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Phosphonic Acid Based Ligand As Potential Chelating Agent For Remediation of Heavier Metal: - Kinetics and Biological Evaluation

Vinay Kr. Singh

Department of Chemistry,Dr.Shakuntala Misra National Rehabilitation University
Mohaan road Lucknow.(India)

ABSTRACT

The tetraphosphonate ligand, trans-1,2-cyclohexylenedinitrilo tetramethylene phosphonic acid was used in the present study was prepared from trans-1,2-cyclohexyldinitrilotetraacetic acid by reaction with phosphorus trichloride in presence of phosphoric acid. The complex formation reaction was acid independent at acidities above 0.05 M while the dissociation reaction had a direct dependence on the acidity over the range of hydrogen ion concentration 0.1-0.01 M. Activation parameters for both formation and dissociation reactions are consistent with an associative mechanism. $\Delta H^{\neq}_f$ *= 58.0 (±1.6) kJ/mol,* $\Delta S^{\neq}_f$ *= -42(±4) J/(mol K),* $\Delta H^{\neq}_d$ *= 40.8 (±2.4) kJ/mol,* $\Delta S^{\neq}_d$ *= -61 (±3) J/(mol K).The effects of in removing heavier metal-235 in rats were examined. Male Wistar rats 8 weeks old were pre-injected with mixed heavier metal oxide in the right muscles and divided into six groups. The activities in the excreta and organs in rats 24 h after injection were measured the results indicate that tetraphosphonate derivatives can remove heavier metal efficiently at pH 6.9 from biological system.*

I. INTRODUCTION

The worldwide use of heavier metal notably as fuel in nuclear power plants (enriched heavier metal) or for armor piercing weapons and also used in civilian fields such as counterweights in airplane construction, (depleted heavier metal) is expanding, thus providing increasing opportunities for occupational and environmental exposure [1-3]. Whatever the chemical form or speciation of the heavier metal compound incorporated, the uranyl ion, UO_2^{2+}, is the most likely form of heavier metal species present in body fluids after contamination [4-5].

Thus, in the case of accidental contamination, an appropriate treatment may be necessary to reduce deposition in target organs.The body content of these radionuclides and the consequences of accidental incorporation may be reduced by treatment with chelating agents. Such agents have been widely studied and have proved to be useful in man. Chelation therapy may also be advantageous in certain cases of heavy metal poisoning. There is still a need to develop new, more efficient and less toxic agents and better therapeutic schedules for using the existing agents.

The testing of a new agent should take place in three stages: Firstly, an initial screening study in vitro and in vivo; secondly a detailed examination in experimental animals for toxicity and the efficacy of procedures that could be applied in the treatment of human exposure; and finally, the testing of the material in man. This stepwise approach has the advantage that much unnecessary work can be avoided if the agent proves to be ineffective or unacceptably toxic at an early stage [6].

Many chelating agents such as 3,4,3-LIHOPO, Tiron, ethane-1-hydroxy-1,1-bisphosphonate (EHBP) , deferiprone (L1) 1,2-dimethyl-3-hydroxypyrid-4-one ,DTPA , CBMIDA , have been examined for the removal of heavier metal [7-15]. Most of them work as soft acid-based concept of HSAB which is an extremely useful qualitative theory that enables predictions of what adducts will form in a complex mixture of potential Lewis acids and bases [16-17]. Since the basic property of soft acid is large ionic size and low charge density. Keeping this fact in our mind we have synthesised multidentate Phosphonate ligand which seems very good candidate for Actinides (which are soft acids) especially for heavier metal.

II. CHEMISTRY

All the chemicals used in this research were of analytical reagent grade, since the ligand requires must be of high purity in potentiometric studies. The stock solution of Eu (III) was prepared by dissolving appropriate amounts of Eu $(NO_3)_3$ in a small excess of HNO_3 (Merck,) to avoid hydrolysis. The concentration of Cu (II) stock solution was checked by EDTA titration. The concentration of free acid in the Cu (II) solution was systematically checked by potentiometric titrations before each series of experiments.

Synthesis of 1, 2-Cyclohexyldinitrilotetramethylene-phosphonic acid

was synthesis by methodology reported in literature [18] by our group. Briefly, to a solution of CDTA dissolved in toluene and phosphorous acid was added. The reaction mixture was refluxed while Phosphorous trichloride was added dropwise to the refluxing mixture. At the end of 3h toluene was removed following addition of deionised water. The filtrate was concentrated under vacuum. The concentrated product was precipitated on addition of methanol/ethanol. (Yield 90-92 %)

Synthesis of Eu (III) and Cu (II) complexes of 1, 2-Cyclohexyldinitrilo--tetramethylene phosphonic acid

was dissolved in water at a concentration of 0.280 mM and hydrated europium chloride 0.242 mM was added under stirring and shielded from light. Reaction was allowed to proceed at 40^0 C for 8h. Completion of reaction was checked by UV visible spectroscopy. (Yield: 74%.)

For Cu Complexation, to a solution of in 25 mL methanol, Cupric chloride dehydrated in methanol 10mL was added under stirring. The solution was stirred for 18 h at room temperature. The product was filtered off and washed with ethanol before drying in vacuo. (Yield; 85 %)

III. KINETIC STUDIES

Potentiometric and Spectroscopic measurements (UV-VIS) of Eu (III) and Cu (II) complexes were made by metrohm potentiometer. The stability constants of complexes and the protonation constants of the ligands were determined by a previously described potentiometric method. At least 3 different potentiometric titrations were carried out for each complex systems in a 1.0 x 10^{-4} to 7.5 x10^{-3} M concentration range . In order to maintain the ionic strength at constant level, 0.1 M KNO_3 was added. pH meter, equipped with a combination electrode, measured the pH values. In all cases the temperature was kept constant at 25.0 ± 0.10 °C.

The optical absorbance spectra of the samples were recorded on a Shimadzu UV-2100 spectrophotometer, with a 1 cm quartz cell, at appropriate pH values. The stoichiometries of these complexes were determined by taking

the spectra of systems including isolated ions and ligands in definite mol ratios in the 200-500 nm ranges. Thus, the results of the potentiometric studies were also validated by UV methods.

IV.PRELIMINARY *IN VIVO* INVESTIGATIONS

4.1 Animals and procedures

Thirty male Wistar rats, 2 months old, weighing 142±7g, were pre-injected intramuscularly with mixed heavier metal oxide at per rat, and divided into six groups (n=6). Rats were kept in individual cages to collect urine and feces separately every 24 hr during this experiment. The feces that had fallen on the net were picked up using a pair of tweezers. The urine on the cage bottoms was gathered with a mixture containing nitric acid, distilled water and hydrofluoric acid (50:125:1). On day 3, the rats were sacrificed to obtain blood, liver, kidney, spleen, femur and muscles of the heavier metal-injected site. The Samples were incinerated at 700 °C for 24 hr in a crucible. Two millilitres of a solution as described above was added to the crucible. Subsequently, the solution was poured into a counting vial with a scintillator.

Group 1(G1): rats injected intraperitonially with heavier metal in a solution of pH 3.5 and injected with sodium bicarbonate 30 min later; Group 2 (G2): heavier metal compounded with chelates before injection; Group 3 (G3): heavier metal in a pH 3.5solution; Group 4 (G4): heavier metal in a pH 3.5 solution 30 min later, Group 5(G5): heavier metal in a pH 6.9 solution; and Group 6(G6): heavier metal in a pH 6.9 .In all the cases the injection of 1000 μmol/kg chelates is given . The heavier metal activities in the excreta and organs in rats 24 hrs after injection were measured.

V. RESULT AND DISCUSSION

The properties of a metal coordination complex are determined by the ligand donor atoms and ions coordinated to the metal center as by the nature of the metal itself. Phosphonate compounds with more than one phosphonate group are effective sequestrants and possess other useful properties such as high water solubility and chemical stability. Multidentate phosphonate ligands exhibit good metal ion control properties and bind strongly to a range of metals due to their high ionisation at physiological pH. Phosphate and phosphonate compounds localize avidly in bone and generally display low toxicity. Moreover Phosphonic acid derivatives and the more elaborated ligands widely used as complexing agents for calcium, zinc and/or a number of bivalent metals. These properties of phosphonate allowed us to identify a new powerful uranyl chelate based on phosphonic acid. We have directed our efforts in the synthesis of a poly α-aminophosphonic acid based on polyaminocarboxylate family with the idea to develop a multidentate ligand with increased complexation properties.

Tetraphosphonate chelator, was synthesized according to the methodology reported in literature[18]. Tetraphosphonic acid was analyzed by different spectroscopic technique such as IR, NMR, and by elemental analysis. The spectral evidence confirms the presence of different functionalities NMR multiplet in the range of 3.59-2.88 (8H, complex multiplet,-CH_2-P-), 2.1(2H, m), 1.74(2H, m), 1.71-1.21 (4H, q).confirms the presence of acyclic rings. It also confirms the proposed stoichiometry and structure for the phosphonic acid derivative. Integral of NMR confirms the number of protons as well as coupling constant confirms the nature of bonds.

A first-order rate law was found to adequately describe the experimental data under all conditions. The calculated first-order rate constants as a function of the ligand concentration are plotted The finite intercepts in each system indicate that the most appropriate analysis is to assume a first-order approach to equilibrium. Within this assumption, the slope corresponds to the rate of complex formation while the intercept defines the rate constant for complex dissociation (at constant acidity)**(Fig 1).**

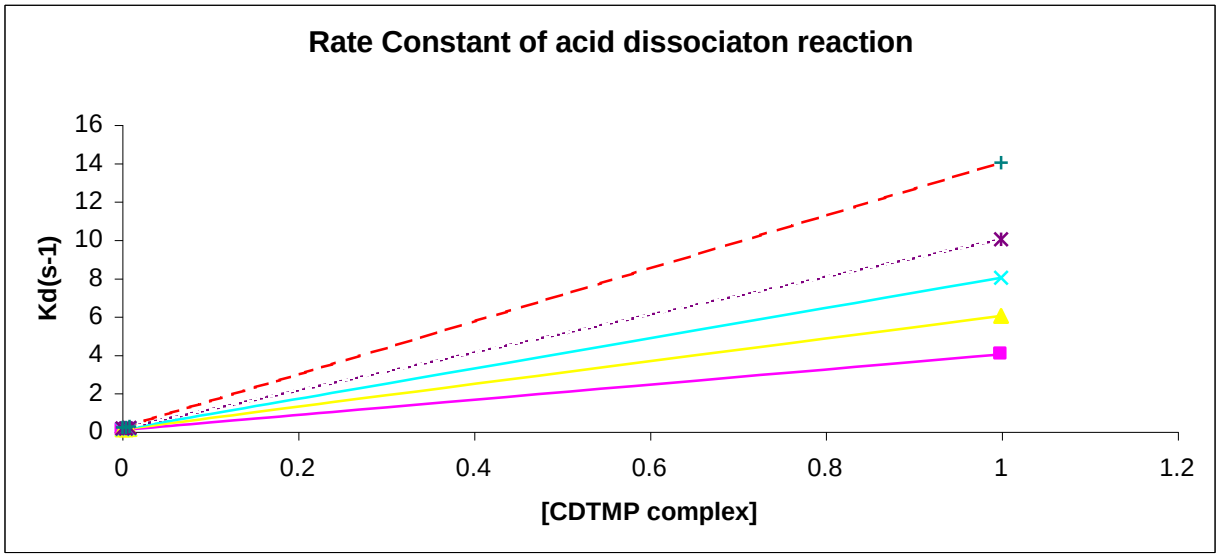

Fig1 : Rate constant of complex for heavier isotope chelation

The lower activation enthalpy for formation of uranyl complexes with the ligands is probably a result of the shorter distance between the phosphonate groups as compared with the case of the 1,2-diphosphonate ligand. It should be noted, although not readily amenable to rationalization, that the activation enthalpies for the forward and reverse reactions are nearly identical for the reaction with but are slightly larger for the forward reactions. The more negative activation entropy for the reverse reaction in all cases implies a greater degree of ordering between the products through precursor complex to the activated complex . This difference may reflect the dehydration of the complex, plus the influence of chelation effects.

Using the Eyring equation, and the resolved rate constants as a function of temperature, the activation enthalpy and entropy for both formation and dissociation reactions were calculated**.** The activation parameters for both formation and dissociation reactions are comparable to previously reported values.

The rates of heavier metal excreted were 1.12 % of the injected dose in group G1, 1.34% in G3, and 0.52% in G5, whereas they were 11.65% in group G2, 9.40% in G4, and 10.15% in G6, respectively . The amount of activity in excreta (urine and feces) was highest in group G2. A significant difference of activity in the excreta between groups G 5 and G6 was found. The retention rates in the liver, femur, and spleen in groups G2, G4, and G6, except for the value of spleen in G4, decreased more than those of the corresponding G1, G3 and G5 groups, respectively. However, the retention rate in the kidney in groups G2 and G4 was higher than that in groups G1 and G3 respectively, but the rate in the G6 group was lower than that of G5.

Table-1 : The rates of heavier isotope excreted in urine and feces 24 hr after injection; the values are presented as percentages of injected doses

Group	Excreted rate in urine (%)	Excreted rate in feces (%)	Total rate in excreta (%)
G1	1.12 ± 0.76	1.85 ± 0.58	2.97
G2	11.65 ± .042	8.77 ± 0.92	20.42
G3	1.34 ± 0.55	14.34 ± 1.32	15.68
G4	9.40 ± 1.1	3.16 ± 0.33	12.56
G5	0.52 ± 0.19	0.15 ± 0.82	0.67
G6	10.15 ± 0.17	0.63 ± 0.51	10.78

The excretion route of the compound administration is through the kidneys and the hepatobiliary system, with the latter being more important **(Table 1)**. The effectiveness of compound on the removal of heavier metal from various critical organs and the blood were clearly observed, with significant differences being detected in the femur.

In conclusion, the results showed that multidentate phosphonic acid based ligand can remove heavier metal from the body. while its has no effect for heavier metal at pH3.5 solution and shows undesirable characteristic of accumulating heavier metal in the kidney. In conclusion, our results indicate that the newly developed complex, offers great potential for its applicability as a decontaminant agent at early times following the radioheavier metal contamination. was effective in removing radioactive heavier metal from the body when administered intraperitoneally.

Although we demonstrated the effects of on removal of heavier metal in the rat bone in the present study, further many examinations might be required to apply this drug for humans. Because it has been argued as to whether a rat is always an appropriate species to use as a model of humans, particularly due to its bone metabolism being very different from that of humans .

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