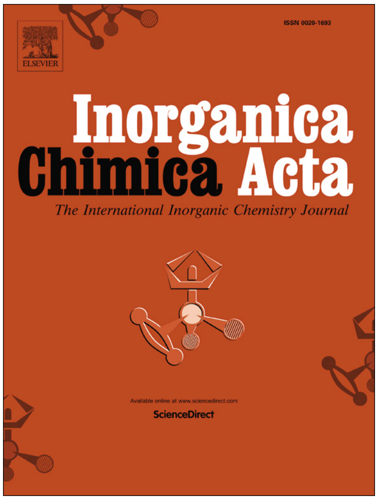

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Interactions of N-heteroalkylaminomethylenebisphosphonic acids with Cd(II) ions: Electrochemical and spectroscopic investigations

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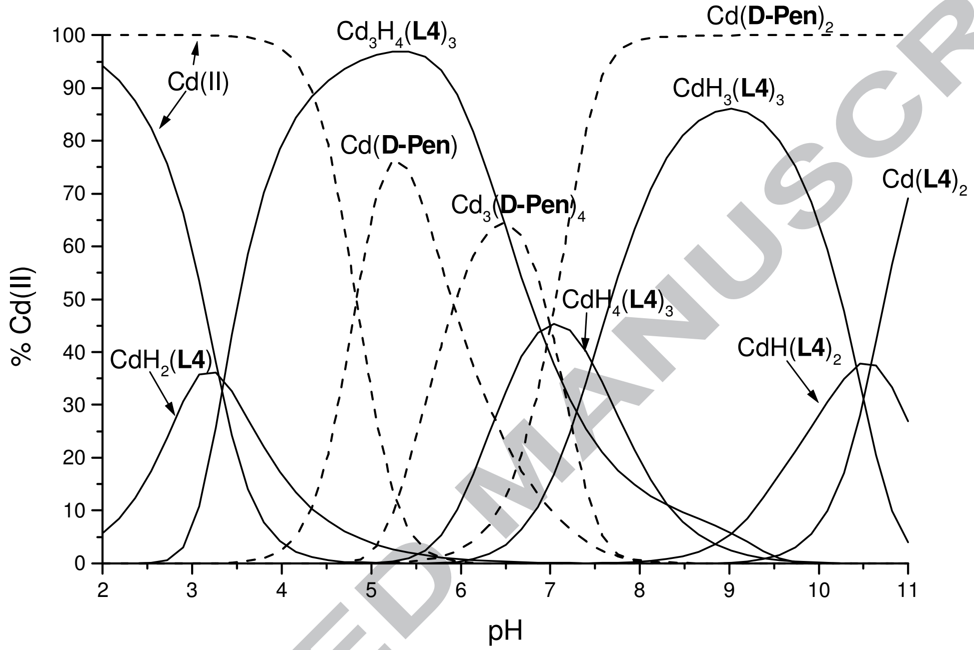
Abstract:

The aminomethylenebisphosphonates are known to be excellent chelators for many metal ions. In this work, the coordination properties of three different aminomethylenebisphosphonates with N-substituted heteroalkyl moieties (**L1, L2** and **L3**) and their N-pyridyl derivative (**L4**) toward cadmium(II) ions are described. Due to their coordination abilities over a broad range of pH, the compounds of this group are good candidates for heavy metal detoxification.

To determine the stability constants and the coordination mode of formed aminomethylenebisphosphonate-cadmium(II) complexes, four analytical methods were employed: potentiometry, pulse polarography (DPP), nuclear magnetic resonance spectroscopy (NMR) and electrospray ionisation mass spectrometry (ESI-MS). The studies performed revealed that ligands **L1, L2** and **L3** possessed similar coordination properties, with Cd(II) ions binding below pH 2 to form equimolar complexes and above pH 6 to form bis-complexes. The ligand **L4** behaved in a different way, forming six complexes of varied stoichiometry: CdH_4L_3, CdH_3L_3, $CdHL_2$, CdL_2, CdH_2L and $Cd_3H_4L_3$. The hypothetical competition plot between common complexone D-penicillamine and the studied ligands showed better complexation properties for the aminomethylenebisphosphonates in the pH range 2-6 than for neutral pH, where above neutral pH D-penicillamine bound Cd(II) ions significantly better.

Keywords: aminobisphosphonates, metal ions, coordination, Cd(II) ions, polarography, potentiometry, mass spectrometry MS, nuclear magnetic resonance NMR

Graphical Abstract:



Highlights:

- Aminobisphosphonic acids revealed strong chelating abilities for Cd(II) ions.
- The potentiometric data were supported with differential pulse polarography, NMR spectroscopy and ESI-MS.
- Binding abilities of aminobisphosphonates were compared to the common metal chelator, penicillamine performing better in the acidic pH range, comparably in the neutral range and worse in the basic pH range.

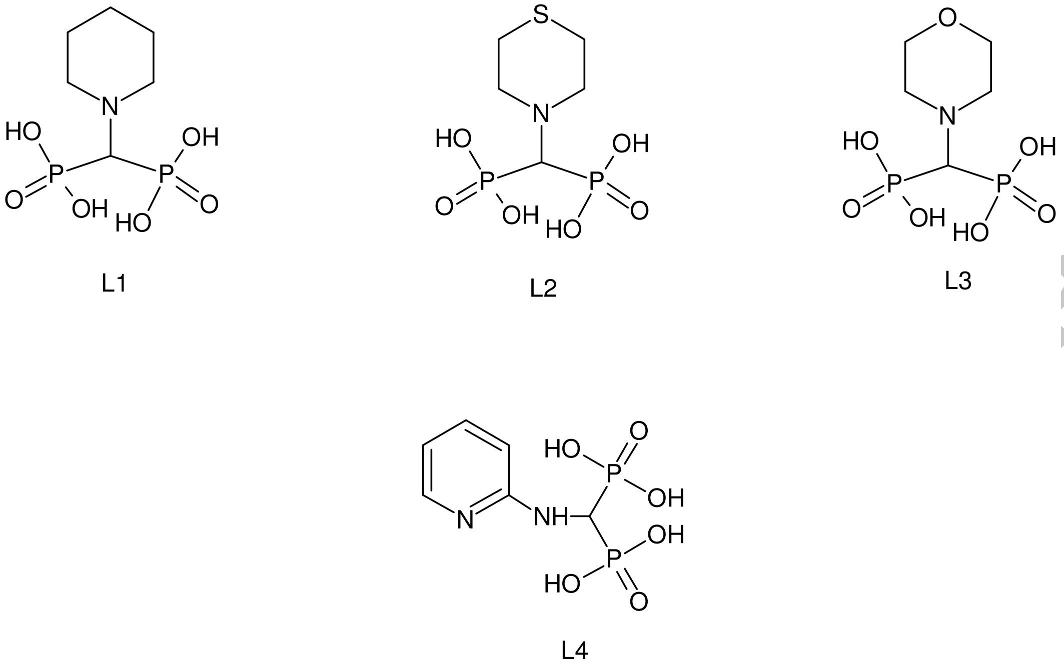
1. Introduction

Bisphosphonates are a group of pyrophosphate analogues containing a P-C-P scaffold in place of the P-O-P fragment. Despite limitations resulting from their low uptake, they are the drugs of choice in curing postmenopausal osteoporosis [1,2]. The most potent bisphosphonates are those containing amino groups in their alkyl side chain. Aminomethylenebisphosphonates are the fourth generation of these drugs, with zolendronate the most successful example [3,4].

Aminomethylenebisphosphonates have been used in medicine as oncological therapeutics because they are able to inhibit tumor cell migration, bone adhesion and apoptosis-limiting properties [5]. These compounds have also been used in cancer immunotherapy as activators of γδ T lymphocytes [6].

Cadmium, a heavy metal, is a significant environmental pollutant [7]. Small quantities of this metal occur naturally in water, soil, air and unfortunately in food [8]. Due to their ubiquity, cadmium ions often pass into the human body. They can be a serious health hazard, not only to humans but to all living organisms, because of their ability to bind certain metalloenzymes and enzyme substrates containing thiol groups [9]. Cd(II) ions are chemically similar to Zn(II) and can replace them in apoproteins, inhibiting or distorting the catalytic activity of the metalloenzymes [10]. The best example of Zn(II)⇆Cd(II) replacement occurs in metallothioneins (MT) [11]. In the absence of cadmium(II) ions, metallothioneins contain seven Zn(II) ions. When Cd(II) ions appear in the human body, Zn(II) ions are substituted with Cd(II) in newly synthesized MT. Cd-MT and Zn-MT have identical stoichiometry [11].

Because of high cadmium toxicity, it is important to have coordinating agents, which can bind cadmium ions to remove them from the environment and living organisms. Bisphosphonates, which are well known to bind Zn(II) effectively and have short residence times in the human body, seem to be obvious candidates for that function. There are numerous literature data describing properties of Zn(II) complexes with aminobisphosphonates but only a few studies demonstrating interaction of Cd(II) ions with these compounds [12,13]. For example in the studies performed by Matczak-Jon et al. the same ligands as in this work (**L1, L2** and **L3**, Scheme 1) were tested towards coordination properties of Zn(II), Mg(II) and Ca(II) ions [14,15].



Scheme1. Studied ligands:

L1 – (piperyd-1-yl)aminomethanebisphosphonic acid;

L2–(thiomorpholin-1-yl)aminomethane-bisphosphonic acid;

L3- (morpholin-1-yl)aminomethanebisphosphonic acid;

L4 - (pyridin-2-yl)aminomethanebisphosphonic acid

There are some crystallographic data available, concerning Cd(II) binding into the phosphonic group. One of them is the work of Li et al. [16] who showed that cadmium(II) ions in triphosphonic-cadmium(II) complexes are mainly coordinated octahedrally by phosphonate oxygen atoms from the chelating agents. On the other hand Sun et al. characterized some cadmium(II) structures of aminodiphosphonates [17].

The main goal of this work was to find both the coordination models and the formation constants of complexes formed between cadmium(II) ions and three aminomethylenebisphosphonates (**L1, L2** and **L3**) to determine the characteristics and strength of the formed complexes. For comparison, the same properties of the intensively studied N-pyridyl derivative (**L4** - Scheme 1) have been determined [18,19,20].

2. Experimental

2.1. Materials and reagents:

General Information

All solvents and reagents were purchased from commercial suppliers, were of analytical grade (A. R.) and were used without further purification. Unless otherwise specified, solvents were removed with a rotary evaporator. ^{1}H, ^{31}P and ^{13}C NMR experiments were performed on a Bruker BioSpin operating at 500.14 MHz (^{1}H), 202.46 MHz (^{31}P) and 125.76 MHz (^{13}C). Measurements were made in D_2O+ NaOD (99.9 at. % D). The solution temperature was 300 K, and the solvent was supplied by ARMAR AG (Dottingen, Switzerland). Chemical shifts are reported in parts per million relative to TMS or 85% H_3PO_4 used as external standards, and coupling constants are reported in Hertz. Melting points were determined on an Electrothermal 9200 apparatus and are reported uncorrected. Elemental analyses were performed at the Department of Chemistry at the University of Wroclaw on a Perkin Elmer 2400 CHN. Electrospray mass spectra were recorded at the Faculty of Chemistry at Wroclaw University of Technology using a Waters LCT Premier XE mass spectrometer with ESI used as the method of ionization.

2.1.1. Bisphosphonate synthesis

The diphosphonic acids were obtained according to the previously described procedure [21]. The purity of the ligands was established by NMR spectroscopy.

General procedure of the synthesis:

A mixture of amine (0.03 mol), trialkyl (preferently triethyl) phosphite (0.062 mol) and trialkyl (preferently triethyl) orthoformate (0.032 mol) was heated with stirring at elevated temperature (80-120°C) for 14 - 18 h. Then, the volatile components of the reaction mixture were evaporated and the resulting crude product was dissolved in chloroform (150 ml). The organic layer was washed with water (150 ml), then with saturated sodium chloride (150 ml) and again with water (150 ml) to remove any excess diethyl phosphite. The chloroform layer was then dried over sodium sulfate. The drying agent was removed by filtration, and the solvent was removed under reduced pressure. Crude esters were then subjected to hydrolysis.

Hydrolysis – general procedure

The obtained ester (0.030 M) was refluxed for 8-12 hours in 20 ml 6M aqueous hydrochloric acid solution. Then, the volatile components were evaporated, and the resulting crude bisphosphonate was purified by recrystallized from water or water-ethanol mixture.

*(Piperyd-1-yl)aminomethanebisphosphonic acid (**L1**)* was obtained in 64% (4.97 g) yield as a white solid; m.p. 242-243°C (lit. 250-255°C [22], 245-247°C [23]); ^{31}PNMR (202.46 MHz, D$_2$O+NaOD, ppm): δ= 8.54; ^{1}HNMR (500.14 MHz, D$_2$O+ NaOD, ppm): δ= 1.47 (2H, d, *J*= 4.65), 1.68 (4H, d, *J*=4.90 Hz,), 2.94 (1H, t, *J*=18,86 Hz, PC$\underline{H}$P), 3.43 (4H, t, *J*=6.35 Hz) ppm; ^{13}CNMR (125.76 MHz, D$_2$O+NaOD, ppm): δ= 21.32, 24.90, 53.51, 66.95 & 66.99 (t, Hz, *J*=114.62 Hz,P$\underline{C}$P); HRMS (ESI-TOF) m/z: [M-H]$^+$ Calcd for $C_6H_{15}NO_6P_2$ 258.0294, Found 258.0288.

*(Thiomorpholin-1-yl)aminomethanebisphosphonic acid (**L2**)* was obtained in 53% (4.41 g) yield as a white solid; m.p. 239-240°C (lit. 250°C [24]); ^{31}PNMR (202.46 MHz, D$_2$O+NaOD, ppm): δ= 16.39; ^{1}HNMR (500.14 MHz, D$_2$O+ NaOD, ppm): δ= 2.57 (4H, t, *J*=4.95 Hz), 2.66 (1H, t, *J*=22.41 Hz, PC$\underline{H}$P),3.18 (4H, t, *J*=4.79 Hz,), ppm; ^{13}CNMR (125.76 MHz, D$_2$O+NaOD, ppm): δ= 27.82 (t, *J*=5.72 Hz) 53.13, 68.44 & 68.49 (t, *J*=123.68 Hz, P$\underline{C}$P); HRMS (ESI-TOF) m/z: [M-H]$^+$ Calcd for $C_5H_{13}NO_6P_2S$ 275.9861, Found 275.9867.

*(Morpholin-1-yl)aminomethanebisphosphonic acid (**L3**)* was obtained in 59%(4.62 g) yield as a white solid; m.p. 247°C (lit. 250-260°C [22], 248-250°C [23]); ^{31}PNMR (202.46 MHz, D$_2$O+NaOD, ppm): δ= 16.39; ^{1}HNMR (500.14 MHz, D$_2$O+ NaOD, ppm): δ=2.71 (1H, t, *J*=22.10 Hz, PC$\underline{H}$P), 3.00 (4H, t, *J*=4.20 Hz,), 3.61 (4H, t, *J*=4.52 Hz) ppm; ^{13}CNMR (125.76 MHz, D$_2$O+NaOD, ppm): δ= 51.12 (t, *J*=4.85 Hz), 66.66 & 66.67 (t, *J*=123.62 Hz, P$\underline{C}$P), 67.57; HRMS (ESI-TOF) m/z: [M-H]$^+$ Calcd for $C_5H_{13}NO_7P_2$ 260.0089, Found: 260.0081. Anal. Calcd for $C_5H_{13}NO_7P_2$: C, 23.00; H, 5.02; N, 5.36; P, 23.72. Found: C, 22.98; H, 4.86; N, 5.27; P, 22.10.

*(Pyridin-2-yl)aminomethanebisphosphonic acid (**L4**)* was obtained in 46% (3.71 g) yield as a white solid; m.p. 280-282°C; ^{31}PNMR (202.46 MHz, D$_2$O+NaOD, ppm): δ= 14.03; ^{1}HNMR (500.14 MHz, D$_2$O+ NaOD, ppm): δ=3.81 (1H, t, *J*=18.67 Hz, PC$\underline{H}$P), 6.40 (1H, t, *J*=6.50 Hz, ArH), 6.83 (1H, d, *J*=9.20 Hz ArH), 7.23(1H, d, *J*=4,85 Hz ArH), 7.56 (1H, t, *J*=8,75 Hz, ArH) ppm; ^{13}CNMR (125.76 MHz, D$_2$O+NaOD, ppm): δ= 51.74 (t, *J*=125.76 Hz, P$\underline{C}$P), 111.47, 136.48, 142.74, 152.96; lit ^{1}H, ^{31}P, ^{13}C NMR [18]; HRMS (ESI-TOF) m/z: [M-H]$^+$ Calcd for $C_6H_{10}N_2O_6P_2$ 266.9936, Found: 66.9928. Anal. Calcd for $C_6H_{10}N_2O_6P_2$x ½ H_2O: C, 26.01; H, 4.00; N, 10.11; P, 22.35. Found: C, 26.18; H, 3.81; N, 10.035; P, 21.82.

2.1.2. Metal ions

$Cd(NO_3)_2 \times 4H_2O$ was obtained from POCH S.A. (Poland). The metal ion stock solution was prepared by dissolving the metal salt in distilled, deionized water. It was used as the source of cadmium(II) ions in the potentiometric, polarographic and NMR studies. All other chemicals used were A.R. grade quality and were obtained from Sigma-Aldrich Chem. Co.

2.2. Potentiometric studies:

Potentiometric measurements were made using a MOLSPIN 1000 automatic titration system with a METTLER TOLEDO InLab 422 microcombined electrode. The titrator and the electrode were calibrated daily using standard buffer solutions. All titrations were performed in a purified argon atmosphere, using aqueous 0.098642 mol • dm^{-3} NaOH as the titrant. Approximately 170 titration data points were measured, covering the pH range of 2.2–11.8 for ligand and metal complexes. The pH meter readings were converted into hydrogen ion concentration by titrating a standard acid solution (HNO_3, c= 0.004 mol • dm^{-3}) with a base solution at 25°C and $I = 0.1$ mol • dm^{-3} KNO_3. The concentration of ligands was 0.002 mol • dm^{-3}, and the molar ratio of M:L was 1:1, 1:2 and 1:3. All the solutions were prepared using deionized, distilled water.

Calculations were performed using SUPERQUAD [25]. The model selected was the one that gave the best statistical fit and was chemically consistent with the titration data. The concentration distribution diagrams were obtained using SPECIES [26].

2.3. Polarographic studies:

Polarographic measurements were performed using a MTM M161 electrochemical analyzer (MTM Anko, Kraków, Poland) interfaced with M164C cell stand (MTM Anko, Kraków, Poland) in a three-electrode cell configuration. A hanging mercury drop electrode (HMDE), an Ag/AgCl/KCl 3 M and a platinum wire served as the working, reference and auxiliary electrodes, respectively. Experiments were carried out under an argon atmosphere at 25°C in 0.1 mol • dm^{-3} KNO_3 as medium. The solutions were purged with argon (Ar-99%) for 10 min before each experiment. The differential pulse polarographic (DPP) measurements on HMDE were performed with a pulse amplitude of 25 mV, a scan range from -0.4 V to -0.8 V and at scan rate of 2 or 5 mV/s.

The cadmium ion concentration was approximately 2 • 10^{-4} mol·dm^{-3}. The metal-to-ligand mol ratios ranged from 1:2 to 1:40. All ligands were diluted in 0.1 mol·dm^{-3} KNO_3. The stability constants from the polarographic data were evaluated according to the Lingane method modified by De Ford and Hume [27].

2.4. Spectroscopic studies:

2.4.1. NMR studies

The ^1H and ^{31}P NMR spectra were collected at 600 MHz using a Bruker Avance instrument at 298 K. The ligands were dissolved in D_2O with trimethylsilyl propanoic acid (TSP), using phosphoric acid as an external standard. NMR pD titrations were made using a SevenMulti (Mettler Toledo) pH-meter with an InLab Micro Pro (Mettler Toledo) combined electrode. The concentration of ligand was 4-5 mM.

2.4.2. ESI-MS measurements

The electrospray ionization mass spectra were recorded on a Waters GCT Premier mass spectrometer. The ligand concentration was $1 \cdot 10^{-3}$ mol·dm^{-3} and the metal-to-ligand ratio was 1:3; 5% of MeOH was added to each sample. Each complex was measured at three different pH (4, 7 and 9) reached using small amounts of formic acid and NH_4OH. These pH values were chosen to maximize species concentration in metal-to-ligand systems. The desolvation temperature was 300°C, while the source temperature was 120°C. The spectra were recorded in positive and negative ion mode.

3. Results and discussion:

3.1. Acid-base properties of compounds **L1-L4**

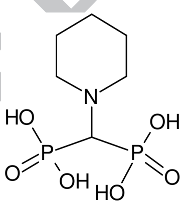
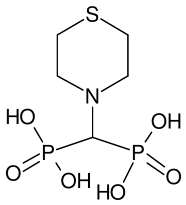
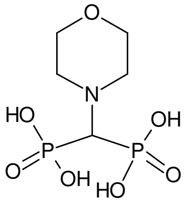
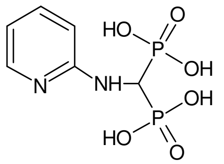
The studied compounds in their fully protonated form possess five dissociable protons $[H_5L]^+$. For ligands **L1**, **L2** and **L3,** four protons can be released from the two phosphonate groups (PO_3H_2) and one from the heterocyclic nitrogen atom, whereas **L4** has an additional pyridine nitrogen.

Ligands **L1, L2** and **L3** have similar structures; thus, their p*K* values are similar to each other (Table 1). Compound **L1** has been previously studied by Matczak-Jon et al., and only two dissociation constants were found for this compound, corresponding to the proton released from the PO_3H^- groups (p*K*=8.16 and p*K*=4.60) [14]. Compounds **L2** and **L3** were also studied by these authors [14], and their p*K* values were similar to those found in our studies. Both phosphonate groups (PO_3H^-), have lower p*K* values than compound **L4** [19].

The first dissociation step can be observed and roughly estimated (p*K* ~ 1.08, Table.1) for only ligand **L4**, which corresponds to the first proton dissociation from PO_3H_2 group, which usually lies in the range of pH 1-2. Measurement difficulty is due to the strong acidity of the first protonation site in phosphonate groups, which is usually unmeasurable. A similarly low pK for this ligand was also noted by Matczak-Jon et al. [18,20]. As shown in Table 1, compound **L4** releases two more protons in the measurable pH range. The proton with pK=5.50 originated from the pyridine nitrogen $NH^{+}_{pyridine}$ at a similar value to those shown in literature data [18]. The second proton is released from a PO_3H_2 group (pK~ 1.08). For this proton, there is also good agreement between the literature and measured dissociation constant values [18,20].

Unlike ligand **L4**, the heterocyclic nitrogen atom of compounds **L1**, **L2**, and **L3** is directly bound to the methanebisphosphonate structural component of the molecule. The strong basicity of the piperidyl nitrogen proton increases this pK. For our titration conditions, it was measured for ligands **L1** (pK ~12.63), **L2** (pK ~12.17) and **L3** (pK~11.63) (see Table.1). Matczak et al. found this constant for compound **L2** to be 11.91 and for **L3** to be 11.65 [14]. The high basicity of the nitrogen can be explained by the presence of an intermolecular hydrogen bond. Such an N-H…O (phosphonate) bond exists, for example, in ligand **L1** [28, 29]. However, protonation values for ligands **L1-L3** seem to be correlated with the electronegativity of the heteroatom in the piperydyl ring when S<O. The same trend in protonation site acidity was also observed in other studies [14].

Table 1. Logarithmic protonation constants (logβ) and pK values for ligands **L1-L4** at 25°C and I=0.1 mol/dm^{-3} KNO_3

	Compound			
Assignment	**L1** 	**L2** 	**L3** 	**L4** 
log β_1	12.63(5)	12.17(2)	11.63(2)	10.32(1)
log β_2	21.35(5)	20.62(3)	19.93(2)	18.13(1)
log β_3	26.26(5)	25.39(3)	24.68(2)	23.63(1)
log β_4	-	-	-	24.71(6)
pK (NH^+)	12.63	12.17	11.63	
pK (PO_3H^-)	8.72	8.45	8.30	10.32

pK (PO_3H^-)	4.91	4.77	3.75	7.81
pK (PO_3H_2)				~1.08
pK (NH^+_{pyr})				5.50

3.2. Cadmium(II) complexes

3.2.1. Potentiometric studies

Potentiometric measurements were used to determine the stability constants of complexes formed between ligands **L1**-**L4** and cadmium(II) ions (Table 2). Subsequently, these stability constants were used to draw speciation diagrams indicating the species formed in solution for a pH range from 2 to 11 (Figure 1).

Table 2. Stability constants (logβ) and pK values for Cd(II) complexes with measured compounds

Species	**L1**		**L2**		**L3**		**L4**	
	Logβ	pK	logβ	pK	Logβ	pK	Logβ	pK
CdH_4L_3	-		-		-		53.47(2)	
CdH_3L_3	-		-		-		46.00(3)	
CdH_2L_2	35.70(7)		34.75(7)		33.62(7)		-	
		10.70		9.48		9.12		
$CdHL_2$	25.00(7)		25.27(7)		24.50(7)		21.86(4)	
				11.71		11.38		10.59
CdL_2	-		13.56(9)		13.12(9)		11.27(4)	
CdH_2L	25.05(2)		24.28(2)		23.68(2)		22.72(2)	
		5.20		4.86		4.79		
$CdHL$	19.85(3)		19.42(3)		18.89(3)		-	
$Cd_3H_4L_3$	-		-		-		67.65(4)	

L1	**L2**

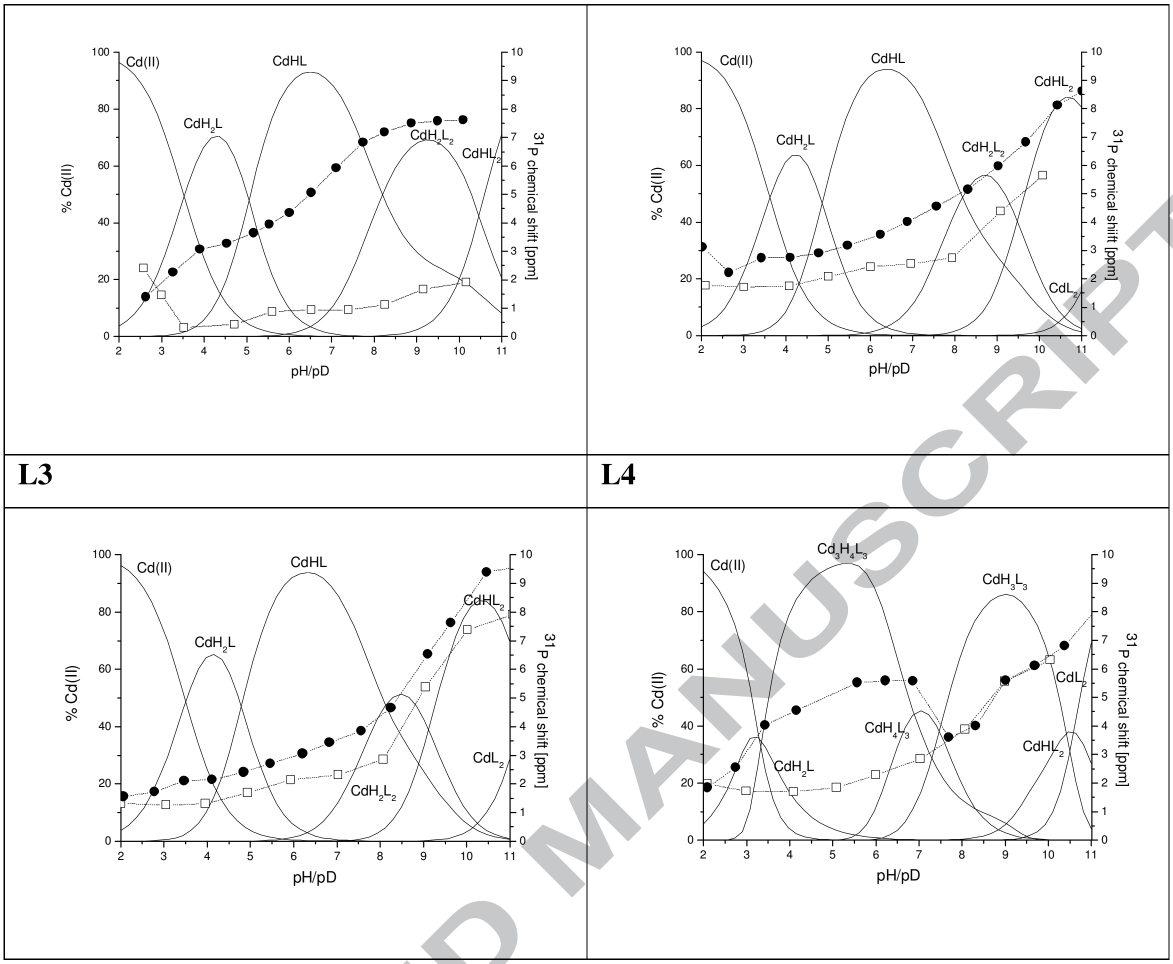


Figure 1. The distribution of complexes for studied ligands and Cd(II) ions in a 3:1 ratio (concentration of 4 mM) versus the chemical shift of bisphosphonate peaks in ^{31}P NMR as a function of pD for free (□) and bound ligands (●)

Titration curves for all studied ligands are present in Figure 2. Due to precipitation, **L3** has only three titration curves present in the diagram (free ligand and 1:2 and 1:3 M:L molar systems). For the remaining ligands, there was no precipitation in the 1:1 molar ratio system but the solution was slightly opalescent between pH 7 and 8, which is not recorded in the diagrams. Due to this opalescence, only the data obtained from the titrations at M:L 1:2 and 1:3 molar ratios were used in the calculations presented in Table 2.

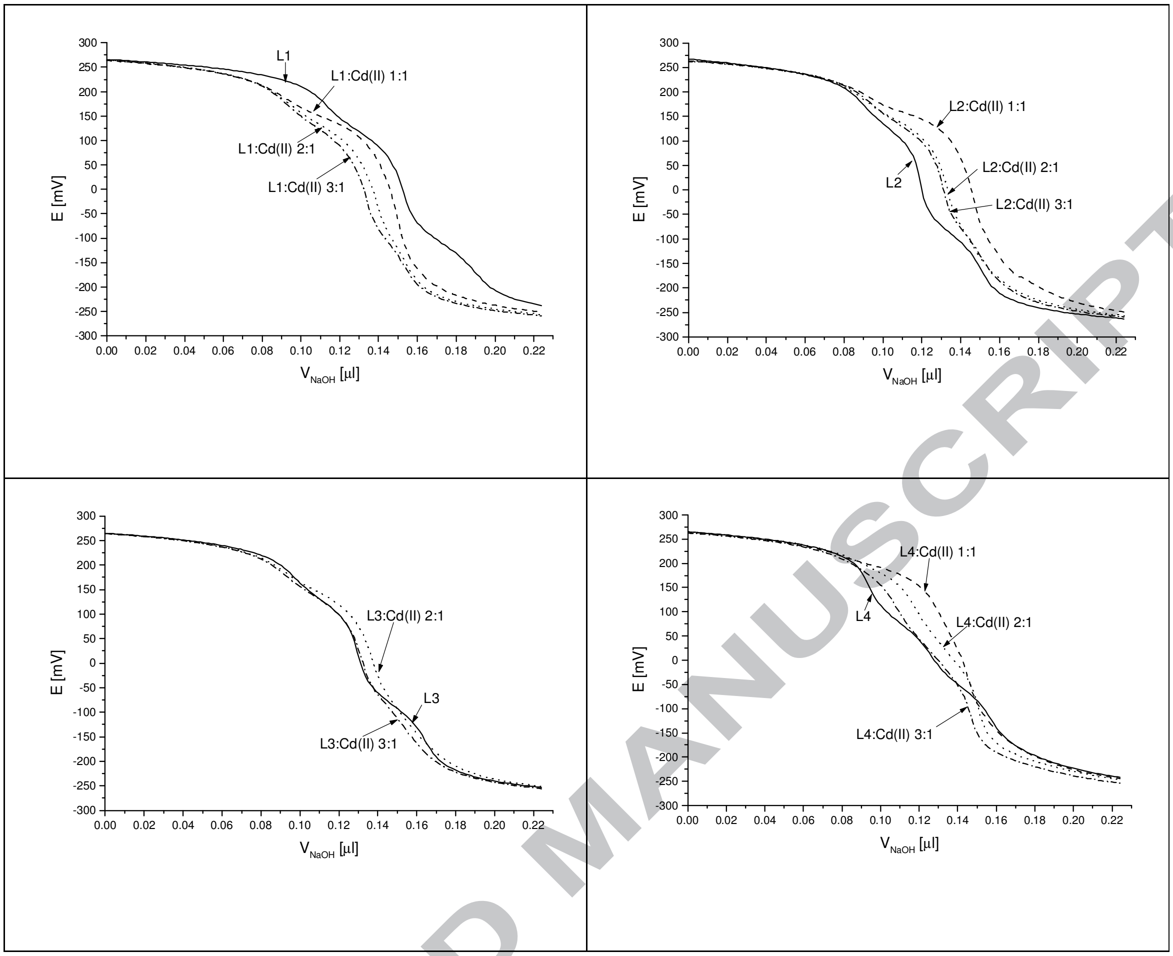


Figure 2. Titration curves of free **L1, L2, L3** and **L4** aminobisphosphonic ligands and their Cd(II) complexes with a metal-to-ligand ratio from 1:1 to 1:3.

The studied compounds represent a class of bisphosphonates in which the nitrogen atom is attached directly to the α-carbon. Such compounds have a tendency to form protonated multinuclear soluble complexes [20]. However, there are also some pyridine bisphosphonates that form complexes with a 1:1 or 1:2 metal-to-ligand ratio with various metal ions [18].

Although the compounds **L1**, **L2**, and **L3** might be considered similar to each other, metal complex speciation differs slightly among the studied ligands. According to the potentiometric results, the Cd(II) ion coordination begins at pH below 2 for all compounds (Figure 1). This result indicates the very strong binding ability of these compounds. The complex formation processes for **L1**, **L2** and **L3** are very similar, and they form almost the same types of complexes (excepting the CdL_2 form for **L1**) (Table 2). All three heteroaliphatic ligands (**L1-L3**) have a strong tendency to form equimolar species CdH_2L and CdHL, which predominate in the pH range of 2-8.5. (Figure 1, Table 2). These common complex forms are the major species in acidic and neutral environments. The ligands may coordinate the Cd(II) ion using oxygen donors originating from phosphonate groups, retaining one protonated oxygen and nitrogen atom. Then, the subsequent deprotonation might lead to engagement of the remaining oxygen in the complex formation or remain it not involved. At pH above 6, bis-complexes begin to appear, forming the following species: CdH_2L_2, $CdHL_2$ and CdL_2 (see Figure 1). However, **L1** is the exception as it does not form a CdL_2 complex. In the first complex, CdH_2L_2, the Cd(II) ion has four oxygen donors, {4O}, and then consecutive deprotonation occurs, most likely with the involvement of at least one nitrogen donor. This phenomenon is justified by the significant reduction in the pK_a of tertiary amine NH^+ groups leading to the CdL_2 complexation form (Table 1,2). The final comparison of the binding properties of ligands **L1-L3** toward Cd(II) ions reveals that the lowest pK_a values, probably caused by the ring electronegative atom, have the most efficient coordination properties (Figure 3).

L4 has quite different coordination behavior from those of **L1**, **L2**, and **L3** because, with the exception of equimolar and bis-complexes, this compound is the only one which forms CdH_4L_3, CdH_3L_3 and the single trinuclear complex with stoichiometry $Cd_3H_4L_3$. The diverse nature of the resulting complexes is not surprising if one considers that **L4** has a substantially different N-substituent and rigid aromatic ring from those of **L1**, **L2** and **L3**, which can prompt the formation in acidic solution of trinuclear complexes similar to these found for Cu(II) ions [30].

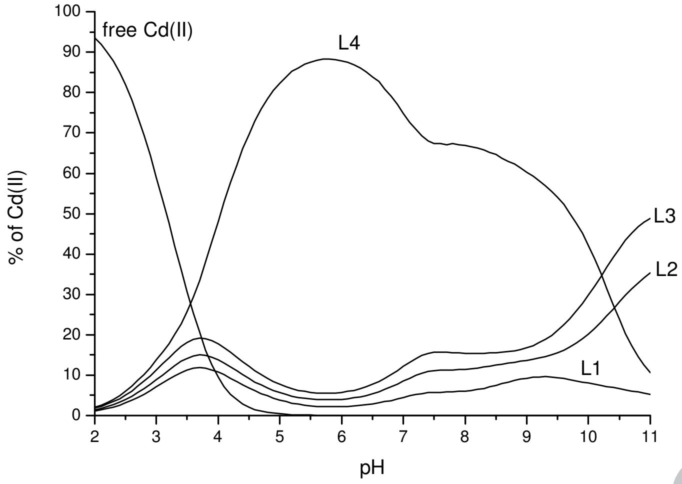


Figure 3. Hypothetical competition plot for the Cd(II)–**L1-L4** system assuming a 1:3:3 molar ratio and the stability constants obtained for binary species; $C_L = 2\times10^{-3}$mol・dm^{-3}

To compare the efficacy of cadmium(II) binding by the studied ligands (**L1-L4**), a hypothetical competition plot was made (Figure 3). This competition plot shows that **L4** is a better chelator for Cd(II) ions than **L1**, **L2** and **L3.**

It was previously established that the presence of a thiol group in the ligand structure increases the binding ability of cadmium(II) ions [31]. The metal ions can be bound by {S,N,O} donor sets with a strong tendency to form oligonuclear species. Therefore, it was interesting to compare our ligands with a common drug containing a thiol group, D-penicillamine (D-Pen). The data obtained in work performed by Kozlowski et. al. [31] was used in a hypothetical competition plot to determine which ligand (thiolate or aminomethylenebisphosphonic acids) is the better chelating agent for Cd(II) ions. Analysis of the figure (Figure 4) showed that at acidic pH, **L4** seems to be the better complexone for Cd(II) ions and that at pH above 7 D-Pen is the more potent chelating agent.

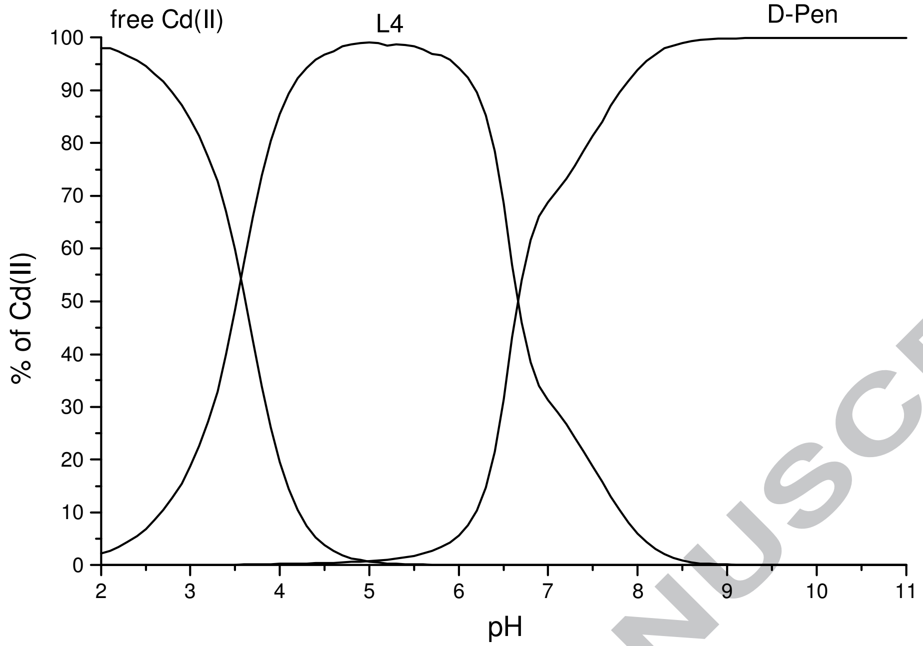


Figure 4. Hypothetical competition plot for the Cd(II)–**L4**-D-Pen system assuming a 1:3:3 molar ratio and the stability constants obtained for binary species; $C_L = 2\times10^{-3}$mol・dm^{-3}

The same study was conducted for the remainder of our ligands. The hypothetical competition plot made for them and for D-panicillamine shows that they are better chelating agents than thiols only at pH 2-5.5 (Figure 5).

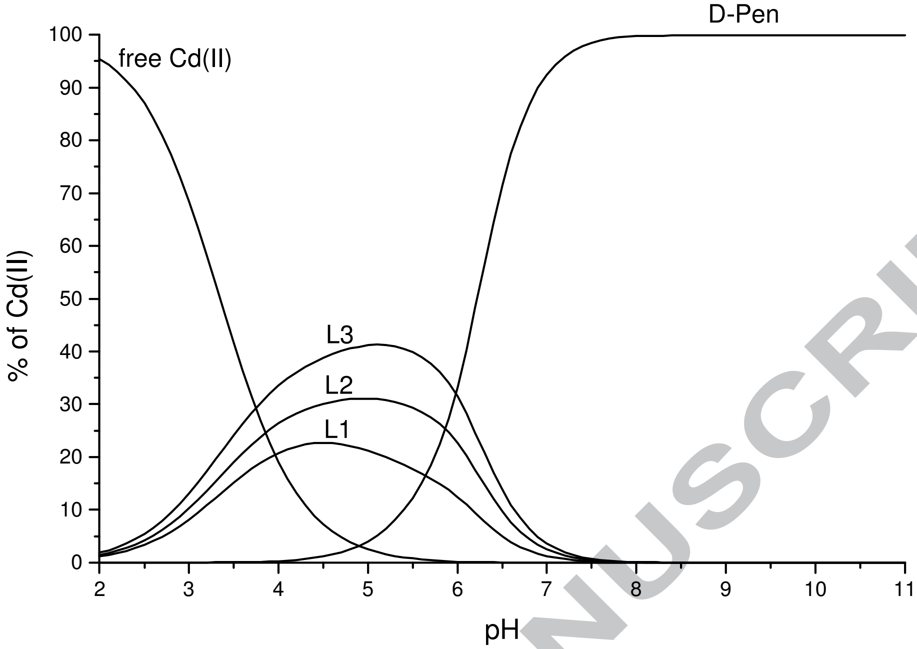


Figure 5. Hypothetical competition plot for the Cd(II)–**L1-L2-L3**-D-Pen system assuming a 1:3:3 molar ratio and the stability constants obtained for binary species; $C_L = 2\times10^{-3}$mol・dm^{-3}

According to the literature data, **L2** and **L3** form dinuclear complexes with Zn(II) ions [14], but 1:1 and 1:2 metal-to-ligand complexes are also present in solution. These complexes are similar to the ones we found for Cd(II) ions with the exception of CdH_2L, which was found in our coordination model. Even the stability constants are quite similar for Zn(II) and Cd(II) systems with **L2** and **L3** [14].

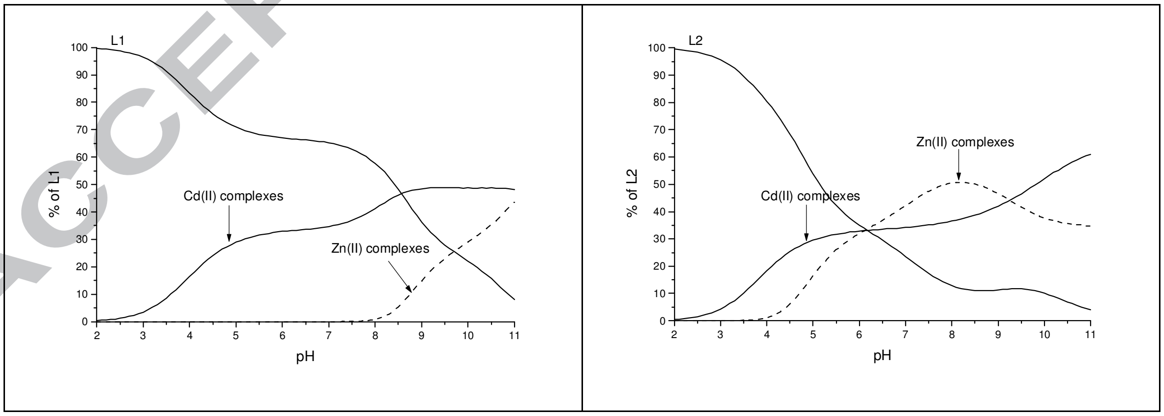
The complexation behavior of some phosphonates toward Cd(II) ions has been observed by Deluchat et al. [13]. This study observed complexes of 1-hydroxyethane-1,1'-diphosphonic acid with cadmium(II) ions at a molar ratio M:L=1:1, so they could observe only equimolar species. They found the same type of cadmium(II) complexes (CdH_2L, CdHL, CdL) but the stability constants were 3 orders of magnitude lower for CdH_2L and CdHL than in this work. Only the CdL species has a similar $\log\beta$ [13]. This may be the result of the involvement of hydroxy- groups present in the ligand in the complex formation.

According to Matczak-Jon et al., at acidic and neutral pH **L4** forms three dinuclear comlexes in which three or four ligand molecules are engaged in Zn(II) ion binding [20].

They also postulated the formation of three protonated bis-complexes and one equimolar species under alkaline conditions. Our investigations with **L4** have shown the formation of 1:1, 3:3, 1:3 and 1:2 metal-to-ligand ratio complexes with Cd(II) ions. However, the results of potentiometric studies of **L4** with Zn(II) ions revealed neither the trinuclear species nor the 1:3 metal-to-ligand ratio species [20].

Other previous results were achieved by Kowalik-Jankowska et al. who also studied complexes of **L4** ligand with copper(II) ions [30]. They suggested the formation of trinuclear and mononuclear Cu(II) complexes. The formation of M_3L_3 species with 1-hydroxyethane-1,1-diphosphonic acid was also suggested by Bouhsina et. al [32].

All of the ligands investigated in our work were studied previously as complexones of Zn(II) ions [14,15,20]. Zn and Cd lie in the same column in the periodic table, and thus it would be interesting to compare the stability constants of formed complexes. By using the stability constants for Zn(II) complexes taken from the literature [14,15,20] and the obtained results for Cd(II) complexes, such a comparison was made and the hypothetical competition plots for each ligand were obtained (Figure 6). The mixture of each ligand and both metal ions was fixed at Zn(II):Cd(II):L 1:1:2 molar ratio. These hypothetical systems revealed that **L2** and **L3** prefer Zn(II) binding in pH 6.5-9.5, but in very acidic pH, they prefer Cd(II) binding. **L4** is the only ligand that prefers Zn(II) binding even at pH 2. In basic solution (approximately pH 8.5), **L4** has a greater affinity to Cd(II) than Zn(II) ions. **L1** prefers Cd(II) binding in the entire pH range studied. The reason for this is probably that the stability constants calculated for Zn(II) complexes of **L1** [15] are significantly lower than the similar Cd(II) complexes calculated in this work.



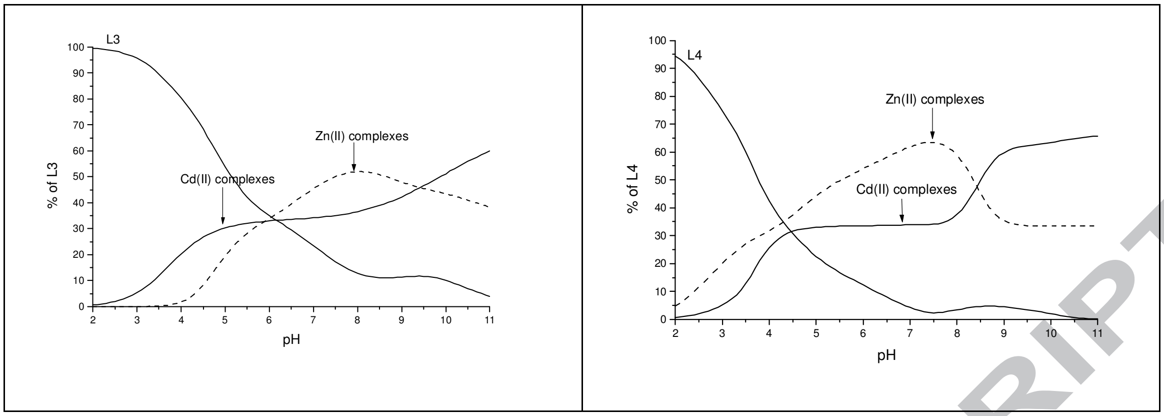


Figure 6. Hypothetical competition plot for 1:1:2 Cd(II):Zn(II):L systems assuming the stability constants obtained for binary species; C_L= 2×10^{-3}mol・dm^{-3}.

3.2.2. Polarographic studies

The data obtained from potentiometry are reliable if they can be supported with studies carried out using different technique. To confirm the coordination model calculated by potentiometry, differential pulse polarography (DPP) was used as a supplementary method. First, the titrations of the compounds **L1-L4** with Cd(II) ions were made. The metal to ligand ratio of 1:75 – 1:77 (c_M=1.0122×10^{-4} mol·dm^{-3}) was used to determine the dominant species at each pH [33,34]. The cadmium(II) reduction potential was measured when each titrant addition was made, followed by plotting the titration curve (Figure 7).

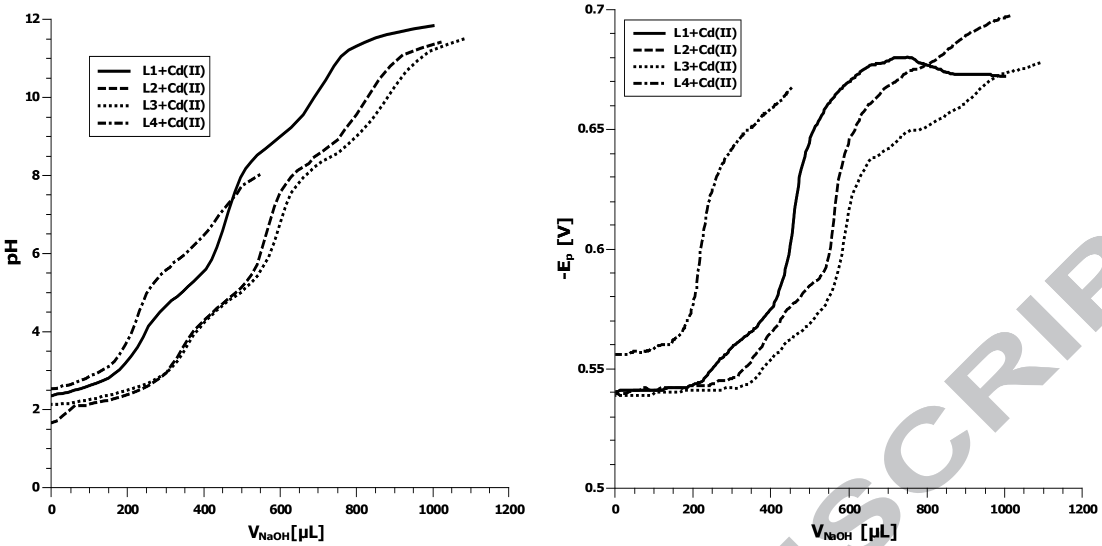


Figure 7. Polarographic titration curves, with the shift in the peak potential measured each time the NaOH was added to a polarographic cell

The curves were analyzed to determine the pH range where the dominant complexes were present (Figure 7) [12]. For every ligand (**L1-L4**) the proper pH values were found, and the remaining DPP measurements were performed at these pH values. The plots in Figure 8 show the dependence of the reduction potential shifts of Cd(II) ions on the concentration of the aminomethanebisphosphonates. When this dependence is linear it confirms that in the pH range under investigation only one complex form is dominant.

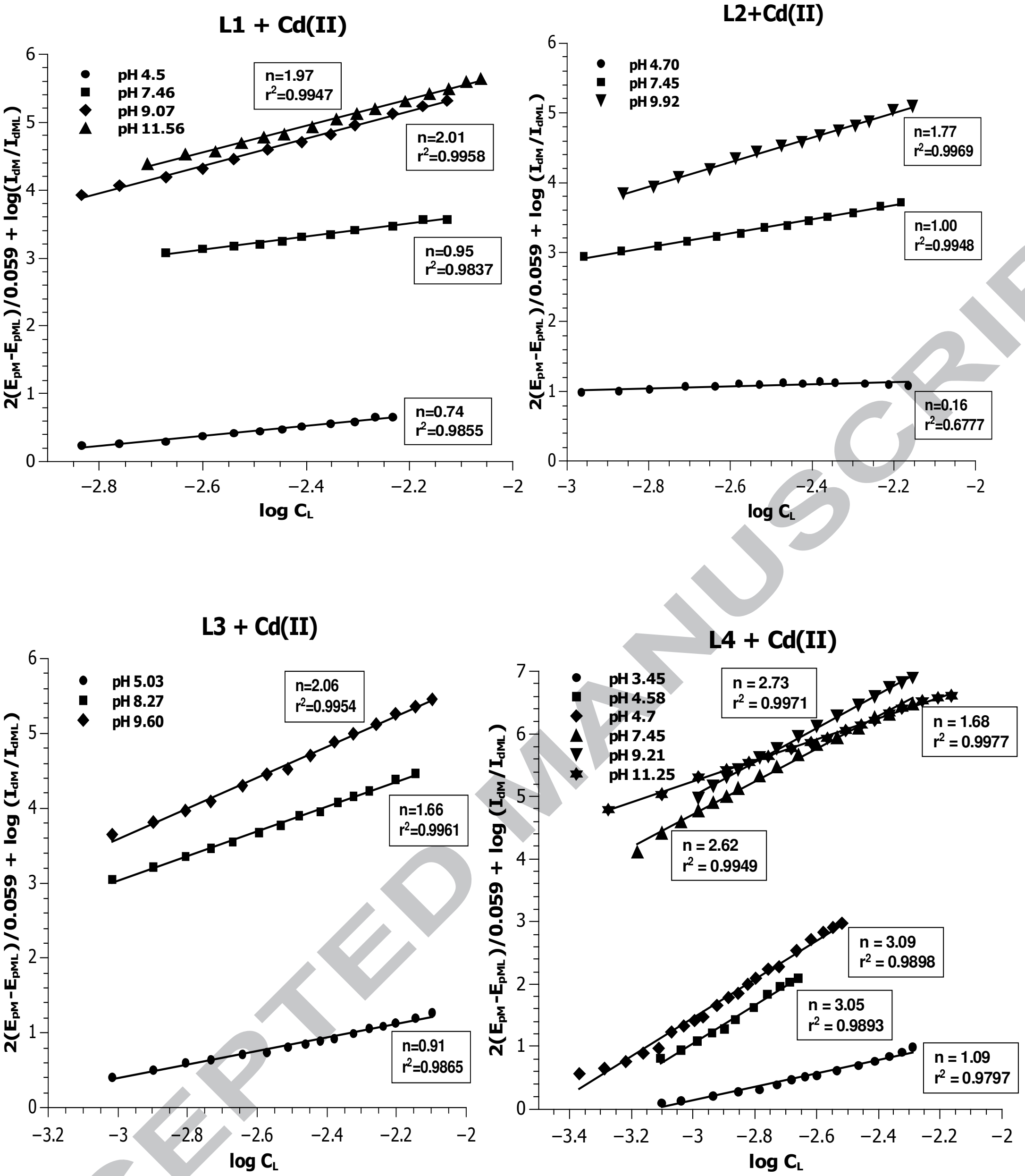


Figure 8. Dependence of the reduction potential shifts of Cd(II) ions on the concentration of the compounds **L1-L4** taken from DPP measurements. Values inside the frames describe the complex stoichiometry (n) and fitting of the measuring points to the regression curves (r^2).

According to the equation of Lingane, modified by De Ford and Hume [27], the stoichiometry of the complexes (n) was determined:

$$\frac{z}{0.059}\left(E_{pM}-E_{pML}\right)+log\frac{I_{dM}}{I_{dML}}=nlog[L]+log\beta_n$$

$$(y = ax + b)$$

Using the series of the measurements where the concentration of metal is constant and the concentration of ligand increases, the above relationship appears in the form of a diagram. The only condition required is the selection of concentrations where $C_M << C_L$ (in this case $[L] \approx C_L$). In the case where only one dominant complex is present in the solution, this relationship is linear (Figure 8). Analysis of the slope and the intersection with the *y*-axis enables determination of n and β_n [27,35].

It was found that all bisphosphonates studied (**L1-L4**) form different complexes in the pH range selected (Figure 8). The first complex present in the solution has the stoichiometry of M:L 1:1. **L1** and **L3** form these complexes in the pH range of 4.5-5 (n=0.74 for **L1** and n=0.91 for **L3**). **L2** behaves slightly differently from **L1** and **L3**, and its equimolar complex 1:1 can be observed only in pH above 7 (n=1). At approximately pH 4, the adsorption process disturbs the reduction potential and no complexes can be observed (n=0.15). This phenomenon may be the result of the presence of the sulfur atom in the pyrimidine ring, which may interact with the hanging mercury drop electrode. In Cd(II)-**L1** and Cd(II)-**L2** systems, when the pH of the solutions increases (pH 7.45), equimolar complexes are still present (n=0.95 and n=1 respectively). In the case of **L3** at pH 8.27, the mixture of equimolar and bis-complexes can be observed (n=1.66). At pH above 9, the stoichiometry of the complexes changes to a 1:2 metal-to-ligand ratio (n=2.01 for **L1**, n=1.77 for **L2**, n=2.06 for **L3**).

The structure of **L4** differs from the rest of the ligands and behaves differently in the solution. The ligand was investigated at different pH values, chosen on the basis of polarographic titration (Figure 7). For this species, the equimolar complexes start to form at a pH one order of magnitude lower than for the other ligands. This complex is dominant and stable, n=1.05 (pH 3.45). At pH above 4.58, the new complex with M:L 1:3 (n=2.91) appears. When the pH increases, this complex is still present (n=2.62 in pH 9.21) but the decrease of n means that a new complex is being formed (n=1.68 in pH 11.25). According to the potentiometric data, this species has a metal-to-ligand stoichiometry of 1:2 and starts to form at pH above 10 (Figure 1).

Potentiometric studies suggest the presence of a polynuclear species in the Cd(II)-**L4** system between pH 3 and 9.5. The polarographic studies can confirm the presence of such species in the solution qualitatively. It is known that the presence of polynuclear species in solution depends on the metal ion concentration. When the concentration of metal ions is very low, there are no polynuclear species in the solution [36]. To remove polynuclear species, one may dilute the complex solution where the metal-to-ligand ratio is constant [37]. When there are polynuclear species in the initial solution, the dilution will cause their rearrangement into mononuclear complexes [36]. Polarography can document such a process by increasing the cadmium ion reduction potential on the hanging drop mercury electrode.

An experiment of this type was performed for the Cd(II)-**L4** system, where the metal-to-ligand ratio was kept at a constant 1:3 and the total concentration was changed up to 40× (Figure 9). The potential of metal ion reduction ($-E_p$) in the monomeric species is independent of the dilution process, whereas the increase of reduction potential is characteristic of the destabilization of the oligomeric species [31]. The results obtained clearly indicate that the dilution of the solution increases the reduction potential from -640 mV to -602 mV (Figure 9). It confirms the presence of a polynuclear species suggested by the potentiometric data ($Cd_3H_4L_3$). The experiment was performed at pH approximately 6.5, where this trinuclear species is present in high concentration.

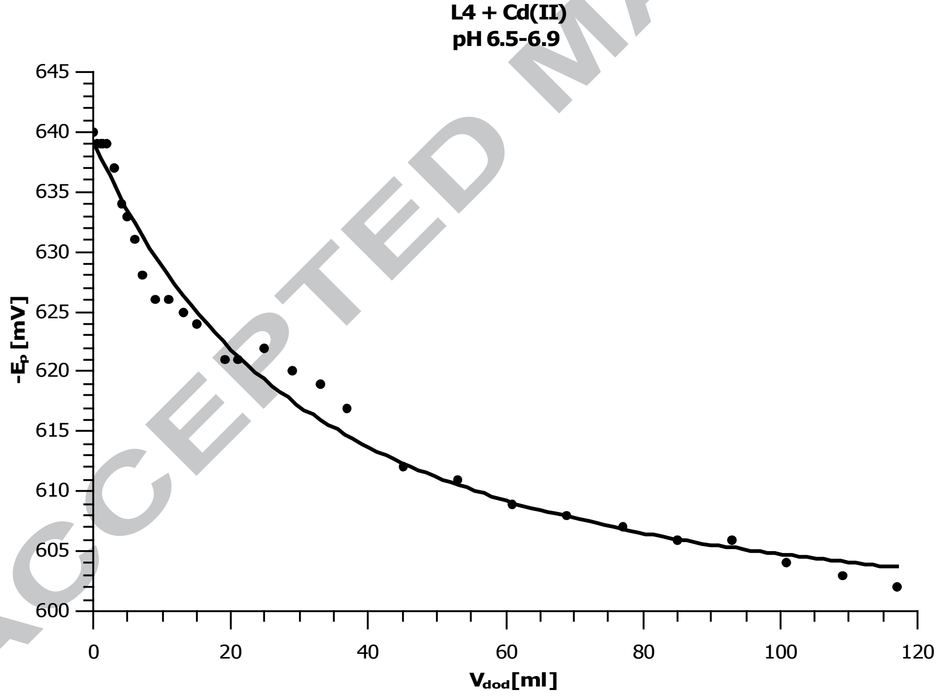


Figure 9. The dilution of Cd(II)-**L4** system up to 40× by 0.1 mol/dm^3 KNO_3. Constant metal-to-ligand ratio 1:3, initial metal concentration $c_{Cd(II)}$=1.69×10^{-3} mol/dm^3.

Similar experiments were performed for the remaining ligands (**L1-L3**), but no changes in the metal ion reduction potential were observed. These results confirm the model calculated by potentiometry (Table 2, Figure 1) where only monomeric species are present in the solution.

Polarographic studies confirmed that all of the studied ligands form stable complexes with Cd(II) ions. These are ML and ML_2 complexes with metal-to-ligand ratios of 1:1 and 1:2. According to the polarographic data **L1**, **L2** and **L3** form complexes that have a high degree of similarity (Figure 7). These data confirm the results received from potentiometry, where these ligands represent the same type of metal binding (Table 2).

L4 has a different chemical structure from the other ligands. It has an additional pyridine nitrogen atom, which is able to bind metal ions. It is possible that the presence of this atom is the reason why the ligand is able to form M_3L_3 and ML_3 complexes. The presence of such complexes in the solution was confirmed by both potentiometry and polarography.

3.2.3. NMR studies

To confirm the aforementioned models of complexation, additional studies were undertaken to relate ^{31}P NMR chemical shift changes of free ligand versus the mixture of 1:3 metal-ligand ratio as a function of pH.

L1 forms four complexes (see ^{31}P NMR chemical shift presented versus potentiometric distribution curves in Figure 1). The CdH_2L complex (1:1 molar ratio) is observed at very low pH, which, upon increase of pH, yields the CdHL species. At the maximal appearance of this species all Cd(II) ions are complexed. At pH over 6.5, only complexes of M:L of 1:2 are observed (CdH_2L_2 and $CdHL_2$). This is supported by NMR titrations, which indicate complexation at low pH. The greatest change in chemical shift is observed at pH 6.5, where CdH_2L_2 species begin to be formed. This may result from the change in type of interaction between the ligand and Cd(II) ions in CdHL and CdH_2L_2 complexes. **L2** and **L3** are similar to **L1**, however the changes in chemical shifts between free and bound ligands are less pronounced (Figure 1).

L4 yields the most complicated distribution of complexes (Figure 1). As in all of the previous cases, they are formed at pH as low as 2. Total disappearance of Cd(II) ions is observed at a pH of 4.5. ^{31}P NMR spectra clearly support the potentiometric data with the presence of 3:3, 1:3 and 1:2 complexes in the studied pH range.

3.2.4. ESI-MS studies

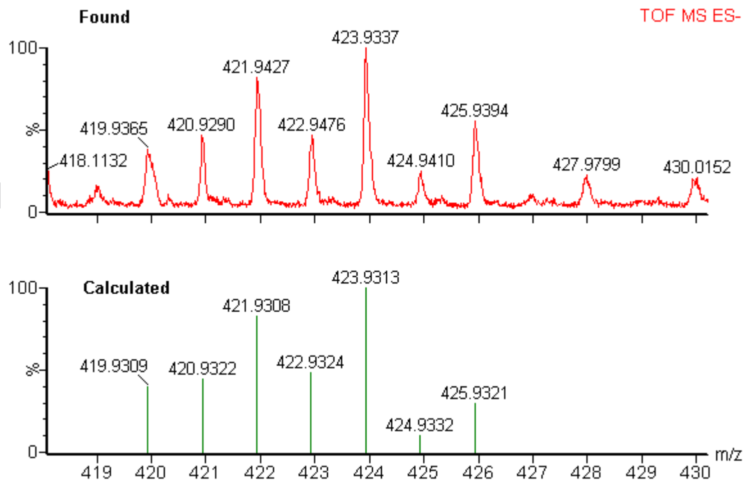
ESI method is a technique widely used to analyze compounds in the solution [38]. Although, this method is not perfect when using solely it can confirm the type of metal complexes formed in the solution because of the presence of characteristic isotopic distribution pattern. Thus, it allows to elucidate the metal-to-ligand stoichiometry.

ESI-MS measurements were made to confirm the stoichiometries of Cd(II) complexes calculated from the studied by means of potentiometry. Both positive and negative spectra were made but because of the ligands acidities the negative ionization method was more effective one. All measurements for all ligands taken into investigation were performed in three different pH values (pH 4, 7 and 9) in order to find major species present in the solution.

During analysis of MS spectra the most abundant signals originated from metal free ligands, their alkali ion adducts and ligand fragments, additionally their dimeric and trimeric forms were found. The less intense peaks were related to Cd(II) complexes which are present in the solution.

Spectra recorded for **L1, L2** and **L3** were investigated in order to confirm the lack of presence of polynuclear Cd(II) complexes in the solution. According to potentiometric studies ligands **L1**, **L2** and **L3** form only mononuclear complexes with dominant 1:1 metal-to-ligand ratio at pH about 4 (Figure 10 a). Such complexes were found for all ligands as mono charged $[CdH_2LCH_3OH]^-$ adducts appearing in a range of 405 to 424 m/z in the spectra. The presence of methanol molecule in the complex is not a surprise and results from its addition to the solution during MS measurements [39].

a



b

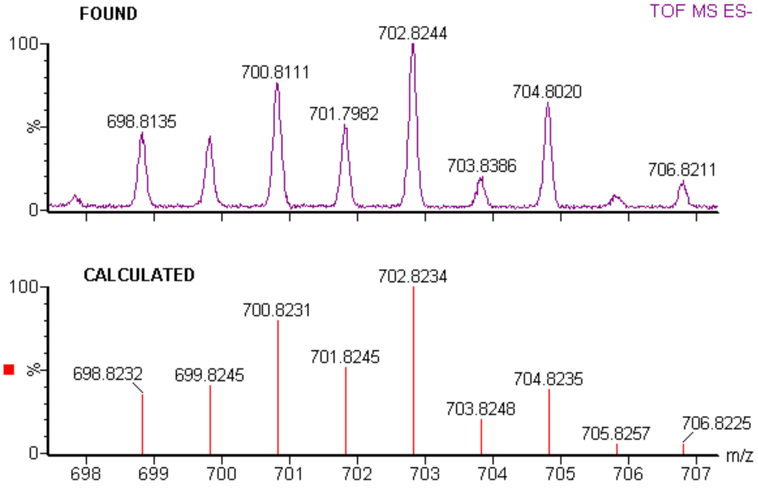


Figure 10. Experimental and calculated isotopic distribution patterns in the ESI-MS spectra performed for Cd(II)-**L2** system in pH 4 (a - $[CdH_2LCH_3OH]^-$ complex) and pH 9 (b - $[CdHL_2K]^-$ complex).

Also metal-to-ligand 1:2 molar ratio species appear in higher pH values (Fig. 10b). They are detected in the spectra as mono charged potassium adducts at the range m/z 666-706 (Fig. 10b). The signals rising from polynuclear species were not observed which is consistent with results obtained by other analytical methods used in this study.

The only ligand which forms polynuclear complexes is **L4**, which is documented by finding of the methanol adduct of CdHL species in the sample at pH 4. In higher pH signals corresponding to polynuclear potassium adduct of $Cd_3H_4L_3$ complex were observed at the m/z 1220 (Figure 11). Additionally CdH_3L_3 - 952 m/z (Figure 12) and CdL_2 (669 m/z) complexes as potassium adduct forms were also detected. Thus, the performed MS experiments confirm the results obtained by other applied techniques.

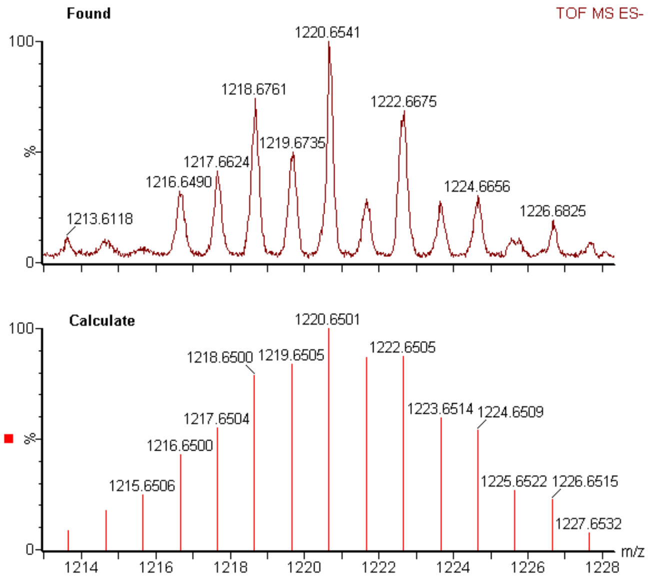


Figure 11. Experimental (a) and calculated (b) isotopic distribution patterns in the ESI-MS spectra performed for Cd(II)-**L4** system in pH 7 for [Cd$_3$H$_4$L$_3$K$_2$]$^-$ complex

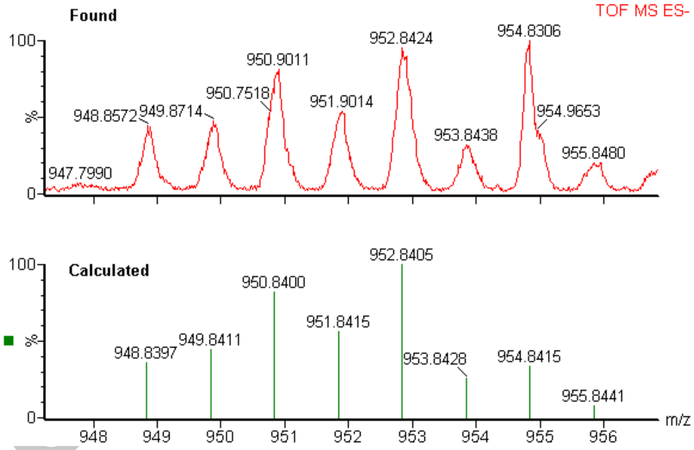


Figure 12. Experimental (a) and calculated (b) isotopic distribution patterns in the ESI-MS spectra performed for Cd(II)-**L4** system in pH 9 for [CdH$_3$L$_3$K]$^-$ complex

4. Conclusions

As shown by the results presented in this paper, compounds **L1, L2, L3** and **L4** are effective ligands for Cd(II) ions. They bind Cd(II) ions even more readily then Zn(II) ions. Their binding properties were demonstrated with solution studies in potentiometry, differential pulse polarography and NMR and ESI-MS spectroscopy. The model of coordination reported in this paper is one which is consistent with all the experimental techniques used.

Acknowledgement

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References

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[1] A. Diez-Perez, Maturitas, 43, Suppl. 1 (2002) S19-S26
[2] Y. Komatsu, Y. Imai, F. Itoh, M. Kojima, M. Isaji, N. Shibata, European Journal of Pharmacology, 507 (2005) 317-324
[3] C. Deal, Clinical Reviews in Bone and Mineral Metabolism 3, No. 2 (2005) 125-141
[4] A.F. Leite, P.T. Figueiredo, N.S. Melo, A.C. Acevedo, M.G.P., Cavalcanti, L.M. Paula, A.P. Paula, E.N.S. Guerra, Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology 102, Issue 1 (2006) 14-21
[5] R. Erez, S. Ebner, B. Attali, D. Shabat, Bioorganic and Medicinal Chemistry Letters 18 (2008) 816-820
[6] C. Belmant, D. Decise, J-J. Fournie, Drug Discovery Today: Therapeutic Strategies, 3, No. 1 (2006) 17-23
[7] M.M. Brzóska, J. Moniuszko-Jakoniuk, Arch. Toxicol. 72 (1998) 63-73
[8] P.L. Goering, M.P. Waalkes, C.D. Klaassen, in: R.A. Goyer, M.G. Cherian (Eds.), Handbook of Experimental Pharmacology, vol. 115, Toxicology of Metals, Biochemical Effects, Springer, New York, 1994, p. 189-214
[9] M.M. Brzóska, J. Moniuszko-Jakoniuk, Food Chem. Toxicol. 39 (2001) 967-980
[10] I.M. Armitage, A.J.M. Schoot Vitercamp, J.R. Chlebowski, J.E. Coleman, J. Magnetic Resonance, 29 (1978) 375-392
[11] J. Ejnik, C.F. Shaw, D.H. Petering, Inorganic Chemistry 49 (2010), 6525-6534
[12] I. Cukrowski, J.R. Zeevaart, N.V. Jarvis, Analytica Chimica Acta 379 (1999), 217-226
[13] V.Deluchat, J-C. Bollinger, B. Serpaud, C. Caullet, Talanta 44 (1997), 897-907
[14] E. Matczak-Jon, B. Kurzak, W. Sawka-Dobrowolska, Polyherdon 31 (2012), 176-187
[15] E. Matczak-Jon, B. Kurzak, P. Kafarski, A. Woźna, Journal of Inorganic Biochemistry 100 (2006), 1155-1166

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[16] H. Li, L. Zhang, G. Li, Y. Yu, Q. Huo, Y. Liu, Microporous and Mesoporous Materials 131 (2010), 186-191

[17] Z-M. Sun, B-P. Yang, Y-Q. Mao, A. Clearfield, Journal of Solid State Chemistry 176 (2003), 62-68

[18] E. Matczak-Jon, B. Kurzak, A. Kamecka, P. Kafarski, Polyhedron 21 (2002), 321-332

[19] E. Matczak-Jon, P. Ślepokura, P. Kafarski, Journal of Molecular Structure 782 (2006), 81-93

[20] E. Matczak-Jon, T. Kowalik-Jankowska, K. Ślepokura, P. Kafarski, A. Rajewska, Dalton Trans. 39 (2010) 1207-1221

[21] J. Sołoducho, R. Gancarz, P. Wieczorek, J. Korf, J. Hafner, B. Lejczak, P. Kafarski, Patent PL 172268 B1, 1997

[22] M. Fukuda, Y. Okamoto, H. Sakurai, Bulletin of the Chemical Society of Japan 48 (1975) p. 1030

[23] H. Gross, B. Costisella, Th. Gnauk L. Brennecke, Journal für Praktische Chemie, 318 (1976) 116 - 126

[24] Leo Pharmaceutical Products Ltd. A/S Bisphosphonic acid derivatives, their production and use, Patent: US4870063 A1, 1989, priority date 12.12.1985

[25] P. Gans, J. Chem. Soc., Dalton Trans. (1985) 1195.

[26] L. Pettit.: University of Leeds, the copy of this program was given to me by an author

[27] Crow D.R., Polarography of Metal Complexes, Academic Press London and New York 1969

[28] E. Matczak-Jon, V. Videnova-Adrabińska, A. Burzyńska, P. Kafarski, T. Lis, Chem. Eur. J. 11 (2005), 2357-2372

[29] P. Młynarz, M. Jewginski, S. Śliwińska, R. Latajka, G. Schroeder, P. Kafarski, Heteroatom Chem. 18 (2007) 774-781.

[30] T. Kowalik-Jankowska, M. Pietruszka, J. Jezierska, E. Matczak-Jon, P. Kafarski, Polyhedron 30 (2011), 1274-1280

[31] H. Kozłowski, J. Urbańska, I. Sovago, K. Varnagy, A. Kiss, J. Spychala, K. Cherifi, Polyhedon 9, No. 6 (1990) 831-837

[32] S. Bouhsina, P. Buglyo, A. Abi Aad, A. Aboukais, T. Kiss, Inorganica Chimica Acta 357 (2004), 305-310

[33] I. Cukrowski, M. Adsetts, Journal of Electroanalytical Chemistry 429 (1997), 129-137

[34] I. Cukrowski, Analytica Chimica Acta 336 (1996), 23-26

[35] Z. Galus, Teoretyczne podstawy elektroanalizy chemicznej, PWN, Warszawa 1977

[36] J. Inczedy, Analytical Applications of Complex Equilibria, Ellis Horwood LTD, 1976

[37] B. Kurzak, J. Spychała, J. Świątek, Journal of Coordination Chemistry 25 (1992), 95-100

[38] V.B. di Marco, G.G. Bombi, Mass Spectrometry Reviews 25 (2006), 347-379

[39] M. Puszyńska-Tuszkanow, M. Daszkiewicz, G. Maciejewska, Z. Staszak, J. Wietrzyk, B. Filip, M. Cieślak-Golonka Polyhedron, 30 (2011) 2016-2025