Complex Formation Equilibria of Monoisopropyl and P,P-Diisopropyl Esters of Clodronic Acid with Calcium and Magnesium Ions in Aqueous (CH₃)₄NCl Solutions

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The complex formation equilibria of the monoisopropyl ester and P,P-diisopropyl ester of clodronic acid with Ca^{2+} and Mg^{2+} ions have been studied by a series of EMF titrations in aqueous solutions at 25°C and $I = 1.0$ (Me₄NCl). Computer analysis of the potentiometric data showed that the complexation scheme can be expressed exclusively in terms of the mononuclear ML species. We found a linear correlation ($r = 0.99$) between β_{101} (ML) and the sum of the logarithm of the acid constants ($\Sigma\, pK_a$) of the studied esters, clodronic and etitronic acids. Also there is a linear correlation ($r = 1.00$) between the logarithm of the overall stability constants of Mg^{2+} and Ca^{2+} complexes, and between log β_{101} (ML) and log β_{011} (HL), of these ligands.

Isopropyl esters of (dichloromethylene)-bisphosphonic acid (clodronic acid) belong to a group of gem-bisphosphonates, which contain a P–C–P bridge. They are chemically related to and mimic the physiolocigal behaviour of P–O–P combounds, pyrophosphates. Most importantly, both pyrophosphate and bisphosphonate ligands have very high affinity for calcium(II) in homogeneous solution and at the surfaces of minerals and bone. Unlike pyrophosphates, bisphosphonates are resistant to chemical and enzymatic hydrolysis and retain their activity in conditions in which pyrophosphates would be destroyed. For this reason bisphosphonates are widely used as therapeutic agents for diseases in the skeletar system and in soft tissues.[1–5]

Clodronic acid, together with conventional cancer therapy, has been used successfully for treatment of hypercalceamia related to osteolytic metastases and malignancies. Clodronic acid has also yielded good results in the treatment of Paget's disease and primary hyperparathyroidism.[6,7] However, the therapeutic index and the absorption of clodronic acid are poor. The clinical properties of methylenebisphosphonates can be modified by varying the subtituents at the bridge carbon and at the phosphate moieties.[8] For this reason the isopropyl ester derivatives of clodronic acid, acting as prodrugs of clodronate or new bisphosphonate drugs, were synthesised. Also there are no data available on the complex formation equilibria of the studied esters with calcium and mag-

nesium ions. The present paper describes study of the complexation of monoisopropyl and P,P-diisopropyl esters of clodronic acid with Ca(II) and Mg(II) ions in aqueous solutions under constant conditions, $I = 1.0$ (CH₃)₄NCl and 25°C by potentiometric measurements.

Experimental

Syntheses. The studied esters were prepared by Leiras Oy according to procedures described elsewhere.[8,9]

Reagents. Stock solutions of tetramethylammonium chloride, tetramethylammonium hydroxide and HCl were prepared and analysed as described previously.[10] Calcium and magnesium stock solutions were prepared by dissolving the corresponding chlorides $CaCl_2 \cdot 2H_2O$ and $MgCl_2 \cdot 6H_2O$ (Merck p.a.). The metal ion content of the solutions was determined by titration with standard EDTA (Merck P.a.).[11]
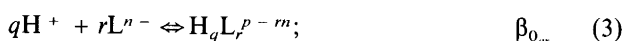
Potentiometric measurements. The potentiometric titrations were performed in a temperature-controlled room (25°C) at 25.0 ± 0.1°C. A locally constructed titration system consisting of a multichannel high-impedance amplifier, a Hewlett-Packard 3478A multimeter and Metrohm 665 Dosimat piston burette was used for the measurements. The equipment was controlled with an Amstrad PC 1640 HD 20 computer using the program TIT3.[12] The indicator electrode was an Orion research

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91-01 glass electrode and the reference electrode an Orion 90-02 double junction Ag,AgCl electrode. The outer mantle of the Ag,AgCl electrode was filled with 1.0 M tetramethylammonium chloride solution. The measurement of the free concentration of H^+ and the calibration of the electrode system were carried out as described in detail earlier.[10]

The measurements were carried out in an argon atmosphere using a constant ionic strength medium of 1.0 M tetramethylammonium chloride by a series of potentiometric titrations. During the titration of acidic three component metal–H^+–ligand solutions, the ratio between the total concentrations of ligand (C_L) and metal (C_M) was held constant, and the total hydrogen ion concentration (C_H) was varied by the addition of tetramethylammonium hydroxide solution [$I = 1.0$, $(CH_3)_4NCl$] from a burette. A total of five different titrations were carried out for each metal–H^+–ligand system within the limits: $0.0174 \leq C_M \leq 0.0059$ M, $0.0078 \leq C_L \leq 0.0128$ M with $0.46 \leq C_M : C_L \leq 2.2$, and $2.9–3.6 \leq -\log[H^+] \leq 6.6–7.6$ (depending on the metal–H^+–ligand system).

Data treatment. The equilibrium reactions in metal–H^+–ligand system can be described by two general reactions (3) and (4):

$$qH^+ + rL^{n-} \Leftrightarrow H_qL_r^{p-rn}; \qquad \beta_{0_{qr}} \qquad (3)$$

$$pM^{2+} + qH^+ + rL^{n-} \Leftrightarrow M_pH_qL_r^{2p+q-rn}; \qquad \beta_{pqr} \qquad (4)$$

where eqn. (3) denotes the acid–base properties of the ligand, eqn. (4) denotes the formation of the metal–ligand complexes and n is 2 (P,P-diisopropyl ester) or 3 (monoisopropyl ester). For reaction (3) the values of the overall protonation constants ($\beta_{0_{qr}}$) were determined earlier[10] and were considered as known in calculations of overall stability constants (β_{pqr}) for reaction (4). The mass balance and equilibrium conditions used in our calculations are as given by eqns. (5)–(7):

$$C_M = [M^{2+}] + \Sigma\Sigma\Sigma p\beta_{pqr}[M^{2+}]^p[H^+]^q[L^{n-}]^r \qquad (5)$$

$$C_H = [H^+] - k_w/[H^+]$$
$$+ \Sigma\Sigma\Sigma q\beta_{pqr}[M^{2+}]^p[H^+]^q[L^{n-}]^r \qquad (6)$$

$$C_L = [L^{n-}] + \Sigma\Sigma\Sigma r\beta_{pqr}[M^{2+}]^p[H^+]^q[L^{n-}]^r \qquad (7)$$

where C_M, C_H and C_L are total concentrations and $[M^{2+}]$, $[H^+]$ and $[L^{n-}]$ are free concentrations of metal, H^+ and ligand, respectively. Generally, in titrations the total concentrations C_M, C_H and C_L are known and the free concentration of H^+ can be measured. In each experimental point $[M^{2+}]$ and $[L^{n-}]$ can be calculated using eqns. (5) and (7) with the assumption of certain values of $\beta_{0_{qr}}$ and β_{pqr}. The search for a model (pqr) and corresponding overall stability constants (β_{pqr}) that give the 'best' fit to experimental data was carried out using modified version SCOGS2[13] of the nonlinear least-squares computer program SCOGS.[14] The error squares

sum $U = \Sigma (V_i^{calc} - V_i^{obs})^2$ was minimized (V_i^{calc} is the calculated and V_i^{obs} the added titrant volume at the point i). The quantities of V_i^{calc} were calculated from input data using current estimates of the constants, and the measured $-\log[H^+]$.[14a] The statistical part of SCOGS2 calculates the P-value as well as an agreement index R for each set of constants. At the 95% confidence level the P-value of the model should exceed 0.05. The P-value is a measure for the normal distribution of the ($V_i^{calc} - V_i^{obs}$)-residuals. The agreement index is calculated from eqn. (8):

$$R = [\Sigma (V_i^{calc} - V_i^{obs})^2/\Sigma (V_i^{obs})^2]^{1/2} \qquad (8)$$

The best set of constants gives the smallest R value. $\bar{n}$, the average number of ligands bound to metal ion, is calculated from eqn. (9):

$$\bar{n} = \{(C_L - (C_H - [H^+] - k_w/[H^+]))/\bar{n}_H\}/C_M \qquad (9)$$

where $\bar{n}_H$, the average number of H^+ bound to each ligand, is calculated using the known values of protonation constants.

Results and discussion

The potentiometric data consisted of five titrations and 109–179 experimental points for each metal–ligand system. The treatment of the potentiometric data was initiated by making Bjerrum plots of $\bar{n}$ vs. log [L] (Fig. 1). The experimental $\bar{n}$-curves seem to be only a function of log [L], and all the curves coincide for the whole $-\log[H^+]$, C_L and C_M region over which the measurements were carried out, and no significant deviation from the calculated $\bar{n}$-curve was observed. This indicates that the values of the stability constants of Mg^{2+} and Ca^{2+} complexes do not depend upon the total concentration of the ligand or the free concentration of the metal and that the system can be described solely by the stepwise formation of ML_r complexes. Further, the plots show that complexes with a ligand-to-metal ratio higher than one are hardly formed, because of the low $\bar{n}$-values. Computer analyses revealed that the ML complexes explain the experimental data fairly well. However, in the case of P,P-diisopropyl ester the precipitation in metal containing solutions limits the operational $-\log[H^+]$ range used in calculations. The precipitation took place at about $-\log[H^+] \geq 7.0$ and the precipitate is probably the ML complex.

The best set of overall stability constants obtained with the program SCOGS2 are given in Table 1. Because the program gave a P-value range 0.051–0.52 and an R-index range 0.0014–0.0062 for stability constants calculated from individual titrations, statistically reliable constants were obtained over the $-\log[H^+]$, C_L and C_M region used in the calculations. The monoisopropyl ester forms stronger complexes with Ca^{2+} and Mg^{2+} than the P,P-
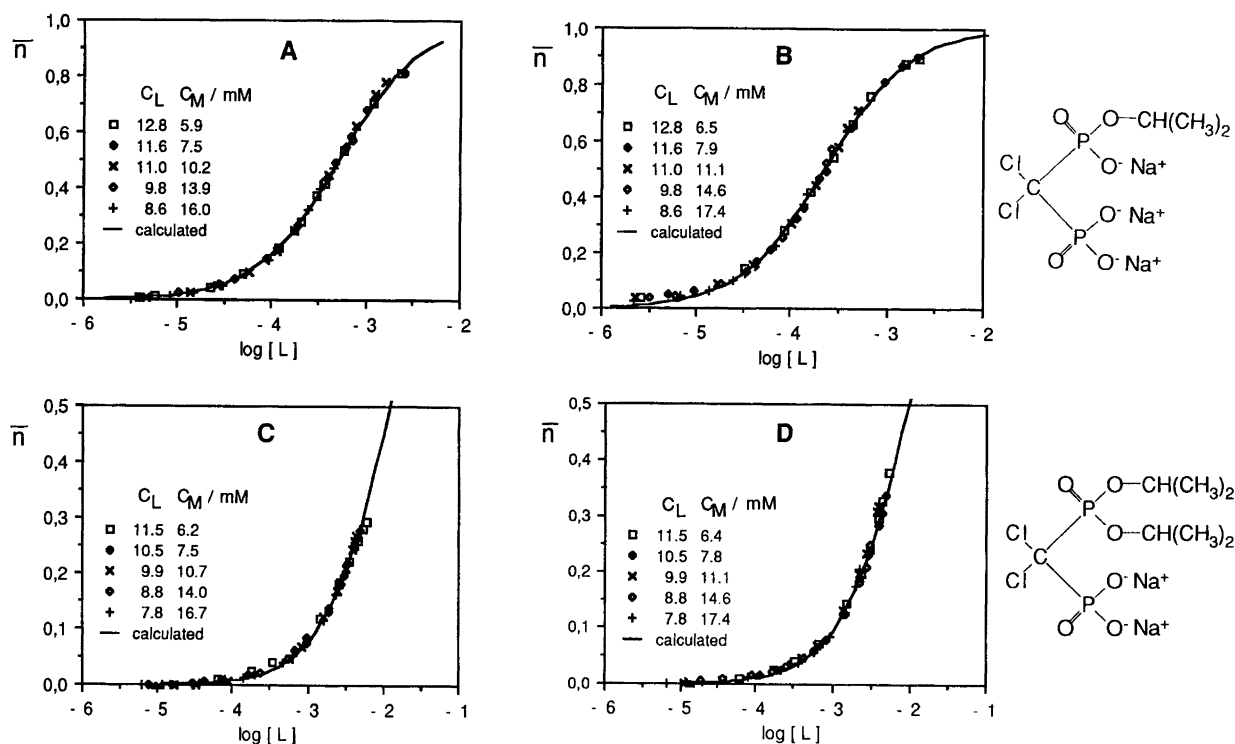
Fig. 1. Experimental and calculated $\bar{n}$ vs. $-\log$ [L] of Ca complexes (A), Mg complexes (B) of monoisoropyl ester of clodronic acid and Ca complexes (C), Mg complexes (D) of P,P- diisopropyl ester of clodronic acid.

diisopropyl ester. Probably only one negatively charged phosphonate group from P,P-diisopropyl ester and both phosphonate groups from monoisopropyl ester are involved in the complex formation, resulting in a stronger chelate effect for the monoisopropyl ester. Also both ligands bind the Mg^{2+} ion more strongly than the Ca^{2+} ion, which is thus in agreement with the Irving–Williams order of stabilities.[15] Figure 2 shows $\log \beta_{101}$ of the Mg^{2+}

and Ca^{2+} complexes as a function of the sum of the logarithm of the acid constants (Σ pK_a) of the monoisopropyl ester, the P,P-diisopropyl ester of clodronic acid,[10] clodronic acid and etitronic acid.[16] The linearity observed in both cases emphasizes the dominance of ionic interactions in these systems.[17] Deviation from unit slope in both cases is attributed to a change in the nature of bonding between the donor group and the metal ions as well as to

Table 1. Logarithm of the overall stability constants of Ca^{2+} and Mg^{2+} complexes ($\log \beta_{101} \pm 3\sigma$), sum of the logarithm of the acid constants (Σ pK_a) and logarithm of the overall first protonation constants ($\log \beta_{011}$) of monoisopropyl ester, P,P-diisopropyl ester of clodronic acid, clodronic acid and etitronic acid.

M^{2+}	$-\log$ [H$^+$]	C_L/mM	C_M/mM	$\log \beta_{101}$	Σ pK_a	$\log \beta_{011}$
P,P-diisopropyl ester of clodronic acid						
Ca	2.9–6.6	7.8–11.5	16.7–6.2	1.91 ± 0.02	6.43^a	6.13^a
Mg	3.0–6.6	7.8–11.5	17.4–6.4	2.02 ± 0.02		
Monoisopropyl ester of clodronic acid						
Ca	3.6–7.4	8.6–12.8	16.0–5.9	3.29 ± 0.02	8.43^a	7.24^a
Mg	3.7–7.6	8.7–12.8	17.5–6.5	3.65 ± 0.02		
Clodronic acid						
Ca				5.4^b	17.71^b	9.55^b
Mg				5.89^b		
Etitronic acid						
Ca				7.17^b	22.13^b	11.14^b
Mg				7.24^b		

^{a} Values are taken from Ref. 10. ^{b} Values are taken from Ref. 16.
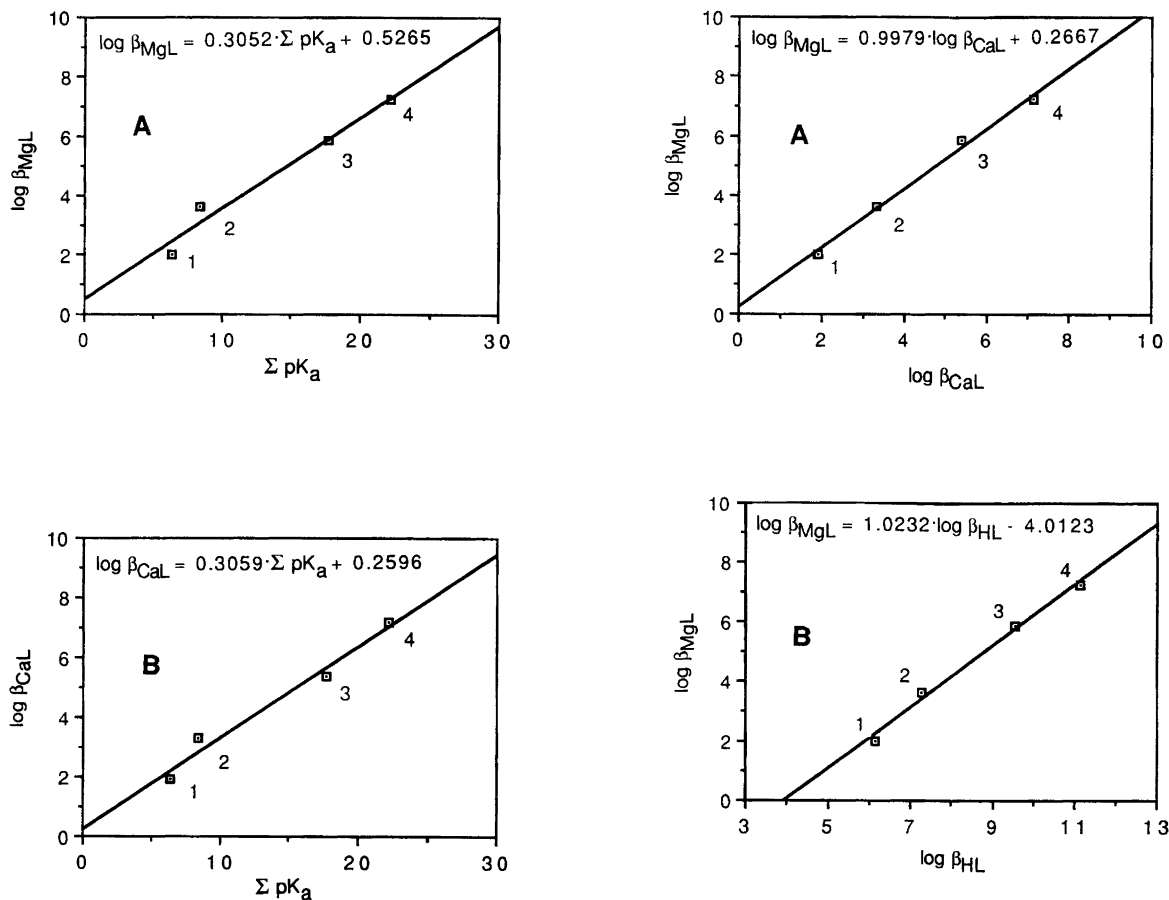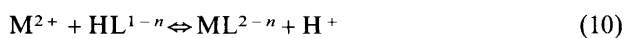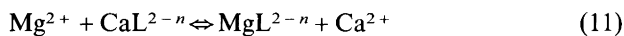
Fig. 2. Log β_{ML} as a function of $\Sigma\, pK_a$ for Mg^{2+} (**A**) and Ca^{2+} (**B**) of (1) the P,P-diisopropyl ester of clodronic acid, (2) the monoisopropyl ester of clodronic acid, (3) clodronic acid and (4) etitronic acid.

solvation effects.[18] A plot of log β_{101}(ML) against log β_{011}(HL) (Fig. 3), and a plot of log β_{101} of Mg^{2+} complexes against those of Ca^{2+} complexes (Fig. 3) gave straight lines of unit slope. These signify that in the first case the equilibrium constants of proton displacement reactions:[19]

$$M^{2+} + HL^{1-n} \Leftrightarrow ML^{2-n} + H^+ \qquad (10)$$

and in the second case the equilibrium constants of central ion exchange reactions:

$$Mg^{2+} + CaL^{2-n} \Leftrightarrow MgL^{2-n} + Ca^{2+} \qquad (11)$$

are equal for these four ligands. The above-mentioned linear relationship equations are these:

$$\log\beta_{101}\,(MgL) = 0.3052\,\Sigma\,pK_a + 0.5265 \qquad r = 0.98 \quad (12)$$

$$\log\beta_{101}\,(CaL) = 0.3059\,\Sigma\,pK_a + 0.2596 \qquad r = 0.99 \quad (13)$$

$\log\beta_{101}$ (MgL)

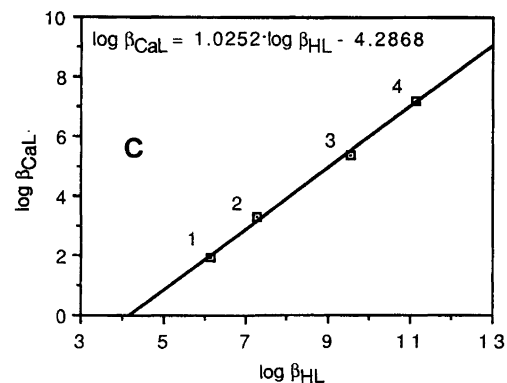$$= 1.0232\,\log\beta_{011}\,(HL) - 4.0123 \qquad r = 1.00 \quad (14)$$

Fig. 3. (**A**) Log β_{MgL} vs. log β_{CaL}, (**B**) log β_{MgL} vs. log β_{HL} and (**C**) log β_{CaL} vs. log β_{HL} for (1) the P,P-diisopropyl ester of clodronic acid, (2) the monoisopropyl ester of clodronic acid, (3) clodronic acid and (4) etitronic acid.

$\log\beta_{101}$ (CaL)

$$= 1.0252\,\log\beta_{011}\,(HL) - 4.2868 \qquad r = 1.00 \quad (15)$$

$\log\beta_{101}$ (MgL)

$$= 0.9979\,\log\beta_{011}\,(CaL) - 0.2667 \qquad r = 1.00 \quad (16)$$

In order to visualize the composition of the solutions studied in this work two distribution diagrams in the $H^+–Mg^{2+}$–monoisopropyl ester/P,P-diisopropyl ester

solutions are given in Fig. 4. The program SOLGAS-WATER[20] was used to calculate these diagrams. Also this program was used to calculate the simplified model of $H^+-Ca^{2+}-Mg^{2+}$-monoisopropyl ester/P,P-diisopropyl ester systems in human blood serum. The protonation constants from Ref. 10 and the stability constants obtained in the present study have been applied together with average concentrations of the studied esters (0.010 mM), Mg^{2+} (0.85 mM) and Ca^{2+} (2.40 mM) ions in blood serum.[16] The distribution diagrams are shown in Fig. 5. The physiological pH of human serum is about 7. As can be seen, at this pH 76% of the monoisopropyl ester is bound to the complexes CaL^- (42%) and MgL^- (34%). In addition there exists free ligand in the forms of HL^{2-} (15%) and L^{3-} (9%). In the case of P,P-diisopropyl ester the situation is totally different: 80% of the ligand is free in the forms of L^{2-} (70%) and HL^- (10%). The relative amounts of the complexes CaL and MgL are 14 and 6%, respectively. The behaviour of the present ligands differ from that of clodronic acid. The distribution diagrams show clearly that for these two esters the calcium containing species are not predominant in human serum. In the case of clodronic acid about 80% of the ligand is bound to Ca^{2+} ion.[16]
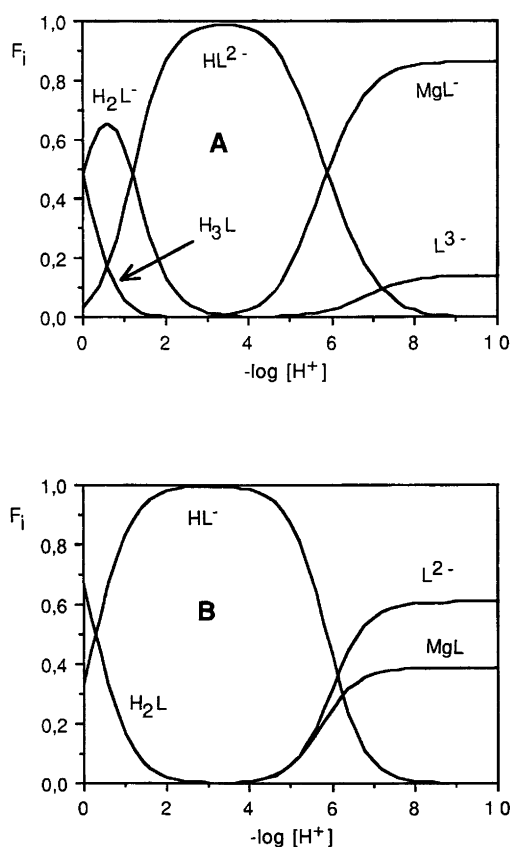

Fig. 4. Distribution diagrams in the (A) H^+-Mg^{2+}-monoisopropyl ester and (B) $H^+-Mg^{2+}-P,P$-diisopropyl ester of clodronic acid solutions ($C_L=10.0$ mM, $C_M=10.0$ mM). F_i is defined as the ratio between the bound ligand content and the total ligand content.
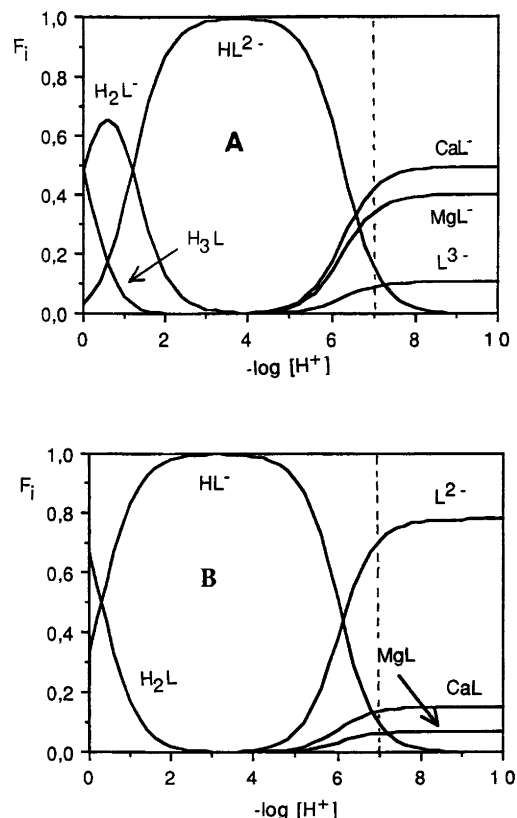

Fig. 5. Simplified model calculation on (A) $H^+-Mg^{2+}-Ca^{2+}$-monoisopropyl ester and (B) $H^+-Mg^{2+}-Ca^{2+}-P,P$-diisopropyl ester of clodronic acid systems in human serum ($C_L=0.01$ mM, $C_{Mg}=0.85$ mM, $C_{Ca}=2.4$ mM). F_i is defined as the ratio between the bound ligand content and the total ligand content. The physiological free hydrogen ion concentration is marked with a dashed line.

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