Complexing Power of Vitamin D 3 Toward Various Metals. Potentiometric Studies of Vitamin D 3 Complexes with Al 3+ , Cd 2+ , Gd 3+ , and Pb 2+ ions in Water-Ethanol Solution

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Complexing Power of Vitamin D_3 Toward Various Metals. Potentiometric Studies of Vitamin D_3 Complexes with Al^{3+}, Cd^{2+}, Gd^{3+}, and Pb^{2+} ions in Water–Ethanol Solution

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This study reports the stability constants of complexes with vitamin D_3 and Al^{3+}, Cd^{2+}, Gd^{3+} and Pb^{2+} ions in a water–ethanol medium (30/70% v/v at 25.0°C). The logarithms of the overall stability constants are: $\beta_1 = 12.4 \pm 0.5$, 7.6 ± 0.3, 9.33 ± 0.07, and 9.1 ± 0.5, respectively, whereas the logarithms of β_2 are 24.4 ± 0.5 (Al^{3+}), 14.3 ± 0.3 (Cd^{2+}), and 15.4 ± 0.5 (Pb^{2+}). Gd^{3+} forms only the 1:1 complex. These values are compared to those reported previously and correlations are established between the stability constants and physical properties, such as the ionization energy.

KEY WORDS: Vitamin D_3; complexes; metal cations; stability; water–ethanol solution.

1. INTRODUCTION

Vitamin D_3 (vit D) (see structure Fig. 1) and its natural hydroxylated derivatives naturally occur in food like fish, egg yolk, meat, milk, and edible mushrooms.(1) Its first therapeutical use is the treatment of osteoporosis and of hyperparathyroidism for patients with chronic renal failure. In the second case, vit D reduces

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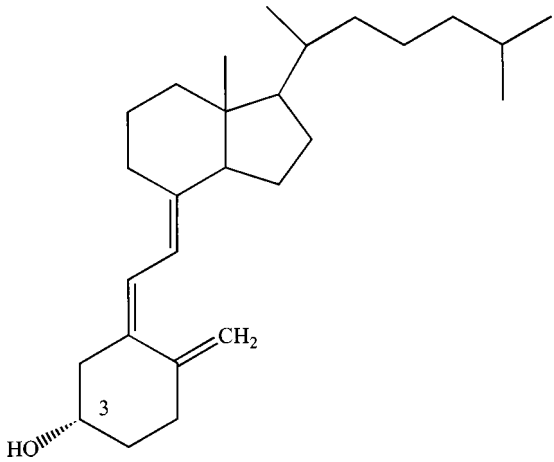

Fig. 1. Chemical structure of vitamin D_3 or cholecalciferol.

hypoplasia of the parathyroid gland, as well as the parathyroid hormone concentration in blood.[2]

Vit D and its hydroxylated derivatives are potent regulators of breast cancer cell development. The ability of 1α,25-dihydroxy vitamin D_3 to inhibit cell growth has been observed in a variety of breast cancer cell lines, including human tumor cells.[3] Recent reports have also described the immunosuppressive properties of this hormone. Although therapeutically effective doses of 1 α,25-dihydroxyvitamin D_3 are known to induce hypercalcemia, the significant potential of this agent has led to a resurgence of interest in the design and synthesis of analogs of 1α,25-dihydroxyvitamin D_3 that retain its therapeutic efficiency and reduce side effects.[4]

As a nonessential element, aluminum may be toxic to human beings at virtually any concentration. The acute toxicity, caused by high therapeutic doses of aluminum, is often described particularly in uremic patients. In addition, recent epidemiological results have confirmed the existence of a relationship between the incidence rate of Alzheimer's disease and the occurrence of aluminum levels above 100 mg-L^{-1} in drinking water poor in silica.[5]

Cadmium is known to be one of the most toxic heavy metals. This metal is an environmental and occupational contaminant and is a serious health hazard to man and animals. The basis for cadmium toxicity is its negative influence on the enzymatic systems of cells, resulting from the substitution of metal ions (mainly Zn^{2+}, Cu^{2+}, and Ca^{2+}) into metalloenzymes and its very strong affinity for biological structures containing thiols groups, for example, proteins, enzymes, and nucleic acids.[6]

Gadolinium(III) binds weakly to serum proteins and can be displaced by ligands such as citrate. The lanthanide ion excretion paths involve both urine and feces

in contrast to manganese where the gastrointestinal elimination route is almost exclusive. As a metal chelate, renal excretion is greatly increased and, consequently, toxicity decreases.[7] Gadolinium complexes of polyaminocarboxylic ligands are useful as contrast agents for magnetic resonance imaging in clinical diagnostics.[8]

Several studies suggesting various mechanisms for lead neurotoxicity have been published: interference with calcium-mediated cellular processes, alterations in neurotransmitter release, activation of protein kinase *c*, inhibition of glutamate uptake into astrocytes, and inhibition of glutamine synthetase activity.[9]

In this paper, we report the stability constants of complexes involving vit D and Al(III), Cd(II), Gd(III), and Pb(II) cations and compare these new results with those previously reported.[10,11] These metal cations were chosen because of their toxicity (Al^{3+}, Cd^{2+}, and Pb^{2+}) or their use in MRI (Gd^{3+}).

2. MATERIALS AND METHODS

2.1. Materials

All chemicals were of analytical grade and were used without further purification. All solutions were prepared with a mixture of bidistilled, deionized, CO_2-free water and absolute ethanol (Merck, Brazil) 30/70% v/v. The vitamin D_3 (cholecalciferol) was purchased from Sigma-Aldrich, France, and its solutions were freshly prepared prior to use.

Metal solutions of anhydrous $Pb(NO_3)_2$ (Merck, Germany) and $Cd(NO_3)_2 \cdot 4H_2O$ (Aldrich, USA) were analyzed by Atomic Absorption Spectroscopy and by titration with sodium EDTA salt (Merck, Germany).[12a] Metal solutions of $GdCl_3 \cdot 6H_2O$ (Aldrich, USA) and $AlCl_3 \cdot 6H_2O$ (J. T. Baker, USA) were used and standardized according to the literature.[12b,13] The 0.1 mol-L^{-1} carbonate-free potassium hydroxide solutions (Merck, Brazil) were standardized against potassium hydrogen phthalate (Carlo Erba, Brazil). KNO_3 (Merck, Brazil) was the supporting electrolyte used to maintain the ionic strength (I) at 0.100 mol-L^{-1}. All procedures have been described in our earlier publications.[10,11] The number of moles of vit D, Gd^{3+}, and Al^{3+} were calculated taking into account their actual weighed masses and the actual volumes of the solutions of either Cd^{2+} or Pb^{2+} added to the reaction vessel in turn.

2.2. Methods

The potentiometric titration studies were performed in aqueous ethanol solutions (H_2O: 30% EtOH: 70%, v/v) because of the low solubility of vit D in water, under an inert atmosphere maintained by a continuous flow of nitrogen saturated with 0.1 mol-L^{-1} KOH (White-Martins, Brazil). The titrations were carried out in a 60-mL (total volume) jacketed reaction vessel with an airtight cap fitted with gas inlet and outlet tubes, and glass and calomel reference electrodes

(Analyser, Brazil). The cell was thermostated at (25.0 ± 0.1)°C (Microquímica, MQBTC 99–20, Brazil). The pK_w of water in this water/ethanol solution was previously determined by potentiometric titration to be 14.71 at (25.0 ± 0.1)°C under the same experimental conditions.[10] A 25-mL Sigma Techware Digitrate piston manual burette was used to deliver the titrant in 0.02-mL increments of standard carbon dioxide-free KOH. The pH (negative logarithm of the concentration of H^+ in mol-L^{-1}) values were directly measured with a Micronal (SP, Brazil) model B-375 pH meter calibrated with standard adjusted ionic strength (I = 0.100 mol-L^{-1} KNO_3) HCl in a water/ethanol (30/70%, v/v) solution before starting the titration. The slope for electrode response was determined using data obtained from potentiometric titrations of a known volume of this standard 0.005 mol-L^{-1} HCl water/ethanol solution with a standard 0.1 mol-L^{-1} KOH in water/ethanol (30/70%, v/v).[10,11]

Vit D was kept under an inert atmosphere in a Schlenke glass and was always transferred to the reaction vessel using another Schlenke glass stoppered with a Suba-Seal (Sigma, USA) inside a glove bag (Sigma, USA). After being accurately, weighed the approximately 40-mg mass of vit D (0.1 mmol) was dissolved in 10 mL of anhydrous ethanol and then transferred with the aid of a syringe to the reaction vessel with all other reactants also under inert atmosphere.

Each potentiometric titration was carried out with approximately 0.1 mmol of vit D and 0.1 and 0.05 mmol of each of the studied metal ions, Al(III), Gd(III), Cd(II), and Pb(II). All titrations were conducted after stabilization of the first pH value of the system by addition of (0.02 ± 0.01) mL successive increments of titrant whereupon the corresponding pH value was read and plotted to calculate the values of the overall formation constants. All titrations were made in the presence of a strong acid, HNO_3 (Merck, Brazil) in 30% v/v aqueous, 70% v/v ethanolic solution, concentration around 0.08 mol-L^{-1}, (determined by a Gran plot) to make sure that all titrations would start from pH values under 2.0. Though the aim of this work was not to study the kinetics of these reactions, the possible delay in reaching equilibrium was taken into account and all potentiometric measurements were completed within 15 min after the addition of the reactants.

2.3. Data Treatment

The equilibrium constants were calculated using the microcomputer program "Best 7."[10,14–16] The basic algorithm is stated as:

$$T_i = \sum_{j=1}^{NS} e_j \beta_j \prod_{K=1}^{i} [C_k]^{e_{ij}}$$

where T_i is a measure of the mass balance of the ith component of the jth species summed over all species present, NS. The concentration of each of the species is the product of the overall stability constant and the individual component concentration

$[C_k]$ raised to the power of the stiochiometric coefficient, e_{ij}. The set of simultaneous equations is solved for each component concentration, $[C_k]$. In the particular case when this represents the calculated concentration of H^+, it is compared with the measured hydrogen ion concentration. The standard deviation, σ_{fit}, in pH units[13] is obtained as follows:

$$\sigma_{\text{fit}} = \sqrt{\frac{U}{N}}$$

where $N = \sum w$ and $U = \sum w(\text{pH}_{\text{obs}} - \text{pH}_{\text{calcd}})^2$ $w = \frac{1}{(\text{pH}_{i+1} - \text{pH}_{i-1})^2}$

The use of this algorithm within the "Best 7" program for computing equilibrium constants involves the following sequence: (1) start with a set of known or estimated overall stability constants (β) and compute $[H^+]$ at all equilibrium points; (2) compute the weighted sum of the squares of the deviations in pH (U); (3) adjust the unknown stability constants and repeat the calculations until no further minimization of U can be obtained, thus giving the final calculated β values. The hydrolysis constants employed for all studied metal ions were obtained from the literature.[16,17] These values, determined in aqueous solutions, assuming that there were no significant differences with the water/ethanol medium, were fully employed in all calculations.

The species' distribution curves were drawn with the microcomputer program, SPE.[10,14–16] In general, three titrations were made for each metal: one with the ligand alone, and two others with the ligand and metal at different metal to ligand ratios. The titration curves are given in Fig. 2. The ligand to metal ratios were organized in such a way to maximize the formation of the 1:1 complex species, $ML^{(n-1)+}$, and the 1:2 complex species, $ML_2^{(n-2)+}$, M being the metal ion employed and L^-, the deprotonated -OH group in vit D. The solution the ratio of 1 Al^{3+} to 3 vit D (10^{-3} mol-L^{-1}) could not be made because of the low solubility of the system at low pH values. All reported results are the average of the three potentiometric titration experiments.

3. RESULTS AND DISCUSSION

The average overall formation constants are calculated from the following equations:[10]

$$M^{n+} + L^- \rightleftharpoons ML^{(n-1)+} \qquad \beta_1 = K_1 = [ML^{(n-1)+}]/[L^-][M^{n+}] \tag{1}$$

$$ML^{(n-1)+} + H^+ \rightleftharpoons MHL^{n+} \qquad K_{MHL} = [MHL^{n+}]/[ML^{(n-1)+}][H^+] \tag{2}$$

$$M^{n+} + 2L^- \rightleftharpoons ML_2^{(n-2)+} \qquad \beta_2 = \left[ML_2^{(n-2)+}\right]/[M^{n+}][L^-]^2 \tag{3}$$

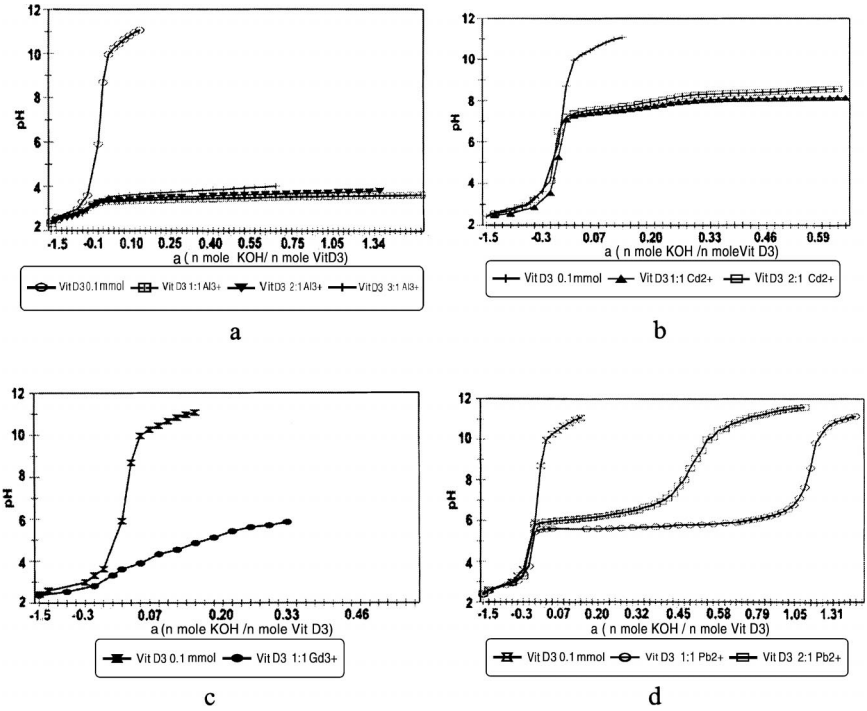

Fig. 2. Titration curves.

where M is the metal ion and L the deprotonated vit D: Vit-O^-. The protonation equilibrium of vit D[10] is given in Eq. (4):

$$\text{Vit} - \text{O}^- + \text{H}^+ \rightleftharpoons \text{Vit} - \text{OH} \qquad \log K = 12.4 \tag{4}$$

The potentiometric pH profiles for each titration of vit D with Gd^{3+} is for a metal to ligand ratio of 1:1 only. For all other metals (Pb^{2+}, Al^{3+}, and Cd^{2+}), metal to ligand ratios are 1:1 and 1:2. Attempts to reach higher metal (Gd^{3+}) to ligand ratio titrations (*e.g.*, 1:2 and 1:3) were unsuccessful and, under the experimental conditions, only the species *ML* (to simplify, the charges will be omitted in the following discussions) was found in quantities higher than 10% of the total species present at the equilibrium.

Table I presents the logarithms of the formation constants for the complexes calculated for the systems described in Eqs. (1–3). The greatest affinity for the binding site of the vitamin D_3 among the metal ions studied was toward the metal ion, Al^{3+}, which is the strongest Lewis acid in the formation of *ML*. The formation constants decrease in the following order: Gd^{3+}, Pb^{2+}, and, finally, Cd^{2+}, which is the weakest Lewis acid toward the deprotonated hydroxyl group of the vit D.

Table I. Logarithmic Values for the Formation Constants of the Complexes of Vitamin D_3 with Various Metal Ions in the Same Experimental Conditions, i.e., $I = 0.1\ M$ and 25°C

	Ref.	$\log K_1 = \log \beta_1$	$\log K_2 (\log \beta_2)$	$\log K_3 (\log \beta_3)$
Al(III)		12.4 ± 0.5	12.0 ± 0.5 (24.4)	n.d.[b]
Cd(II)		7.6 ± 0.3	6.7 ± 0.3 (14.3)	—
Gd(III)		9.33 ± 0.07	n.d.	—
Pb(II)		9.1 ± 0.5	6.3 ± 0.5 (15.4)	—
Mn(II)	11	6.6 ± 0.1	5.8 ± 0.1 (12.4)	—
Fe(II)	11	8.5 ± 0.1	8.0 ± 0.1 (16.5)	—
Fe(III)	11	15.5 ± 0.1	13.0 ± 0.1 (28.5)	6.2 ± 0.1 (34.7)
Co(II)	10	7.6 ± 0.1	6.5 ± 0.1 (14.1)	—
Ni(II)	10	7.8 ± 0.3	6.0 ± 0.3 (13.8)	—
Cu(II)	10	9.3 ± 0.3		—
Zn(II)	10	8.8 ± 0.3	7.7 ± 0.3 (16.5)	—

[a] β_i, Overall stability constant; K_i = stepwise stability constant.
[b] n.d., not detected.

Previous studies have presented ML_3 species only with the very strong Lewis acid, Fe^{3+}.[11]

Figures 3–5 depict the distribution diagrams of the species derived from the formation constants obtained in solutions of vit D and the metal ions Al^{3+}, Cd^{2+}, and Pb^{2+}, respectively, in the metal to ligand ratio of 1:2 and with the metal ion

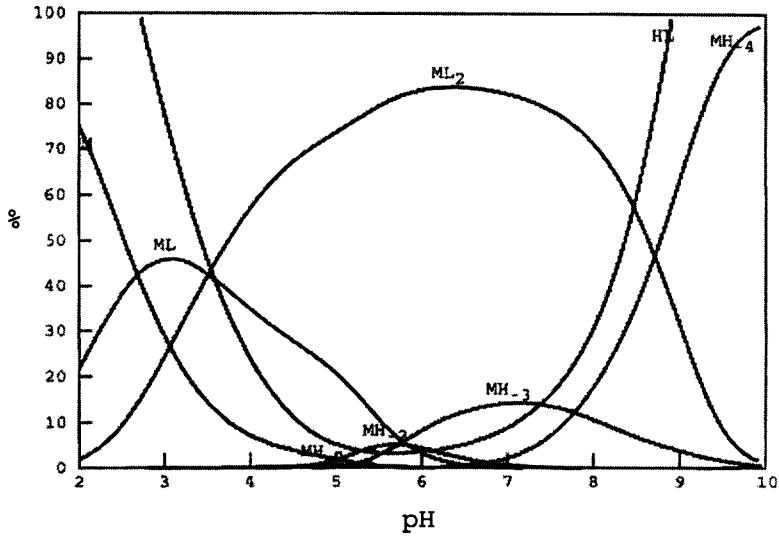

Fig. 3. Species' distribution curve for vitamin D_3 and Al^{3+}.

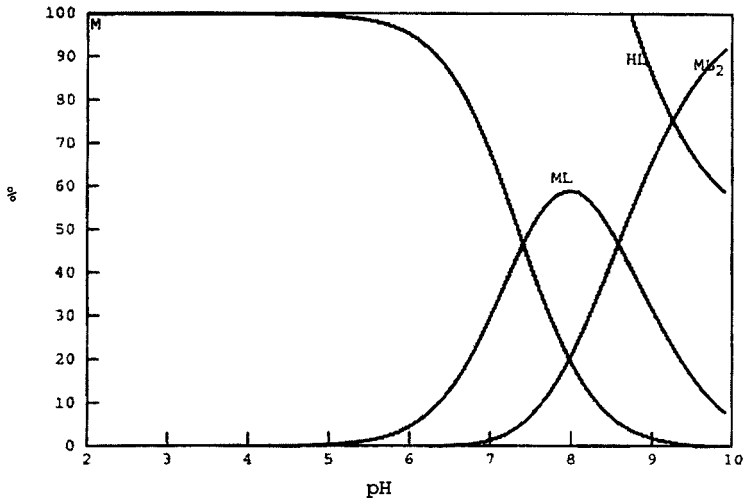

Fig. 4. Species' distribution curve for vitamin D_3 and Cd^{2+}.

concentration set at 100%. Vit D with Al^{3+} (Fig. 3) shows the formation of the *ML* species at a pH value around 1.8 and diminishing around pH $\approx$ 5.5, after reaching its maximum at pH $\approx$ 3.0. The ML_2 species is formed between pH values of 2.5 and 9.5 with a maximum at pH = 6.3. In case of Gd^{3+} and vit D (figure not shown), the only detected *ML* species begins forming at pH = 5.0, reaching its maximum at 8.3, and disappearing around 9.0. Vit D and Pb^{2+} (Fig. 5) showed a distribution

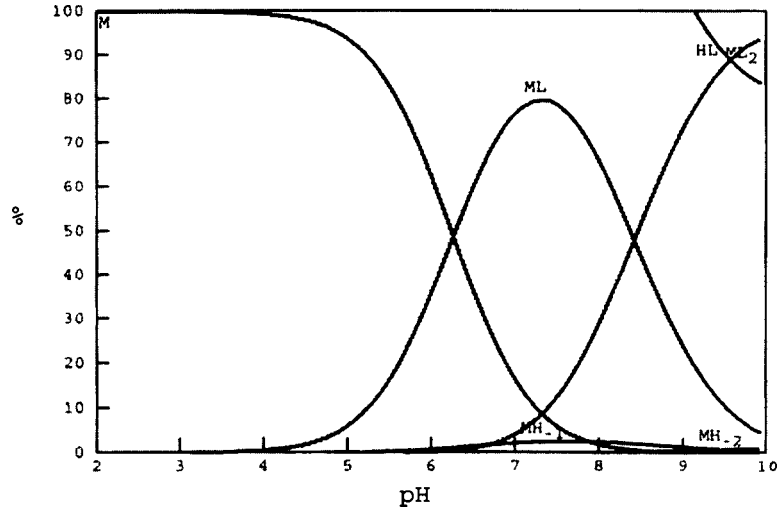

Fig. 5. Species' distribution curve for vitamin D_3 and Pb^{2+}.

diagram where the hydrolytic species were insignificant, although six hydrolysis constants for the six species possibly present were considered in the calculations. It was then possible to calculate the formation constants for the *ML* and ML_2 species involving this a soft Lewis acid. The *ML* complex started forming at a pH around 5.0, reaching its maximum at pH = 7.3 and disappearing above pH = 9.5, while the same steps for ML_2 are at pH values, 7.5, 9.9, and 11.0, respectively. Similar results can be seen in Fig. 4, where Cd^{2+} and vit D also showed little influence of the hydrolytic species in the equilibrium, thus allowing the calculation of the stability of ML_2. The appearance, maximum, and destabilization of *ML* were seen at pH values of 5.5, 8.0, and 9.9, and for ML_2 species: 7.5, 10.0, and 11.0, respectively. Moreover, as the Lewis acids weaken the pH values, the beginning of the formation of the complexes shifted to higher values.

Table I summarizes the logarithmic values for the formation constants of the complexes of vitamin D_3 with all metal ions studied and determined under the same experimental conditions[10,11]. Figure 6 shows the relationship between the stepwise stability constants K_1 and K_2, the overall stability constant β_2, the metal ionization energies, expressed in eV,[18] and the involved metals. These values are consistent with the well-known Irving–Williams series.[19,20] However, we may remark that the complex with Fe^{2+} is more stable than theoretically expected. When observing Fig. 6, we can also notice that lead complexes are more stable

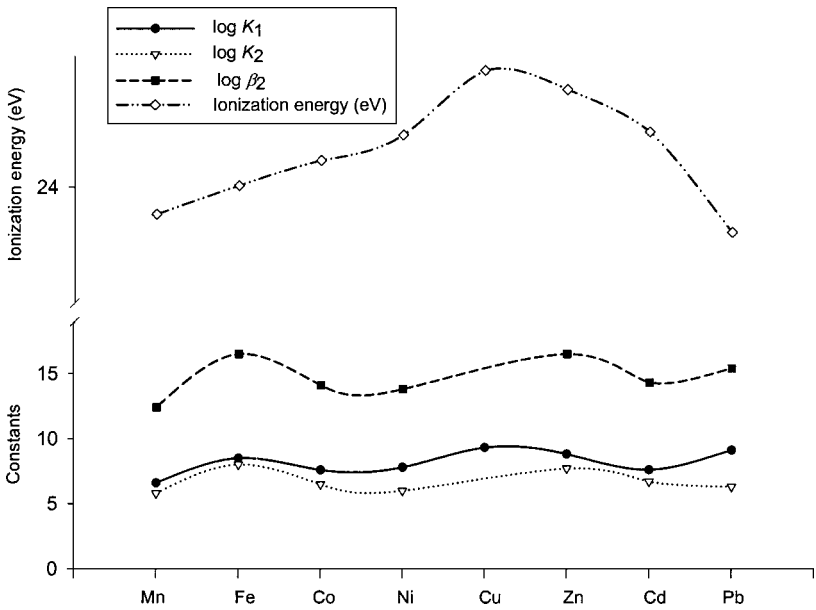

Fig. 6. Relationship between K_1, K_2, β_2, ionization energies (eV), and the nature of the metal.

than the corresponding cadmium species, but it is not possible to conclude anything about their relative toxicities. With regard to the trivalent cations, the three constants could be determined with only Fe(III) and, consequently, it is not possible to obtain any relationship. However, the logarithmic K_1 values increase in the order Gd(III), Al(III), and Fe(III) and for K_2 (or β_2), complexes with Fe(III) are the most stable.

This paper completes our studies on the stability of vit D complexes with various metal cations. The most important fact is that this vitamin could give *ML* complexes with all the studied metals and that these complexes are stable species with log K_1 values ranging from 6.6 for Mn(II) to 15.5 for Fe(III) at 25°C.[10,11] This raises interesting medical questions, such as the possibility of performing MRI for patients with high vit D sera concentration. Furthermore, vit D treatment in case of methemoglobinemy seems quite appropriate as Fe(III) are the most stable species. It may be recalled that for all other metals studied, viz., Mn(II), Co(II), Ni(II), Cu(II), and Zn(II), the values of log β_1 are of the order of 7.9 ± 1.3.[10,11] It is only with Fe(III) that highly stable complexes are formed, log $\beta_1 = 15.5$, a factor that should be taken into account for any further studies in medical applications. This higher stability for iron(II) complexes was also described in the case of hydroxamic siderophores.[21] It has been proves that manganese(II) complexes are equally involved in cadmium(II)-resistant cells[22] and their stability constants lie in the same range (Table I).

It is known that vit D increases intestinal calcium and phosphate absorption. Not so well known, however, is the fact that vitamin D stimulates the coabsorption of other essential minerals like magnesium, iron(II) and (III), and zinc, as well as toxic metals such as lead, cadmium, aluminum, and cobalt and some radioactive isotopes. Vit D may contribute to the pathologies induced by toxic metals by increasing their absorption and retention. Conversely, lead, cadmium, aluminum, and strontium interfere with normal vitamin D metabolism by blocking renal synthesis of 1,25-dihydroxyvitamin D.[23] These facts could be explained by the high values of all our calculated stability constants.

The structure of these complex species could be described on the basis of an octahedral environment (see Fig. 7) as previously reported.[24]

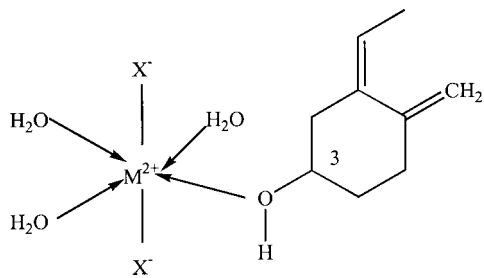

Fig. 7. Proposed structure for 1:1 complexes.

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REFERENCES

1. P. H. Mattila, V. I. Piironen, E. J. Uuso-Rauva, and P. E. Koivistoinen, *Food Chem.* **1**, 95 (1995).
2. S. Tsuruoka, K. Sugimoto, and A. Fujimara, *Life Sci.* **68**, 579 (2000).
3. C. J. Narvaez, G. Zinser, and J. Welsh, *Steroids* **66**, 301, (2001).
4. J. M. Nuss, M. M. Murphy, R. A. Rennels, M. H. Heravi, and B. J. Mohr, *Tetrahedron Lett.* **34**, 3079 (1993).
5. M. Venturini-Soraino, and G. Beryhon, *J. Inorg. Biochem.* **85**, 143 (2001).
6. M. M. Brzoska and J. Moniusko-Jakoniuk, *Food Chem. Toxicol.* **39**, 967 (2001).
7. L. Thunus and R. Lejeune, *Coord. Chem. Rev.* **184**, 125 (1999).
8. K. Kumar, *J. Alloys Compd.* **249**, 163 (1997).
9. S. Raunio and H. Tähti, *Chemosphere* **44**, 355 (2001).
10. A. L. R. Mercê, B. Szpoganicz, R. C. Dutra, M. A. Khan, X. Do Thanh, and G. Bouet, *J. Inorg. Biochem.* **71**, 87 (1998).
11. A. L. R. Mercê, B. Szpoganicz, M. A. Khan, X. Do Thanh, and G. Bouet, *J. Inorg. Biochem.* **73**, 167 (1999).
12. (a) G. Schwarzenbach and H. Faschka, *Complexometric Titrations*, 5th ed. (Methuen, London, 1969), p. 268; (b) p. 184.
13. A. E. Martell and R. J. Motekaitis, *The Determination and Use of Stability Constants*, 2nd edn. (VCH, New York, 1992).
14. Y. J. Li and A. E. Martell, *Inorg. Chim. Acta* **214**, 103 (1993).
15. R. Delgado, Y. Sun, R. J. Motekaitis, and A. E. Martell, *Inorg. Chem.* **32**, 3320 (1993).
16. C. F. Baes Jr. and R. E. Mesmer, *The Hydrolysis of Cations* (Wiley, New York, 1976).
17. E. W. Schwingel, K. Arend, J. Zarling, A. Neves, and B. Szpoganicz, *Brazil. J. Chem. Soc.* **7**, 31 (1996).
18. Handbook of Chemistry and Physics, 69th edn. (CRC Press, Boca Raton, 1989).
19. H. Irving and R. J. P. Williams, *J. Chem. Soc.* p. 3192 (1953).
20. M. A. Khan, G. Bouet, F. Vierling, J. Meullemeestre, and M. J. Schwing, *Transition Met. Chem.* **21**, 231 (1996).
21. Z. D. Liu and R. C. Hider, *Coord. Chem. Rev.* **232**, 151 (2002).
22. S. Himeno, T. Yanagiya, S. Enomoto, Y. Kondo, and N. Imura, *Tohoku J. Exp. Med.* **196**, 33 (2002).
23. J. Moon, *J. Amer. Coll. Nutr.* **13**, 559 (1994).
24. J-F. Gadais, M. A. Khan, and G. M. Bouet, *Transition Met. Chem.* **19**, 651 (1994).