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Speciation of phytate ion in aqueous solution. Sequestration of magnesium and calcium by phytate at different temperatures and ionic strengths, in $NaCl_{aq}$ ☆

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Abstract

The formation and stability of Mg^{2+} and Ca^{2+}–phytate complexes was studied potentiometrically using an ISE-H^+ electrode. Measurements were performed at 10 °C and 25 °C in $NaCl_{aq}$ in the ionic strength range $0.1 \le I \le 0.75$ mol L^{-1}. For both magnesium and calcium systems, the formation of ten $M_i PhyH_j^{(12-2i-j)-}$ species was observed in the range $3 \le pH \le 7$ with $i=1, 2, 3$ and $j=3, 4, 5$ (and $i=3$, $j=2$). These species are quite stable; here we report for example some quantitative data for the species $Ca_i PhyH_3^{(9-2i)-}$, $i=1, 2, 3$ (equilibrium $iCa^{2+} + H_j Phy^{(12-j)-} = Ca_i PhyH_j^{(12-j-2i)-}$: K_{ij}) at $I=0.25$ mol L^{-1} and $t=25$ °C: $\log K_{13}=3.42$, $\log K_{23}=6.47$ and $\log K_{33}=9.41$. The speciation of the Ca^{2+}–phytate system was also checked by ISE-Ca^{2+} measurements. Dependence on ionic strength was modeled using a simple Debye–Hückel type equation and formation constants were calculated at infinite dilution. The stability constants of complexes formed at pH > 7 were estimated using an empirical predictive equation. The sequestering ability of phytate towards Mg^{2+} and Ca^{2+} was calculated in different experimental conditions and compared with those of other chelating agents.

Keywords: Phytic acid; Magnesium and calcium complexes; Complex formation constants; Sequestering ability; Dependence on ionic strength

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1. Introduction

Phytic acid is the common name for *myo*-inositol hexakisphosphate or 1,2,3,4,5,6-hexakis(dihydrogen phosphate), whose structure was first hypothesized about 100 years ago [8] and recently confirmed [9–11]. The pH, the presence of intramolecular interactions between C–H and O–P groups and the formation of hydrogen bonds between the phosphate groups are all factors that affect its conformation, which can be equatorial (five C–O in equatorial position and one in axial position) or axial (five C–O in axial position and one in equatorial position), as reported in a NMR study [12]. The presence of 12 protons in the phytic acid molecule allows the formation of different protonated species as a function of pH; the fully deprotonated form is present in very alkaline media ($\log K^{H}{}_{1} = pK_{a12} = 9.58$ at 25 °C and $I=0.1$ mol L^{-1}, $NaCl_{aq}$). Protonated species interact with cations (including alkali metal cations and ammonium cations), proteins, starch, sugars, etc. Phytic acid represents the main stored inorganic phosphate resource of many vegetables; in cereals and legumes the amount of phytic acid varies from 0.4% to 2.1% of dry weight, according to origin, variety, and growing conditions (climate, irrigation, type of soil) [13]. Phytic acid is considered the principal source of reactive phosphate groups for living organisms; however, being a strong chelating agent towards the multivalent inorganic cations (sodium, calcium, magnesium, manganese, iron, zinc) that form indigestible complexes, it causes a marked reduction in the bioavailability of these dietary elements [11,14–16]. On the other hand, several studies have shown the beneficial effects of phytic acid on human health, mainly due to its role in cancer prevention and/or therapy and its anti-oxidant functions. The results of many experiments on different animals report that phytic acid is chemo-preventive in colon and mammary carcinogenesis, and its presence in the

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☆ Previous contributions to this series: (1–7).

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diets of rats lowers the level of colon carcinogenesis biomarkers [17]. The potential of phytic acid to prevent corrosion in metals has also been investigated [18] along with its capacity for reducing the mobility and bioavailability of cations such as uranium, nickel, lead, chromium and other inorganic contaminants [19–21]. Insoluble Ca^{2+}–phytate complexes have similar applications [22,23].

The literature cited above evidences the level of scientific interest in this ligand. Despite this fervor, however, few papers include quantitative data on the thermodynamic properties of phytic acid–cation interactions. We have reported thermodynamic data on the acid–base properties of phytate in different ionic media [2,4] and their interactions with biogenic polyamines [1], alkali metals [3], dimethyltin(IV) [6] and mercury(II) [7]. Quantitative data on the formation of complex species between phytate (Phy^{12-}), Mg^{2+} and Ca^{2+} are few, and sometimes no distinction is made between soluble and insoluble complexes. Moreover, there are often inconsistencies between data from different laboratories. This lack of information is surprising if compared with the abundance of data existing in extensive compilations on the metal complex formation constants of low molecular weight ligands [24–27]. We have found one paper [28] that reports quantitative data on the soluble complexes in the Ca^{2+}–phytate system (potentiometric measurements, ISE-Ca^{2+}) and more recently another [29] reporting quantitative data (potentiometric measurements, ISE-H^{+}) for the Mg^{2+}–phytate and Ca^{2+}–phytate systems. Other papers are concerned with the formation of solid species [30–33] and neglect interpretation of potentiometric data in the acidic pH range, before the formation of insoluble complexes [32,33]. We have already studied the formation of insoluble Ca^{2+}–phytate complexes [5] and aim, in this paper, to make a contribution to understanding of the complexation equilibria occurring in solution between Ca^{2+} and $PhyH_j^{(12-j)-}$. To this end we used the potentiometric approach in two different ways; firstly, mixtures of phytic acid and Ca^{2+} (ISE-H^{+} measurements) were titrated alkalimetrically at 10 and 25 °C and at different ionic strengths up to 0.75 mol L^{-1} (NaCl); secondly, mixtures of phytate and standard Ca^{2+} (ISE-Ca^{2+} measurements) were titrated at various constant pH values at a temperature of 25 °C and ionic strength 0.1 mol L^{-1} (NaCl). We also used ISE-H^{+} measurements to analyze the formation of complex species of Mg^{2+} and Phy^{12-} in the same experimental conditions as for Ca^{2+}.

2. Experimental section

2.1. Chemicals

Standard phytic acid solutions were prepared by weighing pure dipotassium salt and subsequently running it over a strong cationic exchange resin (Dowex 50W × 8). Concentration was checked potentiometrically, and the absence of potassium was confirmed by flame emission spectrometry. Hydrochloric acid, sodium and tetraethylammonium hydroxide (Et_4NOH) solutions were prepared by diluting concentrated ampoules that were then standardized against sodium carbonate (the acid) and potassium hydrogen phthalate (the hydroxides). A standard solution of NaCl was prepared by weighing the *puriss.* salt, dried in an oven at 110 °C. Magnesium and calcium chloride solutions were prepared from the *puriss.* solid products, previously vacuum-dried, and then standardized by EDTA complexometric titrations; their purity was always $\geq 95.5\%$. All reagents were Aldrich or Riedel de Haen. Grade A glassware and analytical grade water ($R \geq 18$ MΩ) were used for all solutions.

2.2. Apparatus

Potentiometric titrations were carried out at 10.0 and 25.0 ± 0.1 °C using apparatus consisting of a Crison micropH2002 potentiometer (resolution ±0.1 mV, reproducibility ±0.15 mV) equipped, for the alkalimetric measurements, with a model 8101 Orion Ross type glass-electrode and a calomel reference electrode. The titrant solution was delivered by a model 765 Metrohm motorized burette with an estimated reading accuracy of 0.003 mL. The apparatus was connected to a PC and automatic titrations were performed using a suitable computer program to control titrant delivery, to check for e.m.f. stability, and to acquire titration data. The same apparatus was used when titrations were performed to measure Ca^{2+} using an Orion 93-20 ISE-Ca^{2+} electrode and Ag/AgCl reference electrode. All the measurements were carried out under magnetic stirring, and the solutions were preserved from atmospheric O_2 and CO_2 by a stream of purified N_2.

2.3. Procedure

Potentiometric measurements using the ISE-H^{+} electrode were carried out by titrating 25 mL of solutions containing: phytic acid and $MgCl_2$ or $CaCl_2$ with 0.1 mol L^{-1} standard NaOH up to incipient precipitation of the insoluble complexes (pH = 6.8–7.0). NaCl was then added (0.10, 0.25, 0.50, 0.75 mol L^{-1}) to adjust ionic strength. Potentiometric measurements with the ISE-Ca^{2+} electrode were performed by titrating 25 mL of the solution containing phytic acid with 0.05 mol L^{-1} standard $CaCl_2$ up to $p\mathrm{Ca} \approx 2.7$. The pH of the solutions under analysis had previously been adjusted to 5.0, 6.0 or 7.0 by the addition of Et_4NOH and controlled during titration by ISE-H^{+}. All titrations were carried out in duplicate. In order to determine electrode potential (E^0) and acidic junction potential ($E_j = j_a \times [H^+]$) in the alkalimetric titrations, independent titrations of HCl 10 mmol L^{-1} were carried out for each experiment using a standard base in the same experimental conditions as used for the systems under investigation. In this way, the pH scale used was the total scale, $\mathrm{pH} \equiv -\log[H^+]$, where $[H^+]$ is free proton concentration (not activity). Calibration in the alkaline range was checked by calculating pK_w values [34]. As concerns ISE-Ca^{2+} measurements, electrode potential and slope were determined before each titration by adding known amounts of standard $CaCl_2$ to solutions having the same ionic strengths as those under investigation. Some experimental details are reported in Table 1.

Table 1
Experimental details for potentiometric measurements with ISE-H^+ in NaCl/MCl_2 mixtures ($M=Mg^{2+}$ or Ca^{2+}) at $t=10$ and 25 °C and with ISE-Ca^{2+} in NaCl at $t=25$ °C

ISE-H^+

C_{NaOH} [a]	C_{NaCl} [a]	C_{Phy} [b]	C_{MCl_2} [b]	n [c]	pH_{max} (Mg^{2+})	pH_{max} (Ca^{2+})
0.1	0.1	1	2	2	7.1	6.0
0.1	0.1	1	3	2		
0.1	0.1	1	4	2		
0.1	0.1	2	2	2		
0.1	0.1	2	4	2		
0.1	0.1	2	6	2		
0.1	0.25	1	2	2	7.1	6.1
0.1	0.25	1	3	2		
0.1	0.25	1	4	2		
0.1	0.25	2	2	2		
0.1	0.25	2	4	2		
0.1	0.25	2	6	2		
0.1	0.5	1	2	2	7.3	6.4
0.1	0.5	1	3	2		
0.1	0.5	1	4	2		
0.1	0.5	2	2	2		
0.1	0.5	2	4	2		
0.1	0.5	2	6	2		
0.1	0.75	1	2	2	7.5	6.7
0.1	0.75	1	3	2		
0.1	0.75	1	4	2		
0.1	0.75	2	2	2		
0.1	0.75	2	4	2		
0.1	0.75	2	6	2		

ISE-Ca^{2+}

pH	$C^{T}_{CaCl_2}$ [d]	C_{NaCl} [a]	C_{Phy} [b]	n [c]
5	0.05	0.1	2	2
6	0.05	0.1	2	2
7	0.05	0.1	2	2

[a] Concentration in mol L^{-1}.
[b] Concentration in mmol L^{-1}.
[c] n = number of titrations.
[d] $C^{T}_{CaCl_2}$ = concentration of $CaCl_2$ as titrant in mol L^{-1}.

Table 3
Complex formation constants in Mg^{2+}–phytate and Ca^{2+}–phytate systems, at $I=0.25$ mol L^{-1} and $t=10$ and 25 °C

Species	$\log K_{ij}$ [a], Mg^{2+} ($t=10$ °C)	$\log K_{ij}$ [a], Mg^{2+} ($t=25$ °C)	$\log K_{ij}$ [a], Ca^{2+} ($t=10$ °C)	$\log K_{ij}$ [a], Ca^{2+} ($t=25$ °C)
$MPhyH_3^{7-}$	3.79±0.06 [b]	3.60±0.05 [b]	3.52±0.12 [b]	3.42±0.01 [b]
$MPhyH_4^{6-}$	2.82±0.01	2.91±0.01	2.67±0.03	2.06±0.01
$MPhyH_5^{5-}$	2.30±0.08	2.73±0.03	2.14±0.07	2.11±0.02
$M_2PhyH_3^{5-}$	6.40±0.06	6.34±0.03	6.98±0.06	6.47±0.01
$M_2PhyH_4^{4-}$	5.14±0.05	5.24±0.03	5.09±0.09	5.24±0.01
$M_2PhyH_5^{3-}$	4.29±0.09	4.46±0.04	4.18±0.12	4.09±0.02
$M_3PhyH_2^{4-}$	10.57±0.03	10.12±0.02	11.25±0.12	11.23±0.03
$M_3PhyH_3^{3-}$	8.91±0.03	8.80±0.01	9.35±0.11	9.41±0.01
$M_3PhyH_4^{2-}$	7.58±0.02	7.25±0.01	7.70±0.03	7.35±0.01
$M_3PhyH_5^{-}$	6.52±0.04	6.18±0.02	6.01±0.13	6.31±0.01

[a] K_{ij} refers to the reaction: $iM^{2+}+H_jPhy^{(12-j)-}=M_iPhyH_j^{(12-2i-j)-}$.
[b] ±S.D.

2.4. Calculations

To analyze potentiometric data, we used two computer programs, namely BSTAC [35] and STACO [36]. These are able to refine all the parameters of an acid–base titration (analytical reagent concentrations, E^0, slope in the titrations with ISE-Ca^{2+}, pK_w, coefficients of junction potential j_a) and calculate complex formation constants. In addition, the ES4ECI [37] computer program was used to draw distribution diagrams and to calculate species formation percentages. LIANA [38], a general purpose program for the solution of linear and non-linear equations, was used to calculate complex formation constants from ISE-Ca^{2+} potentiometric data, and to determine the dependence of complex formation constants on ionic strength.

Complex formation constants are given according the equilibrium

$$iM^{2+} + H_jPhy^{(12-j)-} = M_iPhyH_j^{(12-2i-j)-} \quad K_{ij} \tag{1}$$

where M^{2+} is Mg^{2+} or Ca^{2+}. Calculations to determine Ca^{2+}–phytate complex formation constants from ISE-Ca^{2+} measurements were carried out using LIANA to solve the mass balance equations relative to the involved equilibria.

The dependence of formation constants on ionic strength was taken into account using a Debye–Hückel type equation, as already proposed by our research group [39]:

$$\log K_{ij} = \log {}^{T}K_{ij} - z^{*}0.51I^{1/2}(1+1.5I^{1/2})^{-1} + CI \tag{2}$$

in which $z^{*}=\sum(\text{charges})^2_{\text{reactants}}-\sum(\text{charges})^2_{\text{products}}$; K = formation constants at I ionic strength and ${}^{T}K$ = corresponding formation constant at infinite dilution. The empirical C parameter can be in some cases considered as a true constant ($z^{*} \leq 8$, $I \leq 1$ mol L^{-1}), but generally is a function of ionic strength: recently we found that the equation:

$$C = c_{\infty} + (c_0 - c_{\infty})(I+1)^{-1} \tag{3}$$

can be used also for different conditions [40].

Table 2
Phytate protonation constants in NaCl, at $I=0.25$ mol L^{-1} and $t=10$ and 25 °C [2], and Na^+ complex formation constants at $I=0.1$ mol L^{-1} and $t=25$ °C [3]

t (°C)	$\log K^{H}_{1}$ [a]	$\log K^{H}_{2}$	$\log K^{H}_{3}$	$\log K^{H}_{4}$	$\log K^{H}_{5}$	$\log K^{H}_{6}$	$\log K^{H}_{7}$
10	9.22	9.51	9.07	7.74	6.12	4.89	2.53
25	9.20	9.46	9.11	7.76	6.15	4.90	2.59

t (°C)	$\log K_{60}$ [b]	$\log K_{51}$	$\log K_{42}$	$\log K_{33}$	$\log K_{24}$	$\log K_{15}$	$\log K_{16}$	$\log K_{25}$	$\log K_{34}$	$\log K_{43}$	$\log K_{52}$	$\log K_{61}$	$\log K_{70}$
25	25.9	17.3	12.2	8.0	4.7	2.1	1.3	3.1	5.4	8.6	12.4	18.1	25.9

[a] $\log K^{H}_{j}$ refers to the equilibrium $H^{+}+H_{j-1}Phy^{(12-j+1)-}=H_jPhy^{(12-j)-}$.
[b] $\log K_{pq}$ refers to the equilibrium $pNa^{+}+H_qPhy^{(12-q)-}=Na_pPhyH_q^{(12-p-q)-}$.

Table 4
Comparison between complex formation constants in the Ca^{2+}–phytate system obtained from ISE-H^+ and ISE-Ca^{2+} measurements, at I=0.10 mol L^{-1} and t=25 °C

Species	$\log K_{ij}$ [a] (ISE-Ca^{2+})	$\log K_{ij}$ [a] (ISE-H^+)	$\log \bar{K}_{ij}$
$CaPhyH_3^{7-}$	4.32±0.01 [b]	4.17±0.01 [b]	4.24±0.07 [c]
$Ca_2PhyH_3^{5-}$	7.38±0.05	7.82±0.01	7.60±0.22
$Ca_3PhyH_3^{3-}$	11.04±0.11	11.22±0.02	11.13±0.09
$CaPhyH_4^{6-}$	3.48±0.03	2.74±0.01	3.11±0.37

[a] K_{ij} refers to the reaction: $iM^{2+}+H_jPhy^{(12-j)-}=M_iPhyH_j^{(12-2i-j)-}$.
[b] ±S.D.
[c] ±δ/2 (deviation from the mean).

Formation constants, species concentrations and ionic strengths are expressed in the molar (mol L^{-1}) concentration scale.

3. Results and discussion

3.1. Stability of Mg^{2+}– and Ca^{2+}–phytate complexes

The potentiometric data derived from the alkalimetric titrations were analyzed using least squares computer programs (see calculation section). In the analysis of experimental data, the previously determined [2] protonation constants of phytic acid in NaCl (shown in Table 2) were taken into account. It is necessary to consider protonation parameters in the same background electrolyte owing to the strong interactions between phytic acid and sodium ion. To identify the number and type of M^{2+}–phytate complexes, different speciation models were considered in least squares computer refinement and formation constants for the best results are reported in Table 3 at I=0.25 mol L^{-1}. The species we found were the same for the two cations considered and for the two temperatures selected, namely, three mononuclear species ($MPhyH_3^{7-}$, $MPhyH_4^{6-}$, $MPhyH_5^{5-}$), three binuclear species ($M_2PhyH_3^{5-}$, $M_2PhyH_4^{4-}$, $M_2PhyH_5^{3-}$), and four trinuclear species ($M_3PhyH_2^{4-}$, $M_3PhyH_3^{3-}$, $M_3PhyH_4^{2-}$, $M_3PhyH_5^{-}$). The potentiometric data derived from titrations carried out at I=0.10 mol L^{-1} and t=25 °C with ISE-Ca^{2+} (in the pH interval between 5 and 7) allowed us to calculate the formation constants of four species, namely $CaPhyH_3^{7-}$, $Ca_2PhyH_3^{5-}$, $Ca_3PhyH_3^{3-}$, and $CaPhyH_4^{6-}$. These data are shown in the first column of Table 4 and, for purposes of comparison, the second column shows the corresponding values determined by ISE-H^+ measurements. We can see that the two sets of data are reasonably consistent. The highest deviation from the mean (δ/2), observed for $CaPhyH_4^{6-}$, remains within acceptable limits and the consistency of results confirms the reliability of the proposed complexation model.

In order to study the dependence of formation constants on ionic strength, we used Eq. (2). In this way we were able to calculate both ${}^{T}K_{ij}$ (formation constants at infinite dilution) for each complex species and the empirical parameters c_8 and c_0. Results are reported in Tables 5 and 6 for Mg^{2+} and Ca^{2+} complex species, respectively. As an example, in Fig. 1 we plotted the dependence of stability constants on ionic strength for the species $CaPhyH_5^{5-}$ and $MgPhyH_5^{5-}$ at t=25 °C; a similar trend can be observed for all the other complexes. In calculations relative to the ionic strength dependence of formation constants we took into account the total formal charge of phytate ion, i.e. $z=-12$, although, owing to the strong interference of sodium ion, effective charge may be

Table 5
Thermodynamic (infinite dilution) formation constants in the Mg^{2+}–phytate system and parameters for their dependence on ionic strength at t=10 and 25 °C

Species	t=10 °C			t=25 °C		
	$\log {}^{T}K_{ij}$ [a]	c_∞	c_0	$\log {}^{T}K_{ij}$ [a]	c_∞	c_0
$MgPhyH_3^{7-}$	7.93 [b]	0.19	5.58	7.82 [b]	0.40	5.12
$MgPhyH_4^{6-}$	6.49	0.45	4.89	6.66	0.29	4.44
$MgPhyH_5^{5-}$	5.47	0.46	4.46	6.03	0.11	3.92
$Mg_2PhyH_3^{5-}$	13.73	0.71	9.95	13.86	0.42	9.09
$Mg_2PhyH_4^{4-}$	11.50	0.98	8.83	11.85	0.23	7.81
$Mg_2PhyH_5^{3-}$	9.67	0.99	7.89	10.18	0.10	6.44
$Mg_3PhyH_2^{4-}$	21.55	1.08	14.96	21.41	0.72	13.53
$Mg_3PhyH_3^{3-}$	18.45	1.33	13.33	18.73	0.41	11.61
$Mg_3PhyH_4^{2-}$	15.67	1.51	11.79	15.83	0.11	9.69
$Mg_3PhyH_5^{-}$	13.13	1.62	10.35	13.41	−0.11	7.71

[a] K_{ij} refers to the reaction: $iM^{2+}+H_jPhy^{(12-j)-}=M_iPhyH_j^{(12-2i-j)-}$.
[b] Errors in the fits: M.D.=0.011–0.014; S.D.=0.017–0.033.

Table 6
Formation constants at infinite dilution for the Ca^{2+}–phytate system and parameters for their dependence on ionic strength at t=10 and 25 °C

Species	t=10 °C			t=25 °C		
	$\log {}^{T}K_{ij}$ [a]	c_∞	c_0	$\log {}^{T}K_{ij}$ [a]	c_∞	c_0
$CaPhyH_3^{7-}$	7.67 [b]	0.26	5.49	7.64 [b]	0.40	5.12
$CaPhyH_4^{6-}$	6.34	0.44	4.92	5.82	0.20	4.52
$CaPhyH_5^{5-}$	5.31	0.46	4.46	5.41	0.11	3.92
$Ca_2PhyH_3^{5-}$	14.31	0.71	9.95	13.99	0.42	9.09
$Ca_2PhyH_4^{4-}$	11.45	0.98	8.83	11.87	0.30	7.72
$Ca_2PhyH_5^{3-}$	9.56	0.99	7.89	9.81	0.10	6.44
$Ca_3PhyH_2^{4-}$	22.23	1.08	14.96	22.52	0.72	13.53
$Ca_3PhyH_3^{3-}$	18.89	1.33	13.33	19.34	0.41	11.61
$Ca_3PhyH_4^{2-}$	15.79	1.51	11.79	15.93	0.11	9.69
$Ca_3PhyH_5^{-}$	12.62	1.62	10.35	13.54	−0.11	7.71

[a] K_{ij} refers to the reaction: $iM^{2+}+H_jPhy^{(12-j)-}=M_iPhyH_j^{(12-2i-j)-}$.
[b] Errors in the fits: M.D.=0.011–0.013; S.D.=0.019–0.032.

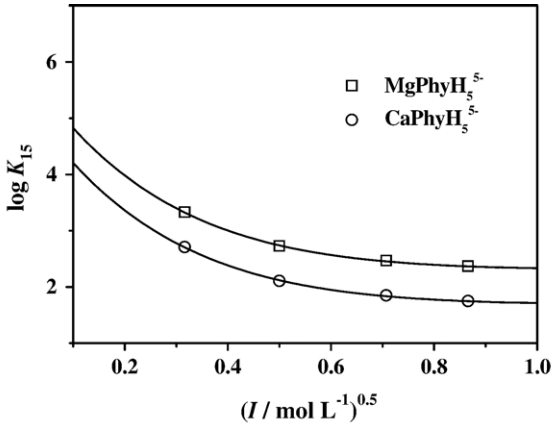

Fig. 1. Dependence of $MPhyH_5^{5-}$ formation constants on ionic strength, at t=25 °C.

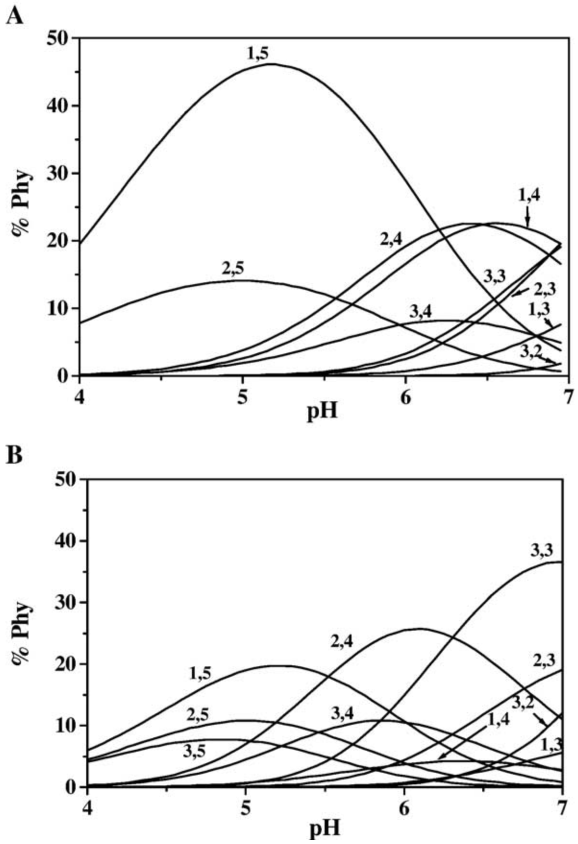

Fig. 2. Speciation diagrams for phytate. Percentages of phytate complexed vs. pH in the Mg^{2+}–phytate (A) and Ca^{2+}–phytate (B) systems at $t=25$ °C and $I=0.10$ mol L^{-1} (NaCl). Indexes (i,j) refer to $M_iPhyH_j^{(12-2i-j)-}$ species. Analytical conditions: $C_{Mg}=3$; $C_{Ca}=3$; $C_{Phy}=1$ mmol L^{-1}.

significantly lower; for example some papers [41–43] have proposed $z=-5$, and this can change the values of formation constants at infinite dilution substantially.

The formation constants of all metal cation–phytate complexes depend strongly on Na^+ concentration, i.e. on the concentration of the supporting electrolyte in our experiments. In fact, Na^+ forms quite stable complexes with phytate, as already reported [3] [for example, $\log\beta$ ($Phy^{12-}+6\ Na^{+}=Na_6Phy^{6-}$)$=25.9$, at $t=25$ °C and $I=0.1$ mol L^{-1}; see also Table 2] and this implies a more marked reduction in the formation percentages of complex species than might be expected on the basis of ionic strength effect alone (see also next section). The formation constants calculated in this study

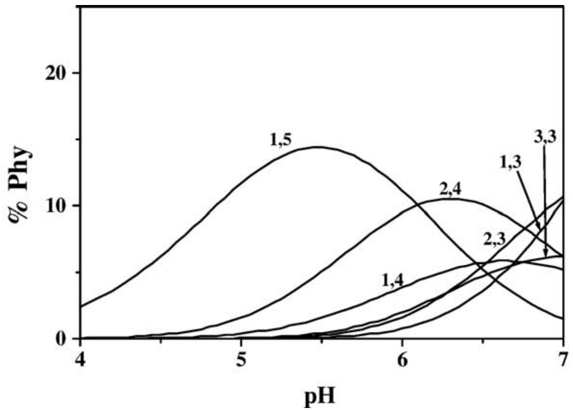

Fig. 3. Speciation diagram for phytate. Percentages of phytate complexed vs. pH in the Ca^{2+}–phytate system at $t=25$ °C and $I=0.10$ mol L^{-1} (NaCl). Indexes (i,j) refer to $Ca_iPhyH_j^{(12-2i-j)-}$ species. Analytical conditions: $C_{Ca}=1$; $C_{Phy}=1$ mmol L^{-1}.

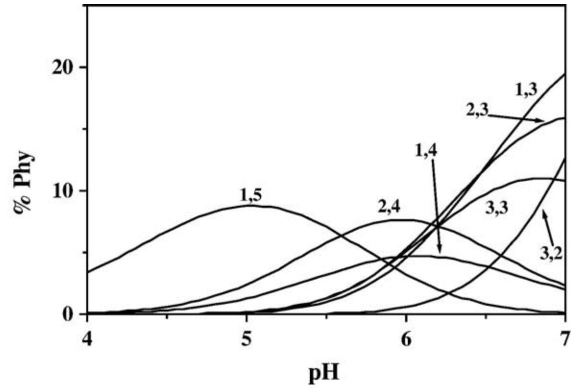

Fig. 4. Speciation diagrams for phytate. Percentages of phytate complexed vs. pH in the Ca^{2+}–phytate system at $t=25$ °C and $I=0.75$ mol L^{-1} (NaCl). Indexes (i,j) refer to $Ca_iPhyH_j^{(12-2i-j)-}$ species. Analytical conditions: $C_{Ca}=3$; $C_{Phy}=1$ mmol L^{-1}.

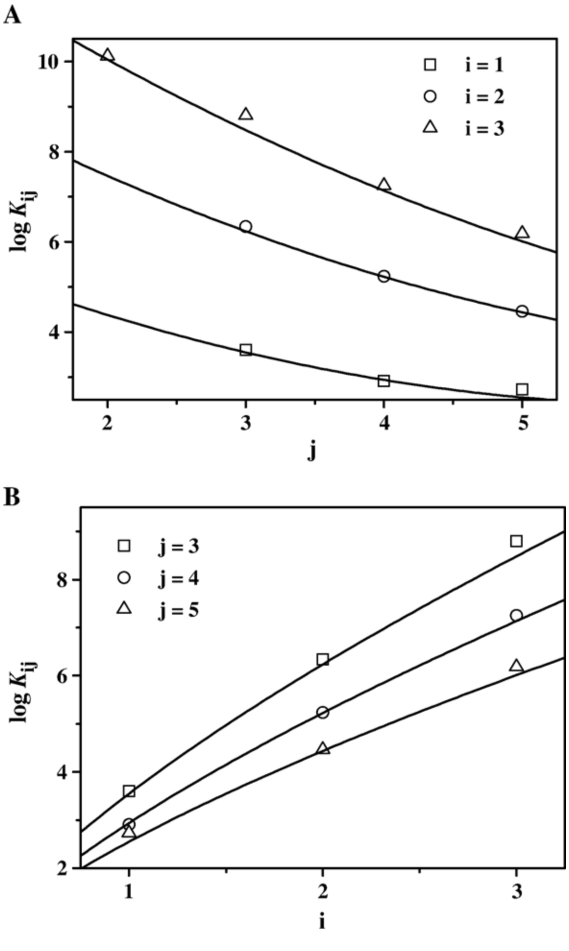

Fig. 5. $\log K_{ij}$ vs. j at $i=$constant (A), and vs. i at $j=$constant (B) in the Mg^{2+}–phytate system at $t=25$ °C and $I=0.25$ mol L^{-1} (NaCl). Indexes (i,j) refer to $Mg_iPhyH_j^{(12-2i-j)-}$ species.

are conditional and do not take the formation of sodium complexes into account. This means that many other complexes may be present in solution, according to the equations:

$$[H_j Phy^{(12-j)-}] = [H_j Phy^{(12-j)-}]_{free} + \sum [Na_k H_j Phy^{(12-j-k)-}] \quad (4)$$

and

$$[M_i H_j Phy^{(12-2i-j)-}] = [M_i H_j Phy^{(12-2i-j)-}]_{free} + \sum [Na_k M_i H_j Phy^{(12-2i-j-k)-}] \quad (5)$$

Eq. (4) indicates a reduction in M^{2+} complex species formation, whilst Eq. (5) implies the stabilization of metal complexes through the formation of mixed metal species. Generally the former effect is more pronounced than the latter. In fact it has been shown that for Na^+ complexes [3] we have: $\log K^{Na} = 0.40.\ \zeta$, where $\log K^{Na}$ refers to the equilibrium $i Na^+ + H_j Phy^{(12-j)-}$, and $\zeta = (|z_{anion}| \sum z_{cation})$; one may consider an analogous empirical relationship also for the formation of mixed Na^+–Ca^{2+} or Na^+–Mg^{2+} species with $\zeta' < \zeta$, and therefore $\log K^{NaM} < \log K^{Na}$.

3.2. Speciation of Phytate in presence of Mg^{2+} and Ca^{2+}

Table 7
Parameters for the dependence of formation constants on proton and cation stoichiometric coefficients in the species $M_i PhyH_j^{(12-2i-j)-}$

M^{2+}, °C	I (mol L^{-1})	P_1	P_2	P_3	P_4
Mg^{2+}, 10	0	13.73±0.03 [a]	−1.04±0.02 [a]	−1.32±0.01 [a]	0.14±0.01 [a]
	0.10	8.1±0.1	−0.80±0.09	−0.76±0.04	0.10±0.02
	0.25	6.9±0.2	−0.77±0.09	−0.62±0.05	0.09±0.02
	0.50	6.4±0.2	−0.8±0.01	−0.53±0.06	0.10±0.03
	0.75	6.3±0.2	−0.9±0.01	−0.48±0.06	0.10±0.03
Mg^{2+}, 25	0	13.8±0.3	−1.2±0.02	−1.36±0.07	0.19±0.03
	0.10	8.0±0.1	−0.81±0.06	−0.80±0.04	0.13±0.02
	0.25	6.7±0.2	−0.69±0.07	−0.69±0.04	0.11±0.02
	0.50	6.2±0.2	−0.61±0.08	−0.67±0.04	0.10±0.02
	0.75	6.1±0.2	−0.57±0.08	−0.69±0.04	0.10±0.02
Ca^{2+}, 10	0	14.7±0.3	−1.3±0.2	−1.52±0.07	0.19±0.04
	0.10	8.9±0.2	−1.0±0.1	−0.95±0.06	0.13±0.03
	0.25	7.7±0.2	−0.9±0.1	−0.80±0.05	0.12±0.03
	0.50	7.2±0.2	−1.0±0.1	−0.70±0.05	0.11±0.03
	0.75	7.1±0.2	−1.0±0.1	−0.65±0.07	0.12±0.03
Ca^{2+}, 25	0	15.1±0.3	−1.77±0.09	−1.48±0.08	0.26±0.03
	0.10	9.2±0.3	−1.4±0.1	−0.90±0.08	0.19±0.03
	0.25	7.9±0.3	−1.3±0.2	−0.80±0.08	0.18±0.04
	0.50	7.4±0.3	−1.3±0.1	−0.78±0.08	0.18±0.03
	0.75	7.3±0.2	−1.2±0.1	−0.80±0.08	0.17±0.03

[a] ±S.D.

Table 8
Formation constants calculated using Eq. (6) in Mg^{2+}–phytate and Ca^{2+}–phytate systems, at $I = 0.25$ mol L^{-1} and $t = 10$ and 25 °C.

Species	$\log K_{ij}$ [a], Mg^{2+} ($t = 10$ °C)	$\log K_{ij}$ [a], Mg^{2+} ($t = 25$ °C)	$\log K_{ij}$ [a], Ca^{2+} ($t = 10$ °C)	$\log K_{ij}$ [a], Ca^{2+} ($t = 25$ °C)
$MPhy^{10-}$	6.86±0.16 [b]	6.72±0.15 [b]	7.71±0.19 [b]	7.92±0.25 [b]
$MPhyH^{9-}$	5.57±0.07	5.46±0.06	6.11±0.07	5.99±0.10
$MPhyH_2^{8-}$	4.46±0.05	4.41±0.03	4.73±0.05	4.43±0.07
M_2Phy^{8-}	10.89±0.25	10.67±0.23	12.25±0.30	12.56±0.40
M_2PhyH^{7-}	9.24±0.13	9.00±0.12	10.17±0.15	10.17±0.20
$M_2PhyH_2^{6-}$	7.77±0.06	7.55±0.05	8.33±0.06	8.14±0.08
M_3Phy^{6-}	14.28±0.33	13.99±0.30	16.05±0.40	16.46±0.53
M_3PhyH^{5-}	12.31±0.18	11.97±0.18	13.58±0.22	13.68±0.30

[a] K_{ij} refers to the reaction: $i M^{2+} + H_j Phy^{(12-j)-} = M_i PhyH_j^{(12-2i-j)-}$.
[b] ±S.D.

Fig. 2 shows two diagrams for phytate speciation in the presence of Mg^{2+} or Ca^{2+}. In these plots the formation percentages of phytate species are reported vs. pH in the range $4 \leq pH \leq 7$, and calculated for a metal/ligand ratio 3/1 ($C_{Phy} = 1$ mmol L^{-1}) in $NaCl_{aq}$ at $I = 0.1$ mol L^{-1} and $t = 25$ °C. In these diagrams the M^{2+}–phytate complexes with formation percentages below 4% and the protonated phytate species are omitted. Phytate and Mg^{2+} form six species with formation percentages >10% and, of these, $MgPhyH_5^{5-}$ is present in the highest concentrations (45% at pH = 5.1). In the Ca^{2+}–phytate system, seven species with percentages >10% are formed and three of these ($CaPhyH_5^{5-}$, $Ca_2PhyH_4^{4-}$ and $Ca_3PhyH_3^{3-}$) are highly representative of the system. The maximum formation percentage for $CaPhyH_5^{5-}$ (20%) is observed at pH = 5.2 and is present in the whole pH range considered. If we compare the two speciation diagrams (obtained under the same analytical conditions), we can see that $MgPhyH_5^{5-}$ is formed in higher percentages than corresponding Ca^{2+} species. Formation percentages for pentaprotonated dinuclear species are similar in both systems. Moreover, formation percentages for $MPhyH_4^{6-}$ and $M_2PhyH_4^{4-}$ species in the Mg^{2+}–phytate system are the same, whereas in the Ca^{2+}–phytate system the percentage of $CaPhyH_4^{6-}$ is significantly lower than that of $Ca_2PhyH_4^{4-}$. Finally, the formation percentages of complex species for Ca^{2+} follow the trend: $Ca_3PhyH_3^{3-} > Ca_2PhyH_4^{4-} > CaPhyH_5^{5-}$, which is the exact opposite of that observed for Mg^{2+} complexes. As expected, when the metal/phytate ratio is reduced to 1 : 1, mononuclear species are favoured (see Fig. 3). Comparison of Figs. 2B and 3 reveals a drastic reduction in the number of tri- and dinuclear species, whilst mononuclear species become

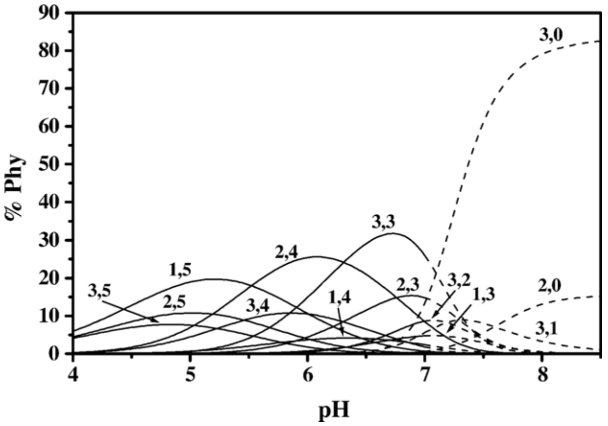

Fig. 6. Speciation diagram for phytate. Percentages of phytate complexed vs. pH in the Ca^{2+}–phytate system at $t = 25$ °C and $I = 0.10$ mol L^{-1} (NaCl). Indexes (i,j) refer to $M_i PhyH_j^{(12-2i-j)-}$ species. Analytical conditions: $C_{Ca} = 3$; $C_{Phy} = 1$ mmol L^{-1}. (—) = experimental data: $6 \leq pH \leq 7$: (- - -) = calculated data: $7 \leq pH \leq 8.5$.

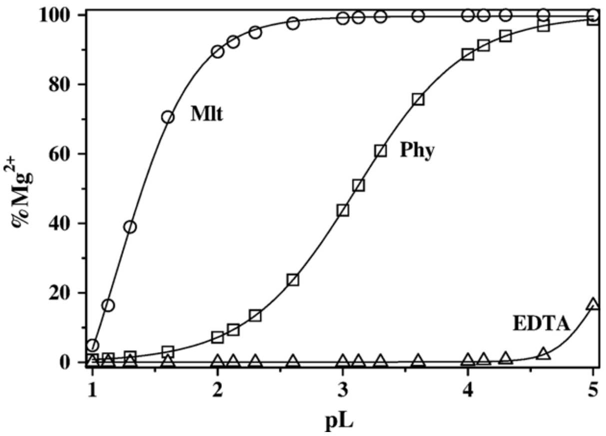

Fig. 7. Percentage of free Mg^{2+} vs. pL (L=Mlt, Phy, EDTA). Experimental conditions: $t=25$ °C, $I=0.25$ mol L^{-1}, $[Mg^{2+}]_{tot}=0.01$ mmol L^{-1}, pH=7.

predominant. The effect of Na^+ on the formation percentages of Ca^{2+}–phytate complexes can be seen by considering a speciation diagram at higher ionic strength, as shown in Fig. 4. A marked difference between percentage curves can be observed in Fig. 2B ($I=C_{NaCl}=0.1$ mol L^{-1}) and Fig. 4 ($I=C_{NaCl}=0.75$ mol L^{-1}), where the yield of all species is reduced by 50%.

3.3. Empirical correlations between the stability and stoichiometry of complexes

The formation constants of different complex species show a regular trend as a function of both proton and metal stoichiometric coefficients. The $\log K_{ij}$ values decrease monotonically as the stoichiometric coefficient j (proton) increases; a reverse trend is observed for $\log K_{ij}$ vs. i (metal ion). This is clearly shown in Fig. 5. After some trials, we found the best empirical equation for these trends to be

$$\log K_{ij} = P_1 i^{2/3} + j(P_2 + P_3 i^{2/3}) + P_4 j^2 \tag{6}$$

where P_1–P_4 are empirical parameters, as reported in Table 7. This equation is quite useful since it allows us to predict the stability of species (in particular unprotonated species) formed outside the pH range ($4 \leq pH \leq 7$) investigated. Table 8 shows some formation constants calculated using Eq. (6). Note that in most cases the standard deviation calculated is less than 0.2 log units, which is a very good result for estimates in a very complex system. We can use these estimates to plot formation percentages at pH>7, as shown in Fig. 6. It is interesting to note that at pH>7.5 the unprotonated species are formed in quite high yields. Results obtained using formation data from empirical equations must obviously be handled with care.

3.4. Sequestering ability of phytate and other chelating agents

The sequestering ability of a ligand L toward a metal cation M^{z+} can be expressed by the function $\%M_{free}$ vs. pL ($p\mathrm{L}=-\log [\mathrm{L}]_{tot}$). Since this function is very similar to a sigmoid curve (or a dose response curve), we can use the Boltzman type equation (with asymptotes of 100 for $p\mathrm{L}\rightarrow+\infty$ and 0 for $p\mathrm{L}\rightarrow-\infty$):

$$\%M_{free} = 100 \times \left[1 - \frac{1}{1+\exp[(p\mathrm{L} - p\mathrm{L}_{50})/S]}\right] \tag{7}$$

where $p\mathrm{L}_{50}$ and S are empirical parameters. In particular, $p\mathrm{L}_{50}$ represents the ligand concentration necessary to sequester 50% of metal ion; this parameter can therefore be used as a measure of the sequestering abilities of different ligands. The parameter S is a measure of the slope in the flex of the function $\%M_{free}$ vs. pL. Moreover, it is evident that $p\mathrm{L}_{50}$ depends on pH values in addition to ionic strength and temperature. Fig. 7 compares the sequestering abilities of phytate, 1,2,3,4,5,6-benzen-hexacarboxylate (mellitate, Mlt) [44] and ethylenediaminotetraacetate (EDTA) [45]. In terms of $p\mathrm{L}_{50}$, this sequestering ability depends strongly on both pH and ionic strength. For the Ca^{2+}–phytate system, one can observe a shift of $p\mathrm{L}_{50}>0.5$ log units from $I=0.1$ to $I=0.75$ mol L^{-1}. Data at pH=9 for phytate are obtained by also using formation constants calculated by Eq. (6), but this implies a greater degree of uncertainty: for $pH \leq 7$ errors in $p\mathrm{L}_{50}$ are ±0.05 whilst at pH=9 we have $p\mathrm{L}_{50} \pm 0.4$.

3.5. Final remarks

The sequestering ability of phytate toward Mg^{2+} and Ca^{2+} was studied in a variety of conditions. Results obtained in this work can be used to study the effective composition of multicomponent systems containing phytate and calcium and/or magnesium in physiological (e.g. blood $I=0.15$ mol L^{-1}, $NaCl_{aq}$) or natural systems (e.g., rivers or lakes $I \ll 0.1$ mol L^{-1}; seawater, $I=0.7$ mol L^{-1}). It must be stressed that reliable

Table 9
Parameters of the sigmoidal Eq. (7) for solution having $[M^{2+}]_{Tot}=0.01$ mmol L^{-1} at $t=25$ °C and different ionic strength and pH values

M^{2+}	L	$p\mathrm{L}_{50}$	S	$p\mathrm{L}_{50}$	S	$p\mathrm{L}_{50}$	S	$p\mathrm{L}_{50}$	S	$p\mathrm{L}_{50}$	S	$p\mathrm{L}_{50}$	S
		$I=0.1$ mol L^{-1}						$I=0.25$ mol L^{-1}					
		pH=7		pH=8		pH=9		pH=7		pH=8		pH=9	
Mg^{2+}	Phy^{12-}	3.643	0.432	4.463	0.500	5.571	0.206	3.108	0.434	3.780	0.432	5.209	0.278
	Mlt^{6-}	2.065	0.276			2.650	0.372	1.439	0.191	1.736	0.253	1.802	0.245
	$EDTA^{4-}$							5.305	0.187	5.349	0.121		
Ca^{2+}	Phy^{12-}	2.729	0.437	4.331	0.464	5.393	0.167	3.145	0.441	4.442	0.520	5.568	0.204
	Mlt^{6-}	2.989	0.397			3.672	0.420	2.188	0.356	2.550	0.382	2.618	0.382

quantitative information is crucial for accurate speciation studies. Data reported in this paper at different pH − t °C − I mol L^{-1} values can be used to model different natural systems (only two temperatures were considered, but, since the differences in formation constants between 10 °C and 25 °C are fairly small, it is possible to extrapolate stability data in the range $5 \leq t$ (°C)≤ 40). The use of Eq. (7) to express the sequestering ability of a ligand towards a cation also gives immediate information comparing different chelating agents, as shown in Table 9. The stability data reported in this paper must be compared with literature data. Graf [28] reported apparent formation constants for calcium–phytate at pH = 4.8, 6.0 and 7.2 obtained by ISE-Ca^{2+} measurements at $t = 20$ °C and at different ionic strengths. To compare Graf's results with ours we calculated calcium complex formation percentages at the above pH values. In the same experimental conditions we report the total formation percentage, $(\%)_T$, obtained by Graf data and ours (in parenthesis) at different pH values: at pH = 4.8, $(\%)_T = 12 \pm 2$ (10 ± 2), 26 ± 3 (24 ± 3) and 39 ± 4 (39 ± 4) for the ratios $C_{Ca}/C_{Phy} = 1$, 2 and 3 respectively, and at pH = 6, $(\%)_T = 88 \pm 9$ (96 ± 9), for $C_{Ca}/C_{Phy} = 2$. These figures indicate quite good agreement between our own and Graf's results, although they were obtained by different techniques. Torres et al. [29] published formation constants based on ISE-H^+ potentiometric data for both Mg^{2+} and Ca^{2+} phytate complexes at $I = 0.15$ mol L^{-1} $NaClO_{4aq}$. The formation constants for both cations seem to be too high (by several orders of magnitude) considering the stabilities of other metal ion complexes. Our last remark concerns the formation of mixed metal species. For the mixed Mg^{2+}–Ca^{2+}–phytate system, since the formation constants of binary systems are fairly similar, one can resort to statistical calculations [46] with reasonable approximation. For other mixed metal systems, experimental measurements are in progress.

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