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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 ionization 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.

1. Introduction

Bisphosphonates are a group of pyrophosphate analogs 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 zoledronate 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

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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 toward coordination properties of Zn(II), Mg(II) and Ca(II) ions [14,15].

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 [18–20].

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

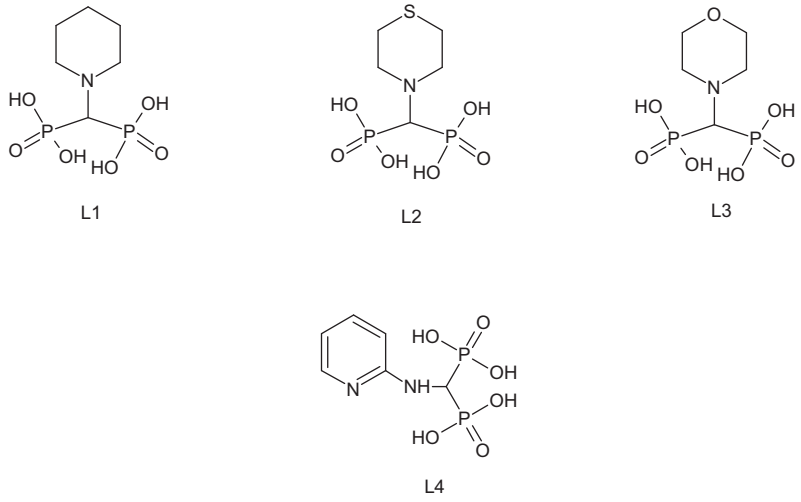
The diphosphonic acids were obtained according to the previously described procedure [21]. The purity of the ligands was established by NMR spectroscopy.

2.1.1.1. General procedure of the synthesis. A mixture of amine (0.03 mol), dialkyl (preferently diethyl) 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 of 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.

2.1.1.2. Hydrolysis – general procedure. The obtained ester (0.030 M) was refluxed for 8–12 h in 20 ml 6 M 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.

*(Piperid-1-yl)aminomethylenebisphosphonic 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_2O + NaOD, ppm): δ = 8.54; ^{1}HNMR (500.14 MHz, D_2O + 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, PCHP), 3.43 (4H, t, J = 6.35 Hz) ppm; ^{13}CNMR (125.76 MHz, D_2O + NaOD, ppm): δ = 21.32, 24.90, 53.51, 66.95 & 66.99 (t, Hz, J = 114.62 Hz, PCP); HRMS (ESI-TOF) m/z: $[M-H]^+$ Calcd for $C_6H_{15}NO_6P_2$ 258.0294, Found 258.0288.

*(Thiomorpholin-1-yl)aminomethylenebisphosphonic 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_2O + NaOD, ppm): δ = 16.39; ^{1}HNMR (500.14 MHz, D_2O + NaOD, ppm): δ = 2.57 (4H, t, J = 4.95 Hz), 2.66 (1H, t, J = 22.41 Hz, PCHP),3.18 (4H, t, J = 4.79 Hz,), ppm; ^{13}CNMR (125.76 MHz, D_2O + NaOD, ppm): δ = 27.82 (t, J = 5.72 Hz) 53.13, 68.44 & 68.49 (t, J = 123.68 Hz, PCP); HRMS (ESI-TOF) m/z: $[M-H]^+$ Calcd for $C_5H_{13}NO_6P_2S$ 275.9861, Found 275.9867.



Scheme 1. Studied ligands: **L1** – (piperid-1-yl)aminomethylenebisphosphonic acid; **L2** – (thiomorpholin-1-yl)aminomethylenebisphosphonic acid; **L3** – (morpholin-1-yl)aminomethylenebisphosphonic acid; **L4** – (pyridin-2-yl)aminomethylenebisphosphonic acid.