization of the differences between the experimental data and theoretical model. This computer program was based on the nonlinear least-squares Gauss–Newton–Marquardt algorithm for a fitting procedure [34]. The gradual K and cumulative β stability constants can be described by the following equations:

$$M^{2+}_{(aq)} + (\text{L-L}) \rightleftarrows [\text{M(L-L)}]^{2+} \quad K_{11} = \frac{\left\{[\text{M(L}-\text{L)}]^{2+}\right\}}{[\text{M}^{2+}] \cdot [(\text{L-L})]} \tag{1}$$

$$[\text{M(L-L)}]^{2+} + (\text{L-L}) \rightleftarrows \left[\text{M(L-L)}_2\right]^{2+} \quad K_{12} = \frac{\left\{\left[\text{M(L}-\text{L)}_2\right]^{2+}\right\}}{\left\{[\text{M(L-L)}]^{2+}\right\} \cdot [(\text{L-L})]} \tag{2}$$

$$\beta_{12} = \frac{\left\{\left[\text{M(L}-\text{L)}_2\right]^{2+}\right\}}{\left\{[\text{M}^{2+}] \cdot [\text{L-L}]^2\right\}} = K_{11}K_{12} \tag{3}$$

2.3. *Potentiometric measurements*

Potentiometric titrations of the ligand and its copper(II) complexes in aqueous solutions in the presence of 0.1 M KNO_3 were performed at 25 °C. The CO_2-free sodium hydroxide solution at 0.036 M was used as a titrant. The samples were titrated using a total volume of 2.5 mL in the pH range 2.4–11.5 for pm, 3.6–11.0 for pn and 2.4–10.0 for pl, respectively. Changes in the pH were monitored with a combined glass/calomel electrode (Mettler Toledo) calibrated in pH standard buffers [35]. The ligand concentration was 10^{-4} M, and metal-to-ligand molar ratios 1 : 1 and 1 : 2 were used. The relationship between the activity and concentration was calculated daily by titration with HNO_3 [36].

The pH-metric titrations were carried out in a thermostated measuring cell, fitted with a magnetic stirrer. The temperature was kept at 25 °C by means of a thermostat system. The pH titrations were carried out with the CerkoLab automatic microtitrator, equipped with the Hamilton's syringe (0.5 mL) and combined glass/calomel electrode (Mettler Toledo). The pH electrode was standardized with aqueous standard buffers (from StanLab). The resolution of the voltage measurements was <0.1 mV.

2.4. *Conductometric titrations*

The conductance measurements were performed using the ELMETRON CC-401 conductivity meter equipped with a glass conductivity cell. The cell was calibrated with a standard KCl solution as described earlier [37] and the cell constant was 1.15 cm^{-1}. The temperature was controlled by the Lauda E100 circulation thermostat. All measurements were carried out at the constant temperature (±0.1 °C) in a jacketed cell by circulating water around the measured solution.

During the conductometric titration the solution of copper(II) perchlorate (1.48×10^{-4} mol dm^{-3}, 5 mL) was placed in the conductivity cell. The solution of vitamin B_6 (1.00×10^{-3} mol dm^{-3}) was added stepwise to the cell using an automatic titrator with the Hamilton's syringe (2.5 mL). After equilibration and thermostating, the conductivity of the solution was measured. The titration curves were carried out at a low-total metal ion concentration to keep the ionic strength during the titration and to minimize an effect of ion association on the calculated equilibrium constants. The procedure was repeated until the desired ligand to metal ratio was attained.

The molar conductivity (Λ_{exp}) of solution containing the Cu^{2+} ion and ligand (L) can be written as:

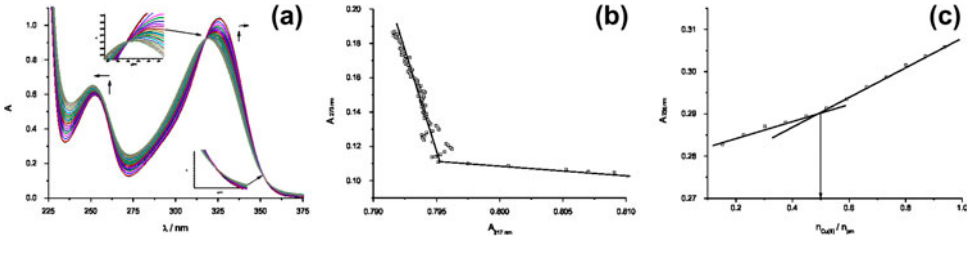

Figure 2. (a) Spectrophotometric titration curves of pyridoxamine hydrochloride (1.00×10^{-4} mol L^{-1}) using a mixture of pyridoxamine at the same concentration and $Cu(ClO_4)_2 \cdot 6H_2O$ (1.48×10^{-5} mol L^{-1}), (b) the A-diagram plot for Cu(II) with pyridoxamine complexation, major stoichiometry 1 : 2, and (c) dependence of absorbance at 235 nm for pm as a function of molar ratio $n_{Cu(II)}/n_{pm}$.

$$\Lambda_{exp} = \Lambda_{Cu^{2+}}\alpha + \Lambda_{CuL}\beta + \Lambda_{CuL_2}\gamma$$

where $\Lambda_{Cu^{2+}}$, Λ_{CuL}, and Λ_{CuL_2} are the molar conductance and α, β and γ are the fractions of the free metal ion, the first and second complex, respectively. By varying the values of K_1 and K_2 and minimizing the square sum of errors between the experimental and the molar conductivities (Gauss–Newton–Marquardt method), the formation constants were calculated.

3. Results and discussion

3.1. *Spectrophotometric measurements*

The interaction of Cu(II) with three forms of vitamin B_6 induces changes in the absorption band positions after complexation. To determine the stability constants of pyridoxamine with copper(II), the ligand studied was titrated by solution of metal perchlorate salt and pyridoxamine. The slight redshift to longer wavelengths corresponding to free ligand is presented in figure 2(a). Two isosbestic points appear at 318 and 353 nm, respectively. These spectral changes suggest the existence of metal ion interactions with the donor of ligand in the system studied.

The A-diagrams (the dependence of the absorbance at 273 nm to the absorbance at 317 nm) were plotted to illustrate specific quantity equilibria constants in the system [figure 2(b)]. Two straight sections presented on the plot confirmed two equilibria during the chelation process.

The dependence of absorbance at 235 nm as a function of $n_{Cu(II)}/n_{pm}$ for pyridoxamine was confirmed [figure 2(c)]. The major stoichiometry of complex formation is 1 : 2, which corresponds to 0.5 M ratio.

During the titration process of pyridoxine by the copper(II) perchlorate solution containing pn a slight blueshift to shorter wavelengths occurs and two isosbestic points at 266 and 304 nm can be observed [figure 3(a)]. The changes in values of absorbance are associated with a chelation of nitrogen and oxygen to copper(II).

The A-diagrams were analyzed to determine the exact number of equilibria present in the system studied. The two straight sections in the dependence of the absorbance at 304 nm as

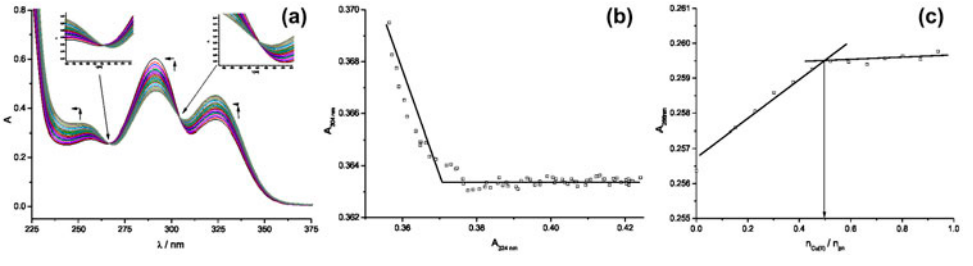

Figure 3. (a) Spectrophotometric titration curves of pyridoxine hydrochloride (1.00×10^{-4} mol L^{-1}) using a mixture of pyridoxine at the same concentration and $Cu(ClO_4)_2 \cdot 6H_2O$ (1.48×10^{-5} mol L^{-1}), (b) the A-diagram plot for Cu(II) with pyridoxine complexation, major stoichiometry 1 : 2, and (c) dependence of absorbance at 266 nm for pm as a function of molar ratio $n_{Cu(II)}/n_{pn}$.

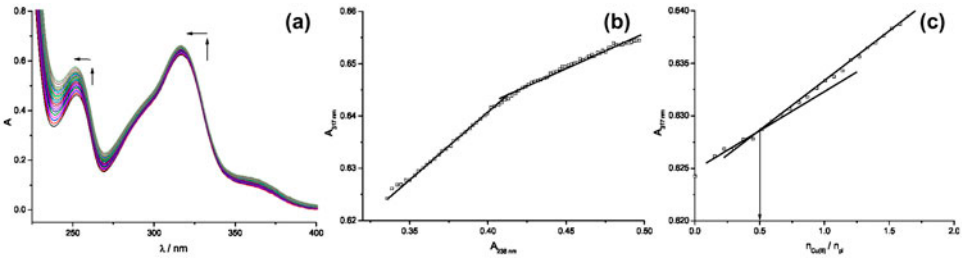

Figure 4. (a) Spectrophotometric titration curves of pyridoxal hydrochloride (1.00×10^{-4} mol L^{-1}) using a mixture of pyridoxal at the same concentration and $Cu(ClO_4)_2 \cdot 6H_2O$ (1.48×10^{-5} mol L^{-1}), (b) the A-diagram plot for Cu(II) with pyridoxal complexation, major stoichiometry 1 : 2, and (c) dependence of absorbance at 317 nm for pl as a function of molar ratio $n_{Cu(II)}/n_{pl}$.

a function of absorbance at 324 nm confirmed the existence of two equilibria during the formation process [figure 3(b)].

The dependence of absorbance at 266 nm for pyridoxine as a function of molar ratio $n_{Cu(II)}/n_{pn}$ is presented in figure 3(c). The tangentials to the diagram intersect at 0.5 value of molar ratio which indicates 1 : 2 stoichiometry of the coordination compounds.

The solution of pyridoxal was titrated by the mixture of copper(II) perchlorate with pl. The intensities of absorption bands gradually increase and hypsochromic shift can be observed [figure 4(a)]. The presented spectral changes are the results of interactions between the metal ion and the donors of the ligand.

To define an accurate number of equilibria presented in the system studied, the A-diagrams were plotted. The dependence of absorbance at 238 nm as a function of absorbance at 317 nm showed two straight sections [figure 4(b)], suggesting that two formation constants could be determined in this case.

These observations were confirmed by the dependence of absorbance at 317 nm for pyridoxal as a function of molar ratio $n_{Cu(II)}/n_{pl}$ [figure 4(c)]. The complexes of Cu(II) were formed according to 1 : 2 metal : ligand stoichiometry, which was consistent with the values of molar ratio equal to 0.5.

3.2. *Potentiometric measurements*

The protonation constants for the pyridoxine, pyridoxamine, and pyridoxal were determined and are given in table 1. Potentiometric titration curve obtained for aqueous solution of pn clearly shows two steps. They corresponded to the protonated nitrogen and the phenolic hydroxyl oxygen of the ligand, respectively. In the case of pm two potentiometric steps

Table 1. Protonation constants of three forms of vitamin B_6 (H_2L^+) at 25 °C.

	CVEQUID		Refs. [38, 39]	
Ligand	pK_1	pK_2	pK_1	pK_2
Pyridoxamine (pm)	3.31 (±0.10)	7.90 (±0.22)	3.40	8.21
Pyridoxine (pn)	4.86 (±0.17)	8.84 (±0.15)	5.00	8.96
Pyridoxal (pl)	4.26 (±0.11)	8.70 (±0.20)	4.20	8.66

Note: Data taken from the literature are given for comparison.

were also observed which related to the protonation of phenolic oxygen of hydroxymethyl group and aminomethyl nitrogen of addend. The obtained experimental values of protonation constants of vitamin B_6 were in agreement with literature data [40, 41].

In the Cu(II)-pm system $Cu(Hpm)_2$ and $Cu(pm)_2$ species were identified. The pyridoxamine can act as a unidentate ligand which binds the metal ion through heterocyclic nitrogen. Additionally, this ligand can chelate the metal ion through the phenolic oxygen and nitrogen or oxygen at one of the adjacent substituents in the four position [42, 43]. The value of first cumulative constant ($\log \beta_1 = 6.37$) shows that pm binds copper(II) bidentate. As the aminomethyl moiety is more basic than the hydroxymethyl group, pm chelates Cu(II) via phenolic oxygen and aminomethyl nitrogen forming a six-membered chelate ring. This mode of coordination of pyridoxamine is observed in the case of Ni(II), Zn(II), and Cd(II), respectively [44]. The $\log K_2$ value of 4.84 indicates the bidentate binding mode of pm. In the case of $Cu(II)(Hpm)_2$ species, the four positions would be occupied by solvent water molecules. In $[Cu(II)(pm)_2]$ species, the ligand occupies the four positions and two water molecules are present in its coordination sphere.

The titration curve obtained for the Cu(II)-pm system is presented in figure 5(a). The experimental potential (E) value (black square) and that fitted with the use of CVEQUID program (red line) are in agreement for the studied system. A comparison of these data leads to the conclusion that there is a good correlation between experimental and calculated results of the examined complex. Moreover, it can also be concluded that spectrophotometric and potentiometric methods are accurate, easy to apply, and suitable for the stability constant determination of coordination compounds.

The species distribution diagram of Cu(II) complex containing pm is shown in figure 5(b). The formation of $Cu(Hpm)_2$ adduct is dominant above pH 4 and its maximum concentration is close to 64%. In the pH range 6.0–11.0 $Cu(pm)_2$ appears and at pH near 9.3 it reaches a maximum concentration of 85%. $Cu(OH)_2$ is also formed above pH 11.5.

In the binary system Cu(II)-pyridoxine, two complex species $Cu(Hpn)_2$ and $Cu(pn)_2$ were observed in solution. $Cu(Hpn)_2$ can be assumed as a monoacid and its $pK_{(Cupn)2}^{H}$ value should be close to the pK_2 value of pyridoxine. The magnitude of $pK_2 - pK_{(Cupn)2}^{H}$ suggested that $Cu(Hpn)_2$ can deprotonate easily. Hence, the complex $Cu(pn)_2$ could be formed more easily or would be the most stable species among complexes studied.

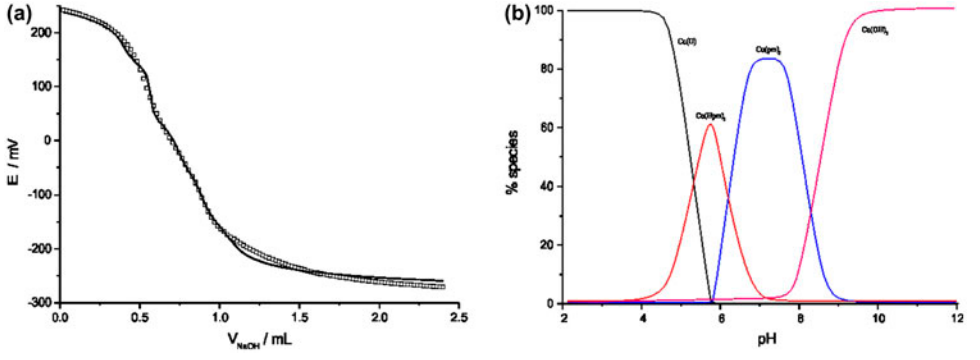

Figure 5. (a) Potentiometric titration curve of a Cu(II) and pyridoxamine hydrochloride solution using NaOH (47 mmol L^{-1}); experimental points and fitting line – the result of calculations, $R^2 = 0.983$ and (b) concentration distribution of various species as a function of volume in the Cu(II)-pyridoxamine system at 0.1 M ionic strength (KNO_3) at 25 °C.

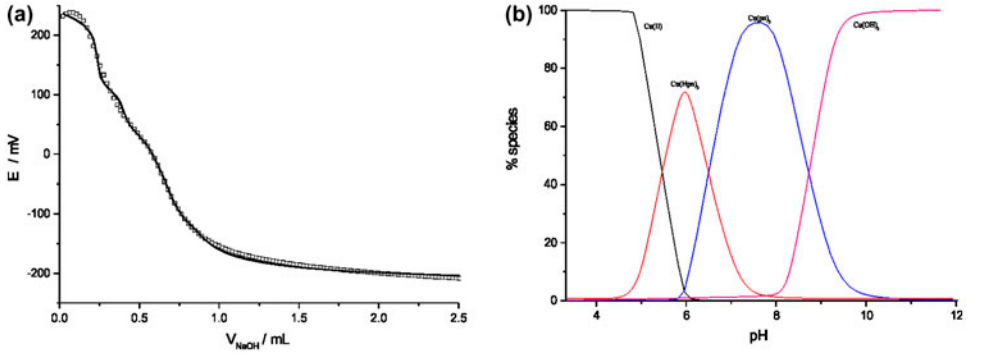

Figure 6. (a) Potentiometric titration curve of a Cu(II) and pyridoxine hydrochloride solution using NaOH (47 mmol L^{-1}); experimental points and fitting line – the result of calculations, $R^2 = 0.997$ and (b) concentration distribution of various species as a function of volume in the Cu(II)-pyridoxine system at 0.1 M ionic strength (KNO_3) at 25 °C.

The literature data propose that heterocyclic nitrogen of pn is a coordination atom in the CuL complex. According to earlier studies, vitamin B_6 exists under a Zwitterionic form around neutral pH. The deprotonation occurs mainly on the C-3 site (phenolic group) and partially on the pyridinium group in the pH range from 2 to 7 [45]. Moreover, the relative position of OH in C-3 site may have a significant influence on the structure and stability of the complex species formed [46]. Furthermore, in pyridoxine, besides phenolic hydroxyl there is an adjacent alcoholic hydroxyl occupying the C-4 site, which should be beneficial to form a more stable six-membered chelate ring. The formation constants of M(II) complexes with pyridine are by 1-2 logarithm units higher than that in the case of free pyridine. This suggests that in the $Cu(pn)_2$ complex the phenolic oxygen in the C-3 position and alcoholic oxygen in the C-4 site of the pyridoxine are involved in complexation of copper(II).

The experimental potential (E) and the value calculated according to the presence of observed complex species are in agreement for the Cu(II) pn system [figure 6(a)]. This confirms veracity and reliability of determined formation constant values.

On the basis of the distribution of the species formation in the Cu(II)-pyridoxine system, shown in figure 6(b), the formation of $Cu(Hpn)_2$ was already observed above pH 5. The maximum concentration of this form did not exceed 75%. The $Cu(pn)_2$ compound started to form from pH close to 6.6 and reached its maximum concentration of 95%. The $Cu(OH)_2$ form appeared at pH 9.

For the Cu(II)-pl system two complex species $Cu(Hpl)_2$ and $Cu(pl)_2$ are formed in solution. The hydroxyl group which is coordinated to copper(II) is the phenolic hydroxyl in the C-3 site of pyridoxal. Moreover, the position of aldehyde moiety existing in the ligand may have a critical influence on the compound structure and on the stability of species formed. In this ligand, besides the phenolic group in the C-3 position, there is an adjacent CHO group occupying the C-4 site, which should be favorable to form a stable chelate (six-membered) ring. The ionization constant of the free aldehyde form is 3.8. The low pK value of this group in the complex is due to the influence of its coordinative bond with copper(II). Furthermore, the aldehyde form (dipolar ion) is stabilized by resonance. The low pK value of phenolic hydroxyl confirms that this group chelates Cu(II). It may result from a lower effective charge separation in this compound. The negative charge of the phenolate ion is distributed in part over the carbons of the pyridine ring. In addition, the pyridoxal can exist

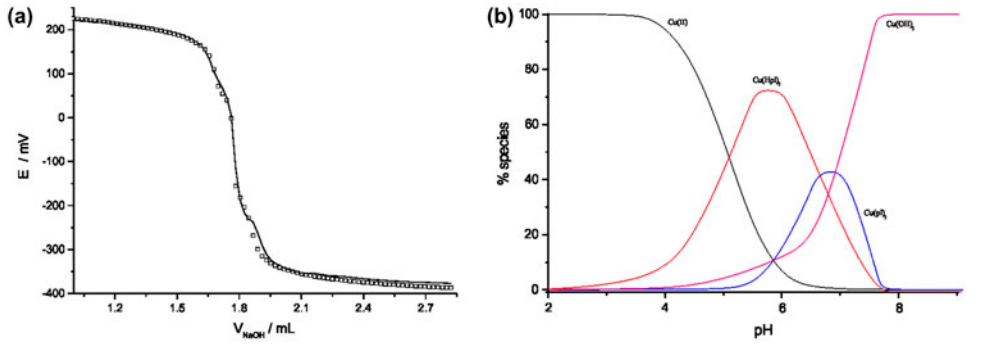

Figure 7. (a) Potentiometric titration curve of a Cu(II) and pyridoxal hydrochloride solution using NaOH (47 mmol L^{-1}); experimental points and fitting line – the result of calculations, $R^2 = 0.991$ and (b) concentration distribution of various species as a function of volume in the Cu(II)-pyridoxal system at 0.1 M ionic strength (KNO_3) at 25 °C.

as a hemiacetal [47]. At pH 1 this form of pl predominates. In alkaline solutions the amount of the free aldehyde form increases [37]. These findings show that estimated coordination sites were consistent with determined values of stability constants.

For the Cu(II)-pyridoxal system, the experimental potential (E) values and those calculated according to the observed complexes are very close [figure 7(a)]; this observation confirms the veracity of selected complexes and the reliability of reported pK values.

The $Cu(Hpl)_2$ forms at pH above 2, as presented in the distribution of species forming in the system [figure 7(b)] with the maximum concentration of 73%. In the pH range 4.5–8.0, the dominant species is $Cu(pl)_2$ which binds about 44% of the total number of ligands. Above pH 8 $Cu(OH)_2$ species are also formed.

3.3. *Conductometric titration*

The stability and selectivity of formed complexes are affected by a number of molecular factors such as the number and character of the donor atoms in the ligand, the polarizability and charge density of the metal ion, the nature of the substituents and the character of the counter anion, and the number and size of chelate rings formed on complexation [48].

Figure 8 shows conductometric titration curves for the reaction of pyridoxamine, pyridoxine, and pyridoxal with copper(II) perchlorate at room temperature in aqueous solutions. The titration of vitamin B_6 produced similar curves. The measurements were performed at constant ligand concentration. This was done by dissolving the metal in the ligand solution to avoid changes in the ligand concentration by dilution. Using a low-metal ion concentration was necessary to insure that the ionic strength remains low and constant during the titration and to facilitate the calculation of the formation constants. Metal salt of the bulky perchlorate ion was used in order to minimize the effect of ion association on the measured conductivities.

The obtained conductometric data (figure 8) reveal that as the metal-to-ligand molar ratio increases, the molar conductivity of the solution decreases. The decrease in molar conductivity starts to descend as the metal to ligand molar ratio of 0.5 is approached for each titration curve. The observed changes in molar conductivity unequivocally indicate that the

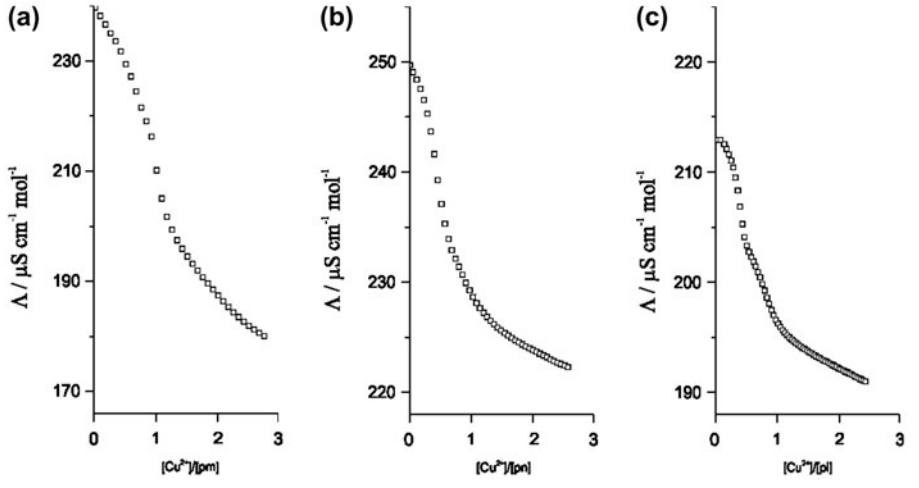

Figure 8. Conductometric titration curves for the reaction of Cu(II) ion with (a) pyridoxamine, (b) pyridoxine, and (c) pyridoxal in aqueous solution at 25 °C.
Notes: The black squares represent the measured molar conductivities and the line is the calculated data.

copper(II) ion reacts with the added vitamin B_6 and can be attributed to the lower ionic mobility of the bulky complexes formed in comparison to that of the free Cu(II) ion. The leveling of the decrease in molar conductivity at metal to ligand stoichiometry of 0.5 indicates that two complexes with formulas of CuL and CuL_2 are formed. The relatively sharp titration end points at Cu(II) to vitamin B_6 molar ratio of 0.5 in the obtained curves of pm, pn, and pl is indicative of the high stability of the complexes formed from the reactions of two ligands with the Cu(II).

The gradual and cumulative stability constants of the complexes formed by the chelation of metal ion by selected ligands were calculated using the procedure outlined in the Experimental section. The measured values of log K_1 and log K_2 are the average of three measurements and the standard deviation of the measurements were less than 0.27.

Our experimental measurements prove that two stability constants can be determined for all system studied. The formation constants of copper(II) complexes with vitamin B_6 obtained by applying the spectrophotometric, potentiometric, and conductometric titration methods are given in table 2.

Inspection of the data indicates very good agreement of results of the gradual and cumulative stability constants of the compounds formed in aqueous solutions obtained using three independent titration techniques. A comparison of the values of the stability constants shows that pyridoxamine complex is the most stable, while that with pyridoxal is least stable. Values of log K_1 and K_2 in table 2 shows also that the CuL_2 complexes are about two orders of magnitude more stable than the CuL complexes, indicating that formation of the first step complex is more favored than the second step complex. This general behavior has been observed in stability constants of a wide range of complexes and has been statistically confirmed by considering the number of sites available for the association and dissociation of the ligands [49].

Table 2. Stability constants of Cu(II) complexes with three forms of vitamin B_6 formed in aqueous solutions determined with the use of three different techniques: potentiometry, spectrophotometry, and conductometry.

	Potentiometric method			Spectrophotometric method			Conductometric method		
Complex cation	log K_1	log K_2	log β	log K_1	log K_2	log β	log K_1	log K_2	log β
$[Cu(pm)_2]^{2+}$	6.37 (±0.17)	11.21 (±0.27)	17.58	6.24 (±0.15)	11.15 (±0.10)	17.39	6.15 (±0.21)	11.05 (±0.12)	17.12
$[Cu(pn)_2]^{2+}$	4.41 (±0.13)	8.29 (±0.25)	12.70	4.53 (±0.15)	8.08 (±0.23)	12.73	4.33 (±0.17)	8.17 (±0.11)	12.50
$[Cu(pl)_2]^{2+}$	2.65 (±0.15)	7.11 (±0.18)	9.76	2.77 (±0.08)	6.94 (±0.12)	9.71	2.81 (±0.13)	6.84 (±0.25)	9.65

Note: All results were determined at 25 °C.

4. Conclusion

The stability constants for complexation of copper(II) with vitamin B_6 were determined spectrophotometrically, potentiometrically, and conductometrically in water. The results obtained from experimental measurements have enabled us to conclude that complexes of Cu(II) are stable in aqueous solutions, as confirmed by values of gradual and cumulative stability constants. They can be ordered according to their increasing stability as follows:

$$\left[\mathrm{Cu(pm)_2}\right]^{2+} > \left[\mathrm{Cu(pn)_2}\right]^{2+} > \left[\mathrm{Cu(pl)_2}\right]^{2+}$$

In this rank copper(II) forms the most stable complex with pyridoxamine, while pyridoxal the weakest one.

The potentiometric titration method supported by the spectrophotometry and conductometry technique has been successfully applied to determine stoichiometry and stability constants of Cu(II) complexes with pyridoxamine, pyridoxine, and pyridoxal. It has been found that formation of binary coordination compounds can be described as a gradual process where the 3-hydroxypyridine derivatives are bidentate ligands.

Conductometric titration has been used to investigate the stability of vitamin B_6 with copper(II) ion. The reported results demonstrate that conductivity can be used to determine the formation constants in aqueous solution and to elucidate the factors that affect the stability of metal complexes.

The determined stability constants have nearly the same values or are close in magnitude although the formation process was evaluated by three independent techniques (spectrophotometric, potentiometric, and conductometric). The slight differences in obtained values of stability constants are due to fact that data were analyzed by three different approaches in the three methods, and each has its own intrinsic error limit.

Our previous study of nickel(II) ion with vitamin B_6 coordination compounds has confirmed that the complex stability increases with the increasing atomic number (Z) of the metal ion and ionic size. The lower stability of analogous Ni(II) complexes with vitamin B_6 can be attributed to the higher tendency of nickel to form covalent bonds with the organic ligands.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was financially supported by the University of Gdansk [grant number BMN 538-8236-B698-15], [grant number BMN 538-8236-B663-15], and [grant number DS/530-8236-D601-15].

References

[1] M. O'Connor, A. Kellett, M. McCann, G. Rosair, M. McNamara, O. Howe, B.S. Creaven, S. McClean, A.F.A. Kia, D. O'Shea, M. Devereux. *J. Med. Chem.*, **55**, 1957 (2010).
[2] K.K. Mukherjea, G. Panda, M. Selim. *Transition Met. Chem.*, **33**, 203 (2008).
[3] J.T. Wang, Q. Xia, X.H. Zheng, H.Y. Chen, H. Chao, Z.W. Mao, L.N. Ji. *Dalton Trans.*, **39**, 2128 (2010).

[4] K.B. Huang, Z.F. Chen, Y.C. Liu, M. Wang, J.H. Wei, X.L. Xie, J.L. Zhang, K. Hu, H. Liang. *Eur. J. Med. Chem.*, **70**, 640 (2013).
[5] A. Prisecaru, M. Devereux, N. Barron, M. McCann, J. Colleran, A. Casey, V. McKee, A. Kellett. *Chem. Commun.*, **48**, 6909 (2012).
[6] S. Roy, R. Banerjee, M. Sarkar. *J. Inorg. Biochem.*, **100**, 1320 (2006).
[7] J. Awapara, R.P. Sandman, C. Hanly. *Arch. Biochem. Biophys.*, **98**, 520 (1962).
[8] C.T. Walsh, W.H. Orme-Johnson. *Biochemistry*, **26**, 4901 (1987).
[9] D.P. Singh, K. Kumar, C. Sharma. *Spectrochim. Acta, Part A*, **75**, 98 (2010).
[10] S.A. Denslow, A.A. Walls, M.E. Daub. *Phys. Mol. Plant Pathol.*, **66**, 244 (2005).
[11] A. Gliszczyńska-Swiglo, H. Szmusiak. *Pol. J. Food Nutr. Sci.*, **57**, 163 (2007).
[12] M.A. Makhyoun, N.A. Al-Salem, M.S. El-Ezaby. *Inorg. Chim. Acta*, **123**, 117 (1986).
[13] J.H.K.A. Acquaye, M.F. Richardson. *Inorg. Chim. Acta*, **201**, 101 (1999).
[14] J.S. Casas, A. Castiñeiras, F. Condori, M.D. Couce, U. Russo, A. Sánchez, J. Sordo, J.M. Varela, E.M. Vázquez-López. *J. Organomet. Chem.*, **689**, 620 (2004).
[15] S. Dey, P. Banerjee, S. Gangopadhyay, P. Vojtíšek. *Transition Met. Chem.*, **28**, 765 (2003).
[16] V.K. Sabirov, A.S. Batsanov, YuT Struchkov, M.A. Azizov. *Russ. Coord. Chem.*, **9**, 1701 (1983).
[17] A.C. Chamayou, M.A. Neelakantan, S. Thalamuthu, C. Janiak. *Inorg. Chim. Acta*, **365**, 447 (2011).
[18] J.H.K.A. Acquaye, M.F. Richardson. *Inorg. Chim. Acta*, **201**, 101 (1991).
[19] J.S. Casas, A. Castiñeiras, F. Condori, M.D. Couce, U. Russo, A. Sánchez, J. Sordo, J.M. Varela. *Polyhedron*, **19**, 813 (2000).
[20] M.S. El-Ezaby, H. Marafie, S. Fareed. *J. Inorg. Biochem.*, **11**, 317 (1979).
[21] M.S. El-Ezaby, T.E. El-Khalafawy. *J. Inorg. Nucl. Chem.*, **43**, 831 (1981).
[22] S. Naskar, S. Naskar, H.M. Mayer-Figge, W.S. Sheldrick, S.K. Chattopadhyay. *Polyhedron*, **30**, 529 (2011).
[23] A.M. Ako, H. Maid, S. Sperner, S.H.H. Zaidi, R.W. Saalfrank, M.S. Alam, P. Müller, F.W. Heinemann. *Supramol. Chem.*, **17**, 315 (2005).
[24] A.E. Martell, R.J. Motekaitis. *The Determination and Use of Stability Constants*, 2nd Edn., VCH Publishers, New York (1992).
[25] C.F. Bell. *Principles and Applications of Metal Chelation*, Clarendon Press, Oxford (1977).
[26] R.J. Motekaitis, A.E. Martell. *Can. J. Chem.*, **60**, 2403 (1982).
[27] W.D. Kim, D.C. Hrncir, G.E. Kiefer, A.D. Sherry. *Inorg. Chem.*, **34**, 2225 (1995).
[28] N. Matsuura, K. Umemoto, Y. Takeda, A. Sasaki. *Bull. Chem. Soc. Jpn.*, **49**, 1246 (1976).
[29] G.A. Lawrance. *Introduction to Coordination Chemistry*, pp. 1939–5175, John Wiley & Sons, Ltd, Chichester (2010).
[30] P. Jiang, Z. Guo. *Coord. Chem. Rev.*, **248**, 205 (2004).
[31] A. Chylewska, M. Ogryzek, R. Hałasa, A. Dąbrowska, L. Chmurzyński, M. Makowski. *J. Coord. Chem.*, **67**, 2885 (2014).
[32] C.J. Marmion, D. Griffith, K.B. Nolan. *Eur. J. Inorg. Chem.*, **2004**, 3003 (2004).
[33] G.Y. An, C.M. Ji, A.L. Cui, H.Z. Kou. *Inorg. Chem.*, **50**, 1079 (2011).
[34] A.I. Vogel. *Vogel's Textbook of Quantitative Inorganic Analysis*, 4th Edn., Longman, London (1978).
[35] H. Irving, M.G. Miles, L.D. Pettit. *Anal. Chim. Acta*, **38**, 475 (1967).
[36] B.K. Sarkar, M.N. Roy, B. Sinha. *Indian J. Chem. A*, **48**, 63 (2009).
[37] D.A. Jose, D.K. Kumar, B. Ganguly, A. Das. *Org. Lett.*, **6**, 3445 (2004).
[38] A.K. Lunn, R.A. Morton. *The Analyst*, **77**, 718 (1952).
[39] V.R. Williams, J.B. Neilands. *Arch. Biochem. Biophys.*, **53**, 56 (1954).
[40] R.M. Smith, A.E. Martell. *Critical Stability Constants*, Plenum Press, New York (1975).
[41] D.E. Metzler, E.E. Snell. *J. Am. Chem. Soc.*, **77**, 2431 (1955).
[42] A. Mosset, F. Nepveu-Juras, R. Haran, J.J. Bonnet. *Inorg. Nucl. Chem.*, **40**, 1259 (1978).
[43] M. Bojczuk-Jeżowska, H. Kozłowski, P. Docock, P. Cerny, T. Trnka. *Carbohydr. Res.*, **216**, 453 (1991).
[44] M.S. El-Ezaby, M. Rashad. *Polyhedron*, **2**, 245 (1983).
[45] E. Chiacchierini, G.D. Ascenzo, G. De Angelis, A.L. Margi, V. Peetrone. *Ann. Chim. (Rome)*, **67**, 195 (1977).
[46] M.M. Shoukry, E.M. Khairy, M.M. Mohamed. *Talanta*, **44**, 1149 (1997).
[47] J. Llor, J.M. Sanchez-Ruiz. *J. Chem. Soc., Perkin Trans.*, **2**, 951 (1988).
[48] V. Gutmann. *The Donor-acceptor Approach to Molecular Interactions*, p. 26, Plenum Press, New York (1978).
[49] H.M. Irving, R.J.P. Williams. *J. Chem. Soc.*, **28**, 3192 (1953).