J. Chem. Eng. Data **2010,** *55,* 4757–4767

Electrochemical Study on the Stability of Phytate Complexes with Cu^{2+}, Pb^{2+}, Zn^{2+}, and Ni^{2+}: A Comparison of Different Techniques[†]

Rosalia Maria Cigala, Francesco Crea, Concetta De Stefano,* Gabriele Lando, Demetrio Milea, and Silvio Sammartano

Dipartimento di Chimica Inorganica, Chimica Analitica e Chimica Fisica, Università di Messina, Viale Ferdinando Stagno d'Alcontres, 31, I-98166 Messina (Vill. S. Agata), Italy

The formation constants of various M/Phy systems (M = Cu^{2+}, Zn^{2+}, Pb^{2+}, Ni^{2+}; Phy = Phytate) were determined in $NaNO_3$ solutions at $0.1 \le I/\text{mol}\cdot\text{L}^{-1} \le 1.0$ and $T = 298.15$ K, by potentiometry and voltammetry. The formation constants of the Cu/Phy and Zn/Phy species, already determined, were reanalyzed together with new voltammetric and potentiometric experiments at low concentrations. A new potentiometric and voltammetric study was carried out on the Ni/Phy and Pb/Phy systems. For all of the investigated systems, the dependence on ionic strength was modeled by the Debye−Hückel and the specific interaction theory (SIT) approaches. The sequestering ability of phytate was evaluated toward the considered cations by calculating the pL_{50} values (i.e., the total ligand concentration necessary to bind 50 % of the cation present in trace) at different ionic strengths and pH. A complete set of "suggested" formation constants was provided. A comparison between the formation constants obtained for the Zn/Phy, Cu/Phy, and Pb/Phy systems reveals that the interactions of phytate with Zn^{2+}, Cu^{2+}, and Pb^{2+} are quite similar, while the Ni/Phy species showed a weaker complexation. For example, for the MH_3Phy species, $\log K_3 = 7.81$, 7.51, 7.10, and 5.97 for Zn^{2+}, Cu^{2+}, Pb^{2+}, and Ni^{2+}, respectively. The same trend is observed concerning the pL_{50}. Their dependence on pH and ionic strength was modeled by two empirical equations.

Introduction

Recently, phytic acid (denoted in this work as Phy) [1,2,3,4,5,6-hexakis(dihydrogen phosphate)myo-inositol] has been studied by many authors in various research fields (see, for example, refs 2 to 6). The increasing interest in the study of this molecule is due to the behavior that phytate plays in aqueous solution (biological fluids, natural and waste waters, soil solutions), where it strongly interacts with many metal and organometal cations, proteins and starch, regulating their bioavilability into living organisms.[3−8] Phytic acid is regarded as the primary storage form of both phosphate and inositol in plant seeds and grains (see, also ref 9 and references therein); it has been reported to be an antioxidant (see also refs 10 and 11). It shows marked anticarcinogenic/antineoplastic properties (e.g., refs 10 and 12), and it may reduce and prevent kidney stone formation.[13] Many of these characteristics are strongly related to the complexing abilities of phytate toward cations essential for biological systems. On the other hand, the characterization of the phytic acid properties represents a challenging task for modern chemists. It is a unique molecule, a dodecanion in its fully deprotonated form; its structure presents a phosphate group linked to each carbon atom of a cyclohexane skeleton[3] (see Figure 1). Phytic acid presents 12 protonation steps over a wide pH range, giving to it the possibility to be a strong complexing agent over all of the pH range. The difficulties in the study of phytic acid are mainly related to its high charge, that may cause variation in the effective value of ionic strength, and to the low solubility of some metal complexes, that restricts the investigable pH range.

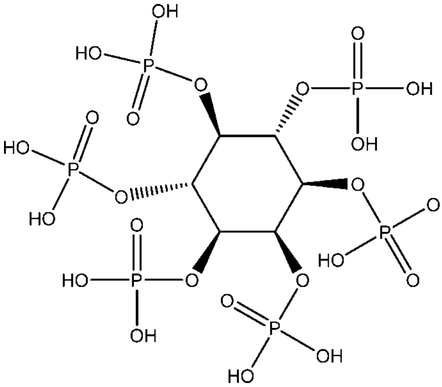

Figure 1. Structural formula of phytic acid.

In the past, many authors[3,14−18] reported studies on the acid−base properties and complexing ability of phytate toward several metal ions. Despite these studies, which were performed in different experimental conditions (ionic medium, temperature, and analytical techniques), the data reported in the literature do not give a clear picture of phytate behavior in aqueous solutions. Moreover, the discordance between the literature data did not allow a definition of the critical values for the protonation and complex formation constants.

For all of these reasons, the aim of the present paper is to define a critical speciation scheme for some M/Phy (M = Cu^{2+}, Pb^{2+}, Zn^{2+}, Ni^{2+}) systems, which is independent from both the adopted experimental conditions and the analytical techniques used to obtain the thermodynamic parameters. To reach this objective, the four M/Phy systems were studied at different ionic strengths and with different analytical techniques, such as ISE-H^+ potentiometry and DP-ASV (differential pulse-anodic stip-

---

[†] Speciation of phytate in aqueous solution. Last contribution to this series: ref 1.

* Corresponding author. E-mail: cdestefano@unime.it. Tel.: +39-090-6765749. Fax: +39-090-392827.

10.1021/je100384f

ping voltammetry), and varying any possible experimental condition (reagent stocks, solution concentrations, operators, etc.). The two cited electrochemical techniques give complementary information concerning solution equilibria. In fact, since potentiometry does not allow performing equilibrium studies at low reagent concentrations (below ~0.5 $\text{mmol}\cdot\text{L}^{-1}$) and since phytic acid forms strong complexes with metal cations, the formation of polynuclear species can be obtained also in relatively dilute solutions (with c_M:c_L molar ratios in favor of the metal); nevertheless, since in this study very low component concentrations were used, we did not observe the formation of polynuclear species (these species were rejected systematically in the refinement procedure). If voltammetry is used as an instrumental technique, much lower reagent concentrations can be used, giving the possibility of an investigation of wider pH ranges, since the formation of sparingly soluble species can be avoided. For Pb^{2+} and Ni^{2+} both potentiometric and voltammetric titrations were carried out at $T = 298.15$ K and $0.1 \leq I/\text{mol}\cdot\text{L}^{-1} \leq 1.0$ in $NaNO_3$, while for Zn^{2+} and Cu^{2+}, only voltammetric measurements were performed at the same temperature and in the same ionic strength range, since potentiometric results were already published.[19,20] The formation constants of the four M/Phy systems, obtained at different ionic strengths, were analyzed to determine the ionic strength dependence parameters according to Debye–Hückel and specific ion interaction theory (SIT) type equations. An analysis of potentiometric, voltammetric, and literature data allowed us to propose "suggested" values for the formation constants of the above cited systems. Speciation and sequestration (by the calculation of pL_{50} values, representing the total ligand concentration necessary to bind 50 % of a cation present in trace) studies were also performed to give information on the complexing ability of this ligand, for possible applications in remediation.

Table 1. Experimental Conditions for the Investigated M/Phy Systems at $T = 298.15$ K in $NaNO_3$

	DPASV						
M	$c_M{}^a$	$c_L{}^b$	$I/\text{mol}\cdot\text{L}^{-1}$	pH range	$E_{dep}{}^c$	$t_{dep}{}^d$	Eq.time^d
Zn^{2+}	1.0–4.0	0.3–2.0	0.15–1.00	3–10	−1.2	40	20
Cu^{2+}	1.0–4.0	0.3–2.0	0.13, 0.50, 1.00	3–10	−0.2	40	20
Pb^{2+}	1.0–4.0	0.1–2.0	0.13–1.00	3–10	−0.8	50	30
Ni^{2+}	1.0–4.0	0.1–2.0	0.13–1.00	3–10	−1.2	45	30

	ISE-H^+			
	$c_M{}^b$	$c_L{}^b$	$I/\text{mol}\cdot\text{L}^{-1}$	pH range
Pb^{2+}	0.8–2.0	1–6	0.12 and 0.50	2.5–8.5
Ni^{2+}	0.8–4.0	1–6	0.13 and 0.50	2–10

[a] In $\mu\text{mol}\cdot\text{L}^{-1}$. [b] In $\text{mmol}\cdot\text{L}^{-1}$. [c] In V (Volt). [d] In s (second). Purge time is 300 s, and step potential is 1.5 mV in all cases for DP-ASV.

Experimental Section

Chemicals. Phytic acid aqueous solutions were prepared by weighing the Aldrich dipotassium salt, $K_2H_{10}Phy$, and passing them over a strong cationic exchange resin (Dowex 50WX8 from Fluka) in H^+ form. The phytic acid concentrations were checked potentiometrically by alkalimetric titrations, while the concentration of potassium in the acid solutions was investigated by flame emission spectrometry, and it resulted always in a value lower than the LOQ (limit of quantification) (< 0.1 $\mu\text{g}\cdot\text{L}^{-1}$). Zn^{2+} and Cu^{2+} were used as nitrate salts (Fluka), while Ni^{2+} was used as the chloride salt (Fluka); Pb^{2+} solutions were prepared from 1 $\text{g}\cdot\text{L}^{-1}$ standard solutions (in nitric acid). Each salt was used without further purification. The metal solutions were standardized against ethylenediaminetetraacetic acid (EDTA) standard solutions,[21] and the purity was always ≥ 99.5 %. Nitric acid and sodium hydroxide solutions were prepared by diluting concentrated ampules (Riedel-deHaën) and were standardized against sodium carbonate and potassium hydrogen phthalate, respectively, previously dried in an oven at $T = 383.15$ K for two hours. The $NaNO_3$ solutions were prepared by weighing the pure salt (Fluka) dried in an oven at $T = 383.15$ K for two hours. All solutions were prepared with analytical grade water ($R = 18\ \text{M}\Omega\cdot\text{cm}^{-1}$) using grade A glassware.

Apparatus and Procedure for Potentiometric Measurements. Potentiometric measurements of Pb/Phy and Ni/Phy systems were carried out (at $T = 298.15 \pm 0.1$ K in thermostatted cells) by two operators using two different setups, to minimize systematic errors and to check the repeatability of the systems. The first setup consisted of a model 713 Metrohm potentiometer, equipped with a half-cell glass electrode (Ross type 8101, from Orion) and a double-junction reference electrode (type 900200, from Orion), and a model 765 Metrohm motorized buret. The apparatus was connected to a PC, and automatic titrations were performed using a suitable homemade computer program to control titrant delivery, data acquisition, and to check for emf stability. The second setup consisted of a Metrohm model 809 Titrando apparatus controlled by Metrohm TiAMO 1.2 software equipped with an Orion (Ross model 8101) glass electrode and an Ag/AgCl reference electrode. Estimated precision was ± 0.15 mV and ± 0.003 mL for the emf and titrant volume readings, respectively, and was the same for both setups. All of the potentiometric titrations were carried out under magnetic stirring and bubbling purified presaturated N_2 through the solution, to exclude O_2 and CO_2. For both the Pb/Phy and the Ni/Phy systems, similar experimental conditions were chosen (summarized in Table 1). The titrand solution consisted of different amounts of phytic acid [(1 to 6) $\text{mmol}\cdot\text{L}^{-1}$], metal cation [(0.8 to 4) $\text{mmol}\cdot\text{L}^{-1}$], and $NaNO_3$ to obtain the pre-established ionic strength values ($0.12 \leq I/\text{mol}\cdot\text{L}^{-1} \leq 0.50$). All of the measurements were performed with an excess of ligand, by titrating 25 mL of the titrand solution with standard NaOH solutions up to pH ~ 10, except that for the Pb/Phy system, where the formation of a scarcely soluble species was observed at lower pH values (pH ~ 8.5). For each experiment, independent titrations of strong acid solutions with standard base were carried out under the same medium and ionic strength conditions as the systems to be investigated, with the aim of determining the electrode potential (E^0) and the acidic junction potential ($E_j = j_a\,[H^+]$). In this way, the pH scale used was the total scale, $\text{pH} \equiv -\log\,[H^+]$, where $[H^+]$ is the free proton concentration. For each titration, (80 to 100) data points were collected, and the equilibrium state during titrations was checked by adopting some usual precautions.[22] These included checking the time required to reach equilibrium and performing back-titrations. For measurements performed at low ionic strengths, the contribution of the ligand to the total ionic strength has to be considered. This topic will be discussed later.

Apparatus and Procedure for Voltammetric Measurements. All M/Phy ($M = Zn^{2+}, Cu^{2+}, Pb^{2+}$, and Ni^{2+}) systems were studied by voltammetric measurements. The DP-ASV experiments were carried out at $T = (298.15 \pm 0.1)$ K in thermostatted cells by a Metrohm 663 VA Stand (Series 05) workstation, equipped with a three-electrode system supplied by Metrohm, consisting of: (i) a multimode mercury electrode (MME, model 6.1246.020) filled with 99.9999 % mercury (electronic grade, from Aldrich) working in SMDE mode (static mercury drop electrode), (ii) a glassy carbon (GC) auxiliary electrode (AE) (model 6.1247.000), and (iii) a double junction

Ag/AgCl/KCl (3.0 mol·L^{-1}) reference electrode (RE) (model 6.0728.000 + 6.1245.000). The workstation was connected to a μAutolab type III potentiostat/galvanostat (Eco Chemie) with an IME663 interface (Eco Chemie). The whole system was controlled by the GPES v. 4.9 software (Eco Chemie). The free hydrogen ion concentration in the DP-ASV experiments was measured before and after each voltammetric run by using the same kind of apparatus and procedure already described in the previous section. The DP-ASV measurements were performed on 25 mL of solution containing known amounts of metal ($10^{-5} < c_M$/mol·L$^{-1} < 10^{-6}$) and $NaNO_3$ to obtain a pre-established ionic strength value ($0.13 \leq I$/mol·L$^{-1} \leq 1.0$). Different amounts of ligand were added to reach a total ligand concentration in the cell ranging between (0.1 and 2.0) mmol·L^{-1}, with five different c_L:c_M molar ratios ($40:1 \leq c_L:c_M \leq 200:1$). For each ratio, at least three voltammetric titrations were carried out by adding standard NaOH to the solution containing M and Phy and recording a voltammogram after each addition. For each titration, 30 to 40 voltammograms (with the corresponding pH values) were recorded in the pH range $3.0 \leq \text{pH} \leq 10.0$. The experimental details are summarized in Table 1. For each metal, the experimental conditions were chosen after preliminary DP-ASV and CV (cyclic voltammetry) measurements, with and without phytate in solution, in which various parameters were systematically varied to select those giving the best performances in terms of signal/noise ratio, single measurement speed, repeatability, and so forth. The electrochemical behavior of phytic acid was also tested, and no electrochemical process was evidenced in the investigated conditions.[23] The reversibility of the redox process was also checked by CV measurements. The processes were reversible in the presence of phytate up to pH = 10.0; only in the case of Zn^{2+} we observed some peak distortions at pH > 10.0. The DP-ASV titrations were carried out automatically; this possibility was guaranteed by the development of a homemade automated system for interfacing the GPES system for voltammetry and the 809 Titrando (for potentiometry). They were connected by a remote box, purchased from Metrohm (model 6.2148.010), and the communication was established by a serial bus port and by setting the parameters on both instruments (in the setting menu of the software). Successively, the methods for the two instruments were created; the whole setup was controlled by the 809 Titrando.

Calculations. The calculation programs are reviewed in ref 24. The nonlinear least-squares computer program ESAB2M was used for the refinement of all of the parameters of the acid−base titrations (E^0, K_w, liquid junction potential coefficient, j_a, analytical concentration of reagents). The BSTAC and STACO computer programs were used in the calculation of complex formation constants. Both programs can deal with measurements at different ionic strengths. The ES4ECI program was used to draw the speciation and sequestration diagrams, and to calculate species formation percentages. The LIANA program was used to fit different equations. The protonation constants of phytate are given according to the equilibria

$$H^+ + H_{(i-1)}Phy^{i-1+z} = H_iPhy^{i+z} \qquad K_i^H \qquad (1)$$

or

$$iH^+ + Phy^z = H_iPhy^{i+z} \qquad \beta_i^H \qquad (2)$$

The complex formation constants are given according to the equilibria:

$$M^{2+} + H_iPhy^{i+z} = MH_iPhy^{i+z+2} \qquad K_i \qquad (3)$$

or

$$M^{2+} + iH^+ + Phy^z = MH_iPhy^{i+z+2} \qquad \beta_i \qquad (4)$$

According to recent work,[25,26] $z = -7$, since the strong interactions between highly charged anions, such as phytate, and the cation of the supporting electrolyte lower the effective charge of the ligand, so that the completely deprotonated phytate form in Na^+ aqueous media is Na_5Phy^{7-}.

It is well-known (e.g., in refs 27 and 28) that, for a ligand L and a metal cation M at fixed c_L:c_M molar ratios and varied pH, the peak potential shift due to the formation of labile complex species at any pH value is given by:

$$\Delta E_p = E_p^{\text{free}} - E_p^{\text{comp}} = \frac{RT}{nF}\ln\frac{c_M}{[M]} + \frac{RT}{nF}\ln\frac{i_p^{\text{comp}}}{i_p^{\text{free}}} \qquad (5)$$

where R is the gas constant, T is the temperature (in K), F is the Faraday constant, n represents the number of electrons involved in the redox process, ΔE_p is the peak shift due to the difference between the peak potential of the free metal (E_p^{free}) and that of the metal in the presence of the ligand (E_p^{comp}), i_p^{comp} and i_p^{free} are the peak heights (current intensities) with and without ligand in solution, respectively, and c_M and [M] are the analytical and the free metal ion concentration, respectively, at any pH value. The left-hand side of the equation and the term relative to the peak intensities are directly obtained from experimental data, while the term containing the free metal concentration is given by solving the correct mass balance equations. Working with high c_L:c_M molar ratios and at relatively low metal concentrations (< 10^{-4} mol·L^{-1}), the assumption that no polynuclear species are formed is reasonable. By taking into account this assumption, the free cation concentration [M] at any pH value is given by:

$$[M] = \frac{c_M}{1 + \sum \beta_{MH_iL_j}[H]^i[L]^j} \qquad (6)$$

with

$$[L] = \frac{c_L}{1 + \sum \beta_i^H[H]^i} \qquad (7)$$

where β_i^H (see eq 2) represents the overall protonation constants of the ligand. Equation 7 is only valid in this form when $c_L \gg c_M$, and complex species can be neglected in the mass balance of the ligand, as in our case. Both the right and the left-hand sides of eq 5 are pH-dependent and are valid for a fixed c_L:c_M molar ratio. The LIANA computer program was used to fit the experimental data and to calculate the formation constants of various MH_iPhy^{i+z+2} species.

The conversion from the molar to molal scale for the different supporting electrolytes was obtained using the appropriate density values.[29]

The dependence on the ionic strength of the formation constants can be obtained by the simple Debye−Hückel type equation

$$\log K_i = \log {}^{\mathrm{T}}K_i - 0.51 \cdot z^* \frac{\sqrt{I}}{1 + 1.5\sqrt{I}} + f(I) \tag{8}$$

$$p^* = \sum (\text{moles})_{\text{reactants}} - \sum (\text{moles})_{\text{products}} \tag{9}$$

$$z^* = \sum (\text{charges})^2_{\text{reactants}} - \sum (\text{charges})^2_{\text{products}} \tag{10}$$

(K_i = formation constant; ${}^{\mathrm{T}}K_i$ = formation constant at infinite dilution.) Equation 8 is also valid for the overall equilibrium constants, β_i. $f(I)$ is a linear function of ionic strength that can be formulated in different ways. The simplest expression for this term is $f(I) = CI$, where C is the only adjustable parameter. Usually, this simple choice is sufficient to explain the experimental data trend in a wide ionic strength range, generally $< 1.0\ \text{mol} \cdot \text{L}^{-1}$. For higher ionic strength values, or supporting electrolytes such as $(CH_3)_4NCl$, C may be dependent on ionic strength, and expressed as:[30]

$$C = c_\infty + \frac{c_0 - c_\infty}{I + 1} \tag{11}$$

in which c_∞ is the value of C at $I \rightarrow \infty$ and c_0 the relative value at $I \rightarrow 0$.

From a general point of view, the protonation steps or the complex formation reactions can be expressed as a function of the activity coefficients as follows (charges omitted for simplicity):

$$\mathrm{H} + \mathrm{H}_{(i-1)}\mathrm{L} = \mathrm{H}_i\mathrm{L} \tag{12}$$

$$\log K_i^{\mathrm{H}} = \log {}^{\mathrm{T}}K_{\mathrm{H}} + \log \gamma_{\mathrm{H}} + \log \gamma_{\mathrm{H}_{(i-1)}\mathrm{L}} - \log \gamma_{\mathrm{H}_i\mathrm{L}} \tag{12a}$$

$$\mathrm{M} + \mathrm{H}_i\mathrm{L} = \mathrm{MH}_i\mathrm{L} \tag{13}$$

$$\log K_{\mathrm{MH}_i\mathrm{L}} = \log {}^{\mathrm{T}}K_{\mathrm{MH}_i\mathrm{L}} + \log \gamma_{\mathrm{M}} + \log \gamma_{\mathrm{H}_i\mathrm{L}} - \log \gamma_{\mathrm{MH}_i\mathrm{L}} \tag{13a}$$

If the molal concentration scale is used, eq 8 becomes the SIT[31] equation, where $f(I) = \Delta\varepsilon_x I$; ε_x is the specific interaction coefficient between the species involved in the equilibria and the ions of the supporting electrolyte. If $NaNO_3$ is used as supporting electrolyte, for the equilibrium in eq 1, it is:

$$\Delta\varepsilon = \varepsilon(\mathrm{H}^+, \mathrm{NO}_3^-) + \varepsilon(\mathrm{Na}^+, \mathrm{H}_{(i-1)}\mathrm{L}^{(i-1+z)}) - \varepsilon(\mathrm{Na}^+, \mathrm{H}_i\mathrm{L}^{(i+z)}) \tag{14}$$

For the equilibrium in eq 3 (metal−ligand complexes), it is:

$$\Delta\varepsilon = \varepsilon(\mathrm{M}^{2+}, \mathrm{NO}_3^-) + \varepsilon(\mathrm{Na}^+, \mathrm{H}_i\mathrm{L}^{(i+z)}) - \varepsilon(\mathrm{Na}^+/\mathrm{NO}_3^-, \mathrm{MH}_i\mathrm{L}^{(i+z+2)}) \tag{15}$$

Table 2. Phytate Protonation Constants at Infinite Dilution and Specific Interaction Coefficients in $NaNO_3$ at T = 298.15 K

	$H_iPhy^{(i-7)}$	
i	$\log {}^{\mathrm{T}}\beta_i^{\mathrm{H}}$ [a]	$\varepsilon(H_iPhy,Na)$ [b]
1	11.20 ± 0.06 [c]	-2.20 ± 0.09 [c]
2	22.47 ± 0.06	-2.55 ± 0.09
3	33.08 ± 0.02	-2.42 ± 0.10
4	42.12 ± 0.04	-2.27 ± 0.11
5	49.21 ± 0.05	-1.96 ± 0.13
6	54.76 ± 0.05	-1.51 ± 0.15
7	57.92 ± 0.05	-1.15 [d] ± 0.17

[a] Refers to eq 2. [b] Parameters of eq 14. [c] ± 95 % C.I. [d] k_m value of the H_7Phy^0 neutral species (see eq 16).

When, from the interaction between the metal ion and the ligand, the formation of a neutral species is observed, it is:

$$\Delta\varepsilon = \varepsilon(\mathrm{M}^{2+}, \mathrm{NO}_3^-) + \varepsilon(\mathrm{Na}^+, \mathrm{H}_{(-2-z)}\mathrm{L}^{2-}) - k_{\mathrm{m}} \tag{16}$$

where k_m is the Setschenow coefficient of the neutral species.[32] As also described for the empirical parameter C in eq 11, ε may be expressed as a function of ionic strength[33] (see eqs 17 and 18).

$$\varepsilon = \varepsilon_\infty + \frac{\varepsilon_0 - \varepsilon_\infty}{I + 1} \tag{17}$$

or

$$\varepsilon = \varepsilon^{(0)} + \varepsilon^{(1)} \ln(I + 1) \tag{18}$$

Generally, for $I < 3.0\ \text{mol} \cdot \text{L}^{-1}$ ($I \leq 1\ \text{mol} \cdot \text{L}^{-1}$ in our case), the single parameter ε is sufficient to fit the experimental data.

Results and Discussion

Protonation of Phytate and Hydrolysis of Metal Ions. Phytate protonation constants were previously determined in $NaNO_3$ at different ionic strengths;[20] the results were reanalyzed by the SIT model, and a paper on phytate protonation is in press.[25] Table 2 reports phytate protonation constant values at infinite dilution and the relative parameters for the ionic strength dependence in $NaNO_3$. With regard to the metal ions, since phytate inhibits strongly their hydrolysis,[19,20,26,34] we used as metal hydrolysis constants the recommended values reported by Baes and Mesmer[35] in perchlorate aqueous solutions: in fact both NO_3^- and ClO_4^- can be considered "weakly interacting" anions toward the metal cations. This approximation does not involve a loss of accuracy in our calculations.

Formation of Copper(II)−Proton−Phytate Species. From the analysis of the voltammetric experimental data by eq 5, in the pH range $3.0 \leq \text{pH} \leq 10.0$, the formation of five mononuclear $CuH_iPhy^{(5-i)-}$ species (with i = 2 to 6) was evidenced, namely, CuH_2Phy^{3-}, CuH_3Phy^{2-}, CuH_4Phy^-, CuH_5Phy^0, and CuH_6Phy^+. The corresponding formation constants at different ionic strengths are reported in Table 3. The formation constant values show a lowering trend with increasing ionic strength. The results were compared and analyzed together with those obtained by Crea et al.[20] (see values in parentheses in Table 3), where the formation constants were derived by both ISE-H^+ and ISE-Cu^{2+} measurements. Two different speciation models were obtained: from ISE-H^+ measurements, the

Table 3. Formation Constants of Cu/Phy Species at $T = 298.15$ K in $NaNO_3$ at Different Ionic Strengths

I/mol·L^{-1}	log K_2[a]	log K_3[a]	log K_4[a]	log K_5[a]	log K_6[a]	technique	ref
0.103				(4.75)[c]		ISE-Cu^{2+}	20
0.130	9.41 ± 0.05[b]	7.53 ± 0.05	5.88 ± 0.05	4.47 ± 0.03	3.28 ± 0.07	DP-ASV	this work
0.134				(5.04)[c]		ISE-H^+	20
0.247				(3.95)[c]		ISE-Cu^{2+}	20
0.291			(5.83)[c]	(3.95)[c]		ISE-H^+	20
0.497				(3.47)[c]		ISE-Cu^{2+}	20
0.500	8.28 ± 0.04	6.69 ± 0.04	5.24 ± 0.04	3.91 ± 0.02	2.68 ± 0.07	DP-ASV	this work
0.538			(5.47)[c]	(3.61)[c]		ISE-H^+	20
0.748				(3.30)[c]		ISE-Cu^{2+}	20
0.770			(5.35)[c]	(3.38)[c]		ISE-H^+	20
0.938				(3.47)[c]		ISE-Cu^{2+}	20
1.000	7.63 ± 0.05	6.12 ± 0.04	4.80 ± 0.04	3.63 ± 0.03	2.60 ± 0.07	DP-ASV	this work
1.019			(5.3)[c]	(3.52)[c]		ISE-H^+	20

[a] Refers to eq 3. [b] ± 95 % C.I. [c] Literature values.

Table 4. Formation Constants of Pb/Phy Species at $T = 298.15$ K in $NaNO_3$ at Different Ionic Strengths

I/mol·L^{-1}	log K_1[a]	log K_2[a]	log K_3[a]	log K_4[a]	log K_5[a]	log K_6[a]	technique
0.120	10.54 ± 0.10[b]	8.95 ± 0.10	7.42 ± 0.09	5.95 ± 0.07	4.52 ± 0.06	3.16 ± 0.06	ISE-H^+
0.135	10.40 ± 0.06	8.17 ± 0.05	6.89 ± 0.05	5.70 ± 0.03	4.73 ± 0.03	3.35 ± 0.07	DP-ASV
0.500	9.04 ± 0.04	7.48 ± 0.04	6.10 ± 0.04	4.88 ± 0.04	3.81 ± 0.02	2.90 ± 0.07	ISE-H^+
1.000	8.21 ± 0.07	7.06 ± 0.05	5.68 ± 0.04	4.08 ± 0.04	2.80 ± 0.03		DP-ASV

[a] Refers to eq 3. [b] ± 95 % C.I.

Table 5. Formation Constants of Zn/Phy Species at $T = 298.15$ K in $NaNO_3$ at Different Ionic Strengths

I/mol·L^{-1}	log K_1[a]	log K_2[a]	log K_3[a]	log K_4[a]	log K_5[a]	log K_6[a]	technique	ref
0.15	10.71 ± 0.08[b]	8.76 ± 0.11	6.99 ± 0.13	5.42 ± 0.12	4.03 ± 0.10	3.30 ± 0.11	DP-ASV	*c*
0.15	(10.80)[d]	(9.73)[d]	(8.3)[d]	(6.75)[d]	(5.24)[d]	(4.62)[d]	ISE-H^+	19
0.50	(9.40)[d]	(8.09)[d]	(6.85)[d]	(5.47)[d]	(4.09)[d]	(3.67)[d]	ISE-H^+	19
0.75	(8.80)[d]	(7.67)[d]	(6.48)[d]	(5.14)[d]	(3.80)[d]	(3.42)[d]	ISE-H^+	19
1.00	8.39 ± 0.09	6.92 ± 0.06	5.56 ± 0.07	4.30 ± 0.07	3.13 ± 0.08	2.03 ± 0.12	DP-ASV	*c*
1.00	(8.40)[d]	(7.60)[d]	(6.75)[d]	(3.86)[d]	(3.49)[d]	(3.51)[d]	ISE-H^+	19

[a] Refers to eq 3. [b] ± 95 % C.I. [c] This work. [d] Literature values.

CuH_4Phy, CuH_5Phy, and Cu_2H_5Phy species were determined, while from ISE-Cu^{2+} titration only the last two species. The differences in the log K_i values and speciation models are due both to the different component concentrations used in the measurements performed by the different instrumental techniques and to the pH range investigated. The formation of sparingly soluble species, at low pH values (4.20 for ISE-H^+, 4.30 for ISE-Cu^{2+}), did not allow the determination of the formation constants of other species. The measurements performed by DP-ASV allowed the investigation of a pH range between $3.0 \leq \text{pH} \leq 10.0$, because: (i) the concentration of copper was lower (~ 3 orders of magnitude) than in the potentiometric measurements; (ii) the high c_L:c_M molar ratios adopted hampered the formation of sparingly soluble species up to pH = 10.0. On the other hand, voltammetric measurements did not allow the determination of the polynuclear species, such as the Cu_2H_5Phy, present in Crea et al.,[20] because c_L:c_M molar ratios necessary to apply eq 5 are too high for the determination of this kind of species.

Formation of Lead(II)−Proton−Phytate Species. In the case of lead, the data from ISE-H^+ measurements were analyzed by the BSTAC computer program, and it was possible to determine, in pH range $2.5 \leq \text{pH} \leq 8.5$, the formation constants of six $PbH_iPhy^{(5-i)-}$ (with $i = 1$ to 6) species, namely: $PbHPhy^{4-}$ PbH_2Phy^{3-}, PbH_3Phy^{2-}, PbH_4Phy^{-}, PbH_5Phy^0, and PbH_6Phy^+. The analysis of DP-ASV data by eq 5 in pH range $3 \leq \text{pH} \leq 10$ allowed the determination of the values of the same species except for the PbH_6Phy^+ species at $I = 1.0$ mol·L^{-1}. All of the results are summarized in Table 4. In this case, the absence of literature data on the Pb/Phy system does not allow us to make a direct comparison with other studies. In any case the log K_i values were studied at different ionic strengths, and the results will be discussed in the next sections.

Formation of Zinc(II)−Proton−Phytate Species. The analysis of the voltammetric data in the pH range $3.0 \leq \text{pH} \leq 10.0$ using the LIANA computer program evidenced the formation of six mononuclear $ZnH_iPhy^{(5-i)-}$ (with $i = 1$ to 6) species, namely: $ZnHPhy^{4-}$ ZnH_2Phy^{3-}, ZnH_3Phy^{2-}, ZnH_4Phy^{-}, ZnH_5Phy^0, and ZnH_6Phy^+. The corresponding formation constants at $I = (0.13$ and $1.0)$ mol·L^{-1} are reported in Table 5. The results were compared and analyzed with data reported in Crea at al.,[19] where a study on the Zn/Phy system was carried out by ISE-H^+ potentiometry. In that case, five ZnH_iPhy species (with $i = 2$ to 6) and four binuclear Zn_2H_iPhy species (with $i = 1$ to 4) were determined. The experimental data at different ionic strengths presented in that paper were reanalyzed together with the new ones obtained by DP-ASV, and in this way it was possible to determine also the ZnHPhy species. Higher differences are present for log K_i values of the Zn/Phy species obtained by the two techniques. They are partially ascribable to the different experimental conditions.

Formation of Nickel(II)−Proton−Phytate Species. Concerning the Ni/Phy system, both ISE-H^+ potentiometry and voltammetry were used as analytical techniques to derive the complex formation constants. The measurements were carried out in the experimental conditions reported in Table 1. Independent of the instrumental technique used, the measurements were carried out in the pH range $2 \leq \text{pH} \leq 10$. The investigation of this pH range allowed us to determine the formation constants of the following six $NiH_iPhy^{(5-i)-}$ species (with $i = 1$ to 6) by both techniques, namely, $NiHPhy^{4-}$, NiH_2Phy^{3-}, NiH_3Phy^{2-}, NiH_4Phy^{-}, NiH_5Phy^0, and NiH_6Phy^+. Table 6 reports the

Table 6. Formation Constants of Ni/Phy Species at $T = 298.15$ K in $NaNO_3$ at Different Ionic Strengths

I/mol·L^{-1}	log K_1[a]	log K_2[a]	log K_3[a]	log K_4[a]	log K_5[a]	log K_6[a]	technique
0.13	8.83 ± 0.06[b]	7.34 ± 0.05	6.11 ± 0.04	4.48 ± 0.04	3.90 ± 0.03	2.28 ± 0.06	ISE-H$^+$
0.13	8.91 ± 0.04	7.34 ± 0.05	6.03 ± 0.02	4.72 ± 0.02	3.41 ± 0.03	2.10 ± 0.04	DP-ASV
0.50	7.55 ± 0.03	6.19 ± 0.02	5.01 ± 0.02	3.93 ± 0.02	2.95 ± 0.02	2.07 ± 0.03	ISE-H$^+$
1.00	6.80 ± 0.04	5.38 ± 0.04	4.35 ± 0.04	3.31 ± 0.04	2.28 ± 0.03	1.75 ± 0.03	DP-ASV

[a] Refers to eq 3. [b] ± 95 % C.I.

Table 7. Some Specific Interaction Coefficients Used in Calculations

M	ε(M, NO_3^-)[a]	ref
H^+ [b]	0.0698/0.0583[b]	38
Cu^{2+}	0.11[a]	39
Pb^{2+}	−0.20	39
Zn^{2+}	0.16	39
Ni^{2+}	0.182	40

[a] Parameter of eq 15. [b] ε_∞ and ε_0 of eq 17.

Table 8. Formation Constants of M/Phy Species at Infinite Dilution and Parameters for Their Dependence on Ionic Strength, at $T = 298.15$ K

i	log ^TK_i[a]	$\varepsilon(MH_iPhy^{(i-5)}, Na^+)$[b]	C[c]
		Cu^{2+}	
2	11.812 ± 0.02[d]	−2.374 ± 0.04	−0.087 ± 0.04
3	9.530 ± 0.02	−2.186 ± 0.03	−0.135 ± 0.03
4	7.479 ± 0.04	−2.171 ± 0.07	0.017 ± 0.07
5	5.391 ± 0.04	−1.588 ± 0.07[e]	−0.268 ± 0.07
6	3.565 ± 0.03	−1.218 ± 0.04[f]	−0.177 ± 0.04
		Pb^{2+}	
1	13.315 ± 0.03	−2.245 ± 0.05	−0.177 ± 0.05
2	11.161 ± 0.03	−2.481 ± 0.06	−0.290 ± 0.06
3	9.151 ± 0.03	−2.282 ± 0.07	−0.348 ± 0.07
4	7.268 ± 0.04	−1.883 ± 0.08	−0.611 ± 0.08
5	5.479 ± 0.05	−1.570 ± 0.07[e]	−0.611 ± 0.07
6	3.789 ± 0.04	−1.301 ± 0.06[f]	−0.421 ± 0.06
		Zn^{2+}	
1	13.898 ± 0.04	−1.477 ± 0.07	−0.594 ± 0.07
2	11.864 ± 0.05	−1.849 ± 0.09	−0.574 ± 0.09
3	9.893 ± 0.05	−1.718 ± 0.11	−0.559 ± 0.11
4	7.808 ± 0.07	−1.262 ± 0.11	−0.884 ± 0.11
5	5.961 ± 0.08	−0.996 ± 0.14[e]	−0.832 ± 0.14
6	4.314 ± 0.06	−0.986 ± 0.10[f]	−0.376 ± 0.10
		Ni^{2+}	
1	11.741 ± 0.03	−1.964 ± 0.06	−0.077 ± 0.06
2	9.852 ± 0.02	−2.020 ± 0.04	−0.375 ± 0.04
3	8.033 ± 0.01	−1.842 ± 0.03	−0.421 ± 0.03
4	6.258 ± 0.02	−1.646 ± 0.05	−0.465 ± 0.05
5	4.511 ± 0.02	−1.370 ± 0.04[e]	−0.428 ± 0.04
6	2.791 ± 0.02	−1.111 ± 0.03[f]	−0.220 ± 0.03

[a] Values at infinite dilution; equilibrium refers to eq 1. [b] Specific interaction coefficients of M/Phy species. [c] Parameters for the dependence of complex formation constants on ionic strength (eq 8). [d] ± 95 % C.I. [e] Setschenow coefficient of neutral species of eq 16. [f] As $\varepsilon(MH_6Phy^+, NO_3^-)$, MH_6Phy^+ is positively charged.

corresponding formation constants at different ionic strengths. By looking at the formation constants of the Ni/Phy system, one can observe that NiH_iPhy species are less stable than those of the PbH_iPhy and CuH_iPhy systems, which are in turn less stable than the ZnH_iPhy species. The observed trend is $Zn^{2+} > Cu^{2+} \approx Pb^{2+} > Ni^{2+}$.

Ionic Strength Dependence. The dependence of the protonation and complex formation constants of phytate can be studied by using both the Debye–Hückel and SIT type equations. In each case, two important aspects must be taken into account in this study. The first one is the strong influence of the ionic strength variation on the equilibria; the second aspect is related to knowledge of the effective charge of the phytate ion in solution. The first one will be discussed in the next sections. Concerning the last aspect, it was already discussed in previous papers:[25,26,36] as is well-known, carboxylates and other anions generally form weak ion pairs with alkali metal cations, and this is confirmed also for phytic acid, whose interactions with Na^+ are reported in ref 37 and where many $Na_iH_{(7-i)}Phy$ complexes were found (with $i = 1$ to 7). This reduces the effective charge of the phytate anion, as a function of the concentration of the interacting cation (Na^+ in this case). In the experimental conditions similar to that of the present paper ($0.1 \leq c_{Na}/mol \cdot L^{-1} \leq 1.0$), it was calculated, by the ionic strength dependence of the solubility product,[25] that the average charge of phytate is $z = -7$. This value was already suggested also by De Stefano et al.[26] The specific interaction coefficient of the completely deprotonated phytate species, Phy^{7-}, is[25]

$$\varepsilon(Phy^{7-}, Na^+) = -2.71 + 1.04 \ln(1 + I)$$

This value was used to fit the phytate protonation constants in $NaNO_3$,[20] to obtain the SIT coefficients relative to the H_iPhy species. These values are reported in Table 2. In calculating the specific interaction coefficients of the different metal–phytate species, we used for $\varepsilon(H^+, NO_3^-)$ the value reported from Bretti et al.[38] (see Table 7). Concerning the SIT coefficients of the metal cations $\varepsilon(M^{2+}, NO_3^-)$ reported in Table 7, they were taken from Grenthe and Puigdomenech,[39] except that for nickel(II). For the $\varepsilon(Ni^{2+}, NO_3^-)$, we used the specific interaction coefficient reported in Gamsjager et al.[40] In this case, it was possible to refine simultaneously the single specific interaction coefficients, considering MH_5Phy^0 as the neutral species and considering the MH_6Phy^+ species as positively charged. To obtain more reliable data for the ionic strength dependence parameters, we used in the calculations the experimental data obtained in this work by different electrochemical techniques together with the literature data reported by Crea et al.[19,20] for log K_i values of Cu/Phy and Zn/Phy systems, respectively.

Values of C and ε (empirical parameters of eqs 11 and 17, respectively) for these species are reported in Table 8 together with log ^TK_i values for Cu^{2+}, Pb^{2+}, Zn^{2+}, and Ni^{2+} complexes, respectively.

Speciation of M/Phy Systems. For the different M/Phy systems ($M = Cu^{2+}, Pb^{2+}, Zn^{2+}, Ni^{2+}$), the distribution diagrams of the MH_iPhy species are reported in Figures 2 to 5 as a function of pH. As can be seen, the different diagrams appear to be similar for the different M/Phy systems, owing to the similarities of both the speciation models and the stability of the metal–ligand complex species. However, some significant differences can be observed for the pH values where 100 % of the metal ions are complexed. As an example, in the case of Cu^{2+}, Pb^{2+}, and Zn^{2+}, they are completely complexed at pH > 5, while for Ni^{2+}, this happens at pH > 6. Other differences are: for Cu^{2+} (Figure 2), the CuH_2Phy^{3-} species reaches formation percentages of (55 to 60) %, while for the other metals the same species has a formation percentage of ∼ 40 %. For Ni^{2+} (Figure 5), the NiH_5Phy^0 and NiH_6Phy^+ species achieve a formation percentages of 45 % and 20 %, respectively, in

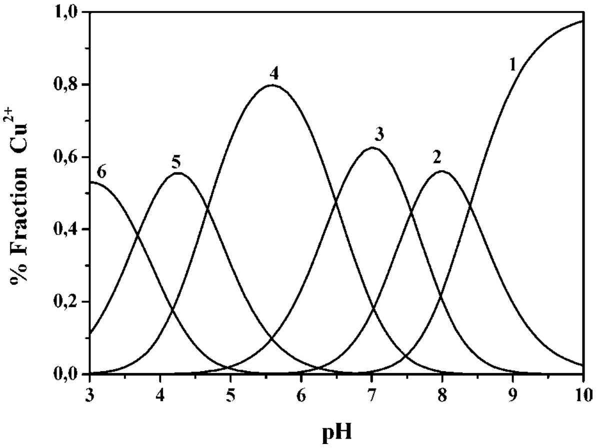

Figure 2. Distribution diagram of Cu/Phy species vs pH. Exp. conditions: $c_{\mathrm{Phy}} = 3\ \mathrm{mmol \cdot L^{-1}}$; $c_{\mathrm{Cu}} = 1\ \mathrm{mmol \cdot L^{-1}}$; $I = 0.15\ \mathrm{mol \cdot L^{-1}}\ \mathrm{NaNO_3}$; $T = 298.15$ K. Species: 1, $\mathrm{CuHPhy^{4-}}$; 2, $\mathrm{CuH_2Phy^{3-}}$; 3, $\mathrm{CuH_3Phy^{2-}}$; 4, $\mathrm{CuH_4Phy^{-}}$; 5, $\mathrm{CuH_5Phy^{0}}$; and 6, $\mathrm{CuH_6Phy^{+}}$.

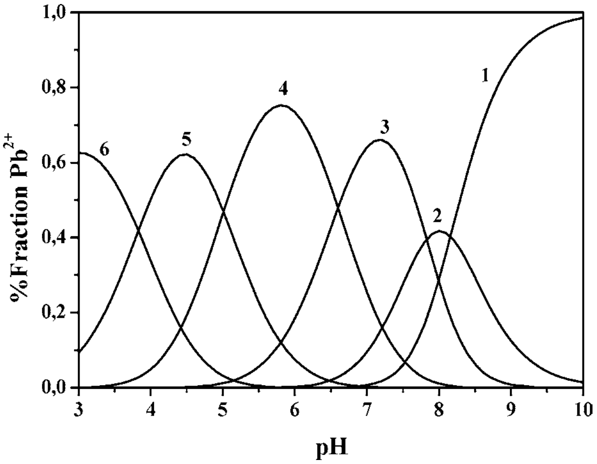

Figure 3. Distribution diagram of Pb/Phy species vs pH. Exp. conditions: $c_{\mathrm{Phy}} = 3\ \mathrm{mmol \cdot L^{-1}}$; $c_{\mathrm{Pb}} = 1\ \mathrm{mmol \cdot L^{-1}}$; $I = 0.15\ \mathrm{mol \cdot L^{-1}}\ \mathrm{NaNO_3}$; $T = 298.15$ K. Species: 1, $\mathrm{PbHPhy^{4-}}$; 2, $\mathrm{PbH_2Phy^{3-}}$; 3, $\mathrm{PbH_3Phy^{2-}}$; 4, $\mathrm{PbH_4Phy^{-}}$; 5, $\mathrm{PbH_5Phy^{0}}$; and 6, $\mathrm{PbH_6Phy^{+}}$.

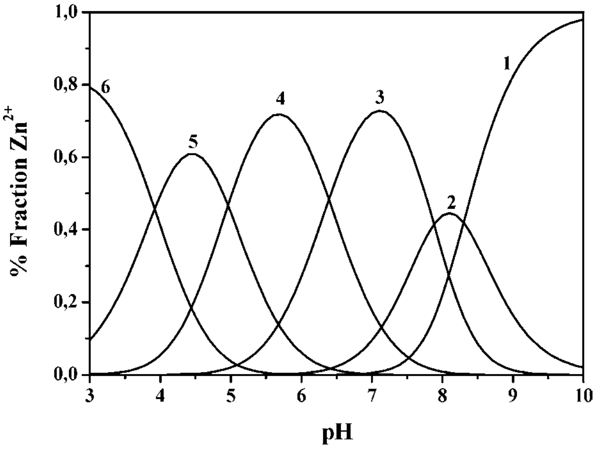

Figure 4. Distribution diagram of Zn/Phy species vs pH. Exp. conditions: $c_{\mathrm{Phy}} = 3\ \mathrm{mmol \cdot L^{-1;}}$ $c_{\mathrm{Zn}} = 1\ \mathrm{mmol \cdot L^{-1}}$; $I = 0.15\ \mathrm{mol \cdot L^{-1}}\ \mathrm{NaNO_3}$; $T = 298.15$ K. Species: 1, $\mathrm{ZnHPhy^{4-}}$; 2, $\mathrm{ZnH_2Phy^{3-}}$; 3, $\mathrm{ZnH_3Phy^{2-}}$; 4, $\mathrm{ZnH_4Phy^{-}}$; 5, $\mathrm{ZnH_5Phy^{0}}$; and 6, $\mathrm{ZnH_6Phy^{+}}$.

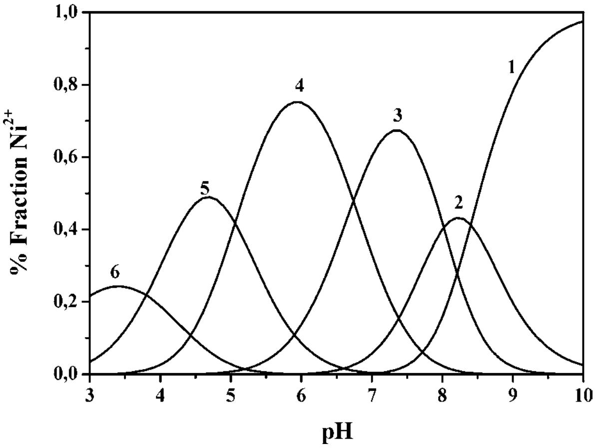

Figure 5. Distribution diagram of Ni/Phy species vs pH. Exp. conditions: $c_{\mathrm{Phy}} = 3\ \mathrm{mmol \cdot L^{-1}}$; $c_{\mathrm{Ni}} = 1\ \mathrm{mmol \cdot L^{-1}}$; $I = 0.15\ \mathrm{mol \cdot L^{-1}}\ \mathrm{NaNO_3}$; $T = 298.15$ K. Species: 1, $\mathrm{NiHPhy^{4-}}$; 2, $\mathrm{NiH_2Phy^{3-}}$; 3, $\mathrm{NiH_3Phy^{2-}}$; 4, $\mathrm{NiH_4Phy^{-}}$; 5, $\mathrm{NiH_5Phy^{0}}$; and $\mathrm{NiH_6Phy^{+}}$.

contrast with the 60 % for both species in the case of $\mathrm{Cu^{2+}}$ and $\mathrm{Pb^{2+}}$ (Figure 3) and (60 to 80) %, respectively, in the case of $\mathrm{Zn^{2+}}$ (Figure 4). In all cases the $\mathrm{MHPhy^{4-}}$ species reaches the formation percentages of 100 % at pH > 9.5. Concerning this last aspect, the formation constant of a completely deprotonated species $\mathrm{MPhy^{5-}}$ could have been determined if a more basic pH range would have been investigated. This would have reduced the formation percentage of the $\mathrm{MHPhy^{4-}}$ species. Unfortunately, higher pH values could not be reached for the loss of reversibility of the redox processes. With regards to the other species not cited in this discussion, they reach a formation percentage of (60 to 65) % at their maximum, and their formation occurs in the same pH range of the analogous species of the different metals, demonstrating that this range mostly depends on the phytic acid protonation constants. In the investigated pH range, metal hydrolysis was inhibited in all cases. As an example in the case of $\mathrm{Pb^{2+}}$ ($c_{\mathrm{Pb^{2+}}} = 10^{-3}\ \mathrm{mol \cdot L^{-1}}$), we calculated that in the absence of phytate, (25, 50, and 100) % of total $\mathrm{Pb^{2+}}$ should be present as $\mathrm{Pb}_j(\mathrm{OH})_i$ species, at pH = (7.0, 8.1, and 10.0), respectively, while in our case, as shown in Figure 3, the hydrolytic species ($\mathrm{Pb}_j(\mathrm{OH})_i$) are not formed. Phytic acid dominates the chemistry of the metal cations over the whole pH range, as also reported in other papers for other cations (e.g., refs 34 and 41).

Sequestering Ability of Phytate. Many times we stressed the importance of defining a correct speciation model obtained by different instrumental techniques in various experimental conditions, to compare the sequestering ability of a ligand toward different metal ions at different ionic strengths, pH, and temperatures. Although this consideration is very important, it is often difficult to compare the complexing ability of a ligand toward one or more metals (different speciation schemes or different side reactions may be present). This problem was overcome by the calculation of $\mathrm{pL_{50}}$, an empirical parameter that, once the experimental conditions (ionic strength, ionic medium, temperature, pH, and metal concentration) are fixed, can give an objective representation of this binding ability. A detailed description of the method is given, for example, in refs 1, 19, and 20. Briefly, $\mathrm{pL_{50}}$ represents the total ligand concentration necessary to bind 50 % of cation in solution and is obtained by a sigmoid equation

$$y = \frac{A_1 - A_2}{1 + 10^{(\mathrm{pL} - \mathrm{pL_{50}})}} + A_2 \qquad (19)$$

where y represents the total percentage of uncomplexed metal, $A_1 = 0$, and $A_2 = 100$. In other words, the higher the $\mathrm{pL_{50}}$ is,

Table 9. pL_{50} Values[a] for M/Phy Systems at $I = 0.15$ mol·L^{-1} in $NaNO_3$ and $T = 298.15$ K

	pL_{50}			
pH	Cu^{2+}	Pb^{2+}	Ni^{2+}	Zn^{2+}
4.0	3.5	3.6	2.6	4.1
5.0	4.6	4.4	3.4	4.9
6.0	5.6	5.3	4.3	5.8
7.4	6.9	6.4	5.4	7.2
8.1	7.5	7.0	6.1	8.0
9.0	8.1	7.6	7.4	8.5

[a] ± 0.1 (95 % C.I.).

Table 10. pL_{50} Values[a] for M/Phy Systems at pH = 8.1 and $T = 298.15$ K in $NaNO_3$

I	pL_{50}			
mol·L^{-1}	Cu^{2+}	Pb^{2+}	Ni^{2+}	Zn^{2+}
0.15	7.5	7.0	6.1	8.0
0.50	7.1	6.5	5.7	7.5
1.00	6.8	6.4	5.3	7.0

[a] ± 0.1 (95 % C.I.).

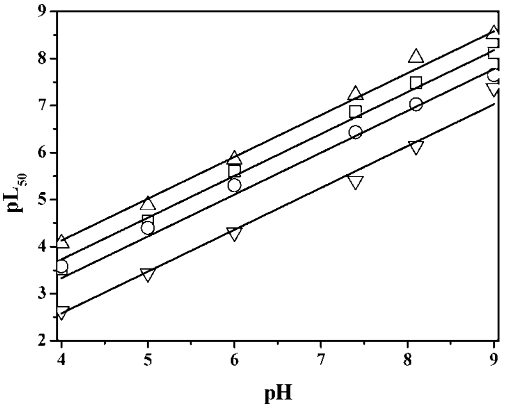

Figure 6. pL_{50} values vs pH for the M/Phy systems. $I = 0.15$ mol·L^{-1} and $T = 298.15$ K. Symbols: □, Cu^{2+}; ○, Pb^{2+}; △, Zn^{2+}; ▽, Ni^{2+}.

the stronger is the binding ability of the ligand at a given pH. Analogously, for a ligand, the higher the pL_{50} is, the stronger is the binding ability toward a given cation.

In this paper the sequestering ability of phytic acid toward copper, lead, zinc, and nickel was evaluated by calculating the pL_{50}; in addition, its dependence on pH and ionic strength was also tested, in the range $4.0 \leq pH \leq 9.0$ and $0.15 \leq I/\text{mol·L}^{-1} \leq 1.00$, respectively. The pL_{50} values, summarized in Tables 9 and 10, show that the sequestering ability of phytate toward the four cations is quite similar. With regards to Pb^{2+} and Cu^{2+}, the differences are negligible, while phytate showed for Zn^{2+} a slightly higher sequestering ability, and for Ni^{2+} lower pL_{50} values at each pH value. The trend reported for pL_{50} for the four cations is the same observed in the case of log K_i values of formation constants, that is, Zn > Cu ≈ Pb > Ni. The diagrams of pL_{50} values versus both pH and ionic strength show a linear trend and are reported in Figures 6 and 7, respectively. In the case of the dependence on I a common slope of −0.92 ± 0.08 (95 % C.I.) was determined for the four metal cations. The intercepts (i.e., the calculated pL_{50} values at $I = 0$ mol·L^{-1}) are 7.64 ± 0.06, 7.16 ± 0.11, 7.99 ± 0.08, and 6.20 ± 0.04 for Cu^{2+}, Pb^{2+}, Zn^{2+}, and Ni^{2+}, respectively. Also in the case of the pL_{50} dependence on pH, the fit was a straight line (as also reported elsewhere[19]), with a common slope of 0.89 ± 0.01 and intercepts: 0.14 ± 0.09, −0.15 ± 0.10, 0.52 ± 0.09, and −1.02 ± 0.08 for Cu^{2+}, Pb^{2+}, Zn^{2+}, and Ni^{2+}, respectively. These two relationships can be useful to determine the pL_{50} values in different conditions, without knowledge of the stability, protonation, and hydrolysis constants.

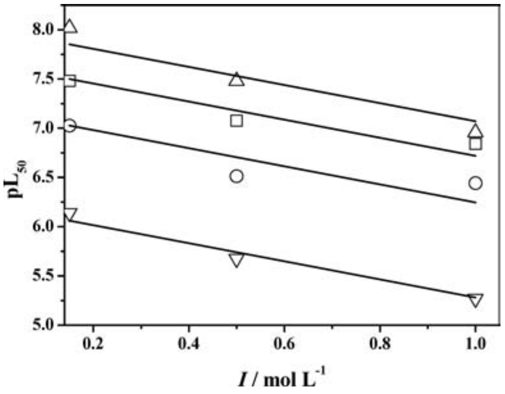

Figure 7. pL_{50} values vs I (in the molar concentration scale) for the M/Phy systems. pH = 8.1 and $T = 298.15$ K. Symbols: □, Cu^{2+}; ○, Pb^{2+}; △, Zn^{2+}; ▽, Ni^{2+}.

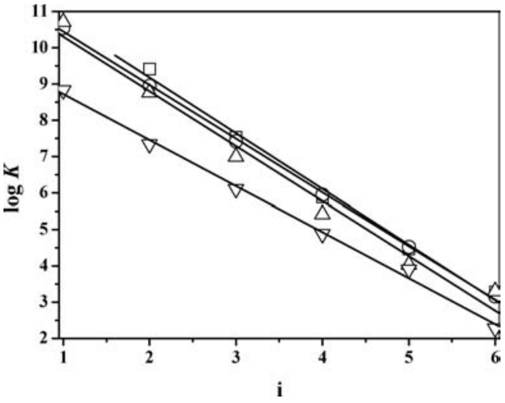

Figure 8. Dependence of log K_i values on the number of protons (i) of the MH_iPhy species at $T = 298.15$ K and $I = 0.13$ mol·L^{-1} in $NaNO_3$. Symbols: □, Cu^{2+}; ○, Pb^{2+}; △, Zn^{2+}; ▽, Ni^{2+}.

Suggested Formation Constants. From the ionic strength dependence analysis of the whole data set presented in this work, it was possible to derive different sets of "suggested" values for the various MH_iPhy ($i = 1$ to 6) species, at $I = (0.15, 0.50,$ and $1.00)$ mol·L^{-1} in $NaNO_3$. These values are obtained by the Debye−Hückel equation and confirmed by the SIT model. As it concerns the $CuHPhy^{4-}$ species, it was not possible to obtain it experimentally. However, an interesting observation can be made by looking at Figure 8, where the log K_i values for the various M/Phy species are plotted versus the number of protons (i) bound to phytate (already described in ref 19). As can be seen, a linear relationship exists. This line can be used for predictive purposes to guess the formation constant of the $CuHPhy^{4-}$ species, as well as those of the ML species. The refined intercepts of these lines are 11.92 ± 0.08, 11.82 ± 0.39, 12.24 ± 0.33, and 9.98 ± 0.15 (± 95 % C.I.), while the slopes are −1.48 ± 0.02, −1.51 ± 0.10, −1.53 ± 0.08, and −1.26 ± 0.04 for Cu^{2+}, Pb^{2+}, Zn^{2+}, and Ni^{2+}, respectively. In all cases the correlation coefficient $R = -0.99$. All of the "suggested values" of the formation constants are summarized in Table 11.

Discussion on Errors. An extensive discussion on error associated with highly charged molecules should be done, to give appropriate weight to the results presented in this paper. One of the most important aspects of the solution equilibria is the evaluation of the errors associated with the determination of the formation constants. Usually this quantity is dependent on two main contributions: (i) the experimental uncertainty of the instruments and (ii) the error associated with the least-

Table 11. Suggested Formation Constants for the M/Phy Systems at $T = 298.15$ K in $NaNO_3$

M	I/mol·kg^{-1}	log K_1[a]	log K_2[a]	log K_3[a]	log K_4[a]	log K_5[a]	log K_6[a]
Cu^{2+}	0.15	(10.74 ± 0.22)[b,c]	9.30 ± 0.27	7.51 ± 0.31	5.98 ± 0.34	4.35 ± 0.37	3.04 ± 0.38
	0.50	(9.33 ± 0.08)	8.27 ± 0.09	6.66 ± 0.10	5.38 ± 0.11	3.86 ± 0.11	2.77 ± 0.11
	1.00	(8.82 ± 0.06)	7.65 ± 0.06	6.13 ± 0.07	5.04 ± 0.07	3.49 ± 0.07	2.57 ± 0.07
Pb^{2+}	0.15	10.29 ± 0.22	8.62 ± 0.26	7.10 ± 0.30	5.68 ± 0.33	4.39 ± 0.35	3.23 ± 0.35
	0.50	9.02 ± 0.08	7.51 ± 0.10	6.17 ± 0.12	4.86 ± 0.14	3.78 ± 0.15	2.88 ± 0.17
	1.00	8.24 ± 0.05	6.79 ± 0.07	5.53 ± 0.09	4.21 ± 0.11	3.24 ± 0.13	2.56 ± 0.15
Zn^{2+}	0.15	10.81 ± 0.22	9.28 ± 0.27	7.81 ± 0.31	6.18 ± 0.34	4.84 ± 0.36	3.77 ± 0.38
	0.50	9.40 ± 0.08	8.08 ± 0.11	6.81 ± 0.14	5.27 ± 0.17	4.15 ± 0.20	3.43 ± 0.22
	1.00	8.40 ± 0.06	7.21 ± 0.09	6.06 ± 0.12	4.48 ± 0.14	3.50 ± 0.17	3.13 ± 0.20
Ni^{2+}	0.15	8.73 ± 0.22	7.30 ± 0.26	5.97 ± 0.30	4.69 ± 0.33	3.45 ± 0.34	2.26 ± 0.35
	0.50	7.50 ± 0.08	6.17 ± 0.10	5.03 ± 0.12	3.93 ± 0.14	2.90 ± 0.15	1.98 ± 0.17
	1.00	6.77 ± 0.05	5.40 ± 0.07	4.35 ± 0.09	3.35 ± 0.11	2.46 ± 0.13	1.76 ± 0.15

[a] Refers to eq 3. [b] Values in parentheses are calculated by fitting log K_i vs i, as reported in Figure 8. [c] ± 95 % C.I.

Table 12. Literature Selected Values of Formation Constants of Various M/Phy Species

M	log K_0[a]	log K_1[a]	log K_2[a]	log K_3[a]	log K_4[a]	log K_5[a]	log K_6[a]	log K_7[a]	I[c]	medium	T/K	ref
Cu^{2+}						2.63			3.0	$NaClO_4$	298.15	18
Cu^{2+}				13.5	12.15	9.07	5.73		0.15	$NaClO_4$	310.15	17
Cu^{2+}		17.33[b]	28.43[b]	37.74[b]	45.27[b]	51.29[b]	56.18[b]	58.82[b]	0.17	$(C_2H_5)_4NClO_4$	293.15	14
Pb^{2+}						3.87			3.0	$NaClO_4$	298.15	18
Zn^{2+}				11.3	10.3	8.54	6.94		0.15	$NaClO_4$	310.15	17
Zn^{2+}	5.4[b]	17.29[b]	27.95[b]	37.42[b]	45.00[b]	50.80[b]	55.87[b]	58.5[b]	0.17	$(C_2H_5)_4NClO_4$	293.15	14
Ni^{2+}						3.78			3.0	$NaClO_4$	298.15	18
Ni^{2+}					8.78	8.44	7.20		0.15	$NaClO_4$	310.15	17
Ni^{2+}	9.23	10.03	9.78	9.27	7.96	7.18	7.05	7.24	0.1	KCl	309.15	15
Ni^{2+}		14.74[b]	27.04[b]	36.71[b]	44.56[b]	51.06[b]	56.28[b]	59.14[b]	0.17	$(C_2H_5)_4NClO_4$	293.15	14

[a] Refers to eq 3. [b] log K_i refers to the equilibria: $M^{2+} + H_2L + (i - 2)H^+ = M(H_iL)$. [c] In mol·L^{-1}.

squares regression. These considerations are often enough to establish the standard deviation on the value of the formation constants. Recently, this research group proposed an empirical relationship (eq 20) for the evaluation of the maximum error on the value of a formation constant. Although it is a rough approximation, it often helps to judge the accuracy of a formation constant:

$$\sigma(\log K) \le a(\sum p) + b\sum z^2 \tag{20}$$

where p are the stoichiometric coefficients of the species involved in the reactions and z are the charges of reactants, with $a = 0.001$ (excellent data), $a = 0.02$ (good data), and $a = 0.05$ (fair data) and $b = 3 \cdot 10^{-4}$. This empirical rule has been derived from a great deal of formation data obtained in this laboratory (refs 37 and 42). In this paper we considered an additive term, which is dependent on the variation of the ionic strength during a titration. This topic is very important when a highly charged molecule, as phytic acid, is present in solution; in fact as an example the ionic strength of a 1 mmol·L^{-1} solution of $Na_{12}Phy$ at pH = 12 (where $Na_{12}Phy = Na_5Phy^{7-} + 7Na^+$) is $I = 28$ mmol·L^{-1}, while at pH = 1 (where $Na_{12}Phy + 7H^+ = Na_5PhyH_7 + 7Na^+$) is $I = 7$ mmol·L^{-1}. In light of these considerations, we modified eq 20 to eq 21.

$$\sigma^2(\log K) \le [a(\sum p) + b\sum z^2]^2 + \sigma^2(\Delta I) \tag{21}$$

where $\sigma(\Delta I)$ depends on the ionic strength variation due to the concentration of the phytate (or other components). A rough calculation of $\sigma(\log K)$ suggested that a value of 0.3 to 0.4 can be considered acceptable for the equilibria considered in this paper. In addition, higher errors should be associated with lower ionic strength values, because the ionic strength contribution due to the reagent concentration has more weight. Higher errors are also present in correspondence of the boundaries of the investigated pH range, since in the acidic range the stoichiometry is characterized by six protons, while in the basic pH region, the charge of phytic acid is higher. On the light of the above considerations, we can affirm that the error on the log K_i values for the boundary terms log K ($MHPhy^{4-}$) and log K (MH_6Phy^+) at $I = 0.15$ mol·L^{-1} should be higher than that on the value of log K (MH_3Phy^{2-}) at $I = 1.0$ mol·L^{-1}. The errors on the formation constants were used to calculate the errors on pL_{50} values by error propagation; this calculation resulted in a value of 0.1 (95 % C.I.).

Literature Comparisons. Many papers are published on the topic of phytate and its role on the bioavailability of various cations. Despite this high number of papers, to our knowledge just a few deal with thermodynamic parameters. In addition, those papers are usually difficult to compare among each other and with the present one, because different experimental conditions were adopted. In any case in Table 12 some selected values of formation constants taken from literature data are reported.

Phytic acid was mainly studied by potentiometry,[14,15,17,18] spectrophotometry,[14,15] NMR,[14] and in a few cases voltammetry.[17,23] Except for the latter, all of the other techniques require a relatively high concentration of metal ion, (as an example, 10^{-3} mol·L^{-1} for potentiometry and NMR, 10^{-4} mol·L^{-1} for spectrophotometry). The utilization of these metal concentrations allows the formation of scarcely soluble species, generally at pH < 6.[20] One of the most interesting papers reporting formation constant values was published by Bebot-Brigaud et al.[14] In that paper, the formation constant values of various M/Phy systems (M = Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+}) were determined by ^{31}P NMR, potentiometry, and spectrophotometry in $(C_2H_5)_4NClO_4$ at $I = 0.17$ mol·L^{-1} and $T = 293.15$ K. Owing to a very low metal-to-ligand concentration ratio, they reported only the formation of variously protonated 1:1 metal phytate complexes, avoiding the formation of polynuclear species and favoring the investigation of wider pH ranges.

For the above cited conditions of temperature and ionic medium, consistent comparisons were hard to make with values reported here. Anyway, bearing in mind that Ni^{2+} and Co^{2+}

usually behave in the same way, also interacting with phytic acid (as reported in ref 14), the reported speciation diagram for cobalt appears similar to the speciation diagram reported for the Ni/Phy system in this work (Figure 5), with low formation percentages in the acidic pH range (the free Ni^{2+} is present also in our condition but is not reported in the figure). The formation pH of the species is shifted in the Bebot-Brigaud diagram with respect to our speciation diagram, probably due to the different phytate protonation constant values in $(C_2H_5)_4NClO_4$ with respect to $NaNO_3$. Also in ref 14, Ni^{2+} shows lower values for the different NiH_iPhy formation constants with respect to analogous Cu^{2+} and Zn^{2+} species, that show similar values for the same species.

Persson et al.[16] studied the interaction of Cu^{2+} toward phytate in $NaClO_4$ at $I = 0.1$ mol·L^{-1} and at $T = 298.15$ K at different pH values and demonstrated that at pH ~ 5.5 phytate binds ~50 % of total copper. In other studies carried out by Martin and Evans[43,44] in KNO_3 at $I = 0.2$ mol·L^{-1} and $T = 298.15$ K, the concentration of metal ion bound to ligand was calculated as a function of pH at $1:1 \leq Cu^{2+}:Phy \leq 6:1$ molar ratios, and they determined the apparent binding heat, which indicates that the complexation reaction is endothermic for these cations. Torres et al.[17] and Vasca et al.[18] used in their investigations different experimental conditions, namely, ionic strength and temperature (Vasca et al.[18] used $NaClO_4$ at $I = 3.0$ mol·L^{-1} and $T = 298.15$ K, whereas Torres et al.[17] carried out their measurements at $I = 0.15$ mol·L^{-1} and $T = 310.15$ K, in the same ionic medium); this does not allow satisfactory comparison between the stability constants and the speciation models obtained. However, the speciation model proposed by Torres et al.[17] is in agreement with that proposed by Bebot-Brigaud et al.,[14] owing to the fact that similar metal/ligand ratios were used. On the other hand, Vasca et al.[18] obtained the CuH_5Phy^{5-} and $Cu_2H_3Phy^{5-}$ species with $\log K_{15} = 2.63$ and $\log K_{23} = 8.97$, respectively. Martin and Evans[45] carried out a calorimetric and titration study on the interaction of phytate with Zn^{2+} in the range $2.5 \leq pH \leq 11.0$, in KCl at $I = 0.2$ mol·L^{-1} and $T = 298.15$ K. The phytate complex formation was obtained at pH < 6, with ~3.5 mol of Zn^{2+} bound per mol of phytate. By means of potentiometric titrations, they also determined the formation constants per phosphate group bound by Zn^{2+} at different metal-to-ligand ratios, while the apparent binding heats at four molar ratios were determined from calorimetric titrations carried out in the range $2.6 \leq pH \leq 6.1$.

Investigations of Ni/Phy system were carried out also by Torres et al.[17] and De Carli et al.[15] in different ionic media (KCl at $I = 0.1$ mol·L^{-1} for De Carli et al.) and temperature ($T = (310.15$ and $309.15)$ K, respectively). Torres et al.[17] found $\log K_4 = 8.78$, $\log K_5 = 8.44$, and $\log K_6 = 7.20$, while De Carli et al.[15] provided a scheme of seven NiH_iPhy (with $i = 1$ to 7), summarized in Table 12. Although the differences in $\log K_i$ values occur, due to the different experimental conditions, the speciation diagram reported in De Carli et al.[15] traces the one reported in this paper for the Ni/Phy system (see Figure 5), with a low formation percentage for the NiH_2Phy^{3-} (40 %) species and a high formation percentage for the $NiHPhy^{4-}$ and NiH_4Phy^{-} ((70 to 80) %) species. Higher differences are present for NiH_6Phy^{+} species; in De Carli et al.[15] it reaches 85 %, while in our case it is (25 to 30) %. Other data were reported by Vasca et al.[18] in $NaClO_4$ at $I = 3$ mol·L^{-1} and $T = 298.15$ K. They reported values of $\log K_{15} = 3.78$ (compared with 3.45 "suggested" in this paper for NiH_5Phy^0 species in $NaNO_3$ at $I = 0.15$ mol·L^{-1}) and $\log K_{23} = 11.85$, owing to the higher metal-to-ligand ratio used.

Very poor literature data are present on Pb/Phy complexes, only Vasca et al.[18] found [in $NaClO_4$ at $I = 3.0$ mol·L^{-1} and $T = 298.15$ K] $\log K_{15} = 3.87$ (compared to 3.24 "suggested" in our paper in $NaNO_3$ at $I = 1.0$ mol·L^{-1}) and $\log K_{23} = 12.87$.

Conclusions

Different M/Phy (M = Zn^{2+}, Cu^{2+}, Pb^{2+}, Ni^{2+}) systems were studied in this paper by both DP-ASV and ISE-H^+ potentiometry at $T = 298.15$ K and at different ionic strengths. The results reported here were analyzed together with other literature data published by Crea et al.[19,20] at different ionic strengths. We considered an average charge for the deprotonated phytate ($z = -7$) as calculated in ref 26 and confirmed elsewhere[25] in Na^+ solutions. The average charge was used to calculate the ionic strength dependence parameters for the complex formation constants, by using the Debye−Hückel and SIT type equations.

In addition the speciation of each M/Phy system and the sequestering ability (by calculating pL_{50} values) were evaluated as a function of pH and ionic strength. Moreover, for each M/Phy system a comprehensive set of "suggested" formation constants was proposed in $NaNO_3$ at $I = (0.15, 0.50,$ and $1.00)$ mol·L^{-1}. In these conditions the speciation diagrams in the investigated pH range ($3.0 \leq pH \leq 10.0$) were drawn, and the pL_{50} values at different pH and ionic strength values were calculated.

We now want to stress again the importance of the utilization of different analytical techniques. The results obtained by potentiometry and voltammetry showed always a reasonable agreement, demonstrating that their simultaneous utilization, if data are critically analyzed, is a useful tool in the study of complex systems. Moreover, coupling the information obtained by these two electrochemical techniques, we can better define the interaction scheme in a metal−ligand system in wider experimental conditions. Although the formation constant values may appear slightly different between the different metal cations, the speciation diagrams reported show that in all cases phytic acid binds almost 100 % of metal over pH ~ 5 to 6. In the selected experimental conditions, only Ni^{2+} showed a weaker complexation in the pH range $3.0 \leq pH \leq 5.5$. The formation of the different MH_iPhy species followed the trend: $Zn \geq Cu \approx Pb > Ni$. The sequestering ability of phytic acid toward the different metal cations was studied, and the results obtained reveal that it follows the same trend as the formation constants. The dependence of pL_{50} was also modeled as a function of pH and ionic strength, by simple linear equations.

Concerning the literature data analysis, the different experimental conditions used by the various authors made the comparisons hard. In any case, a satisfactory accord is obtained with data presented by Vasca et al.,[18] where for Ni^{2+} and Pb^{2+} the formation constants in $NaClO_4$ at $I = 3.0$ mol·L^{-1} and $T = 298.15$ K of the MH_5Phy species are similar with the data reported in this paper. An important aspect that should be also underlined is that phytate complexation inhibited the metal hydrolysis up to pH = 10, as also shown in the reported speciation diagrams (Figures 2 to 5). This behavior already observed in other cases, such as alkaline earth metal cations,[41] Hg^{2+},[34] Cd^{2+},[2,26] Zn^{2+},[19] Cu^{2+},[20] and dimethyltin(IV),[46] and can be considered very important in the study of sediment equilibria (e.g., Fe^{3+} in marine sediments). It should be finally noted that in many cases, dealing with M/Phy complexes, it is possible to analyze different systems simultaneously, reducing the need of further experimental data, to model the dependence on ionic strength and pH (or other variables) of some thermodynamic parameters. This was pointed out, as an example, when the pL_{50} was modeled as a function of pH and ionic strength with the same slope for Cu^{2+}, Pb^{2+}, Zn^{2+}, and Ni^{2+}. This allows the formulation of some models that have simplicity as their main purpose.

Literature Cited

(1) Crea, F.; De Stefano, C.; Milea, D.; Sammartano, S. Thermodynamic parameters for lanthanoid(III) sequestration by phytate at different temperatures. *Monatsh. Chem.* **2010**, *141* (5), 511–520.

(2) De Stefano, C.; Lando, G.; Milea, D.; Pettignano, A.; Sammartano, S. Formation and stability of cadmium(II)/phytate complexes by different electrochemical techniques. Critical analysis of results. *J. Solution Chem.* **2010**, *39*, 179–195.

(3) Crea, F.; De Stefano, C.; Milea, D.; Sammartano, S. Formation and stability of phytate complexes in solution. *Coord. Chem. Rev.* **2008**, *252*, 1108–1120.

(4) Harland, B. F.; Narula, G. Food phytate and its hydrolysis products. *Nutr. Res. (N.Y., NY, U.S.)* **1999**, *19*, 947–961.

(5) Maga, J. A. Phytate: its chemistry, occurrence, food interactions, nutritional significance, and methods of analysis. *J. Agric. Food Chem.* **1982**, *30*, 1–9.

(6) Oatway, L.; Vasanthan, T.; Helm, J. H. Phytic acid. *Food Rev. Int.* **2001**, *17*, 419–431.

(7) Konietzny, U.; Jany, K. D.; Greiner, R. Phytate-an undesiderable constituent of plant-based foods. *J. Ernaehrungsmed.* **2006**, *8* (3), 18–28.

(8) Shears, S. B. Assessing the omnipotence of inositol hexakisphosphate. *Cell. Signalling* **2001**, *13*, 151–158.

(9) Lott, J. N. A. In *Seed Physiology*; Murray, D. R., Ed.; Academic Press: New York, 1984; p 139.

(10) Graf, E.; Eaton, J. W. Suppression of colonic cancer by dietary phytic acid. *Nutr. Cancer* **1993**, *19*, 11–19.

(11) Park, H. R.; Ahn, H. J.; Kim, J. H.; Yook, H. S.; Kim, S.; Lee, C. H.; Byun, M. W. Effects of Irradiated Phytic Acid on Antioxidation and Color Stability in Meat Models. *J. Agric. Food Chem.* **2004**, *52*, 2572–2576.

(12) Vucenik, I.; Shamsuddin, A. M. Protection against cancer by dietary IP6 and inositol. *Nutr. Cancer* **2006**, *55*, 109–125.

(13) Munoz, J. A.; Valiente, M. Effects of trace metals on the inhibition of calcium oxalate crystallization. *Urol. Res.* **2005**, *33*, 267–272.

(14) Bebot-Brigaud, A.; Dange, C.; Fauconnier, N.; Gérard, C. ^{31}P NMR Potentiometric and spectrophotometric studies of phytic acid ionization and complexation properties toward Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} and Cd^{2+}. *J. Inorg. Biochem.* **1999**, *75*, 71–78.

(15) De Carli, L.; Rosso, N. D.; Schnitzler, E.; Carneir, P. I. B. Estudo da estabilidade do complexo àcido fitìco e o ìon Ni(II). *Cienc. Tecnol. Aliment. (Campinas, Braz.)* **2006**, *26*, 19–26.

(16) Persson, H.; Turk, M.; Nyman, M.; Sandberg, A. S. Binding of Cu^{2+}, Zn^{2+}, and Cd^{2+} to Inositol Tri-, Tetra-, Penta-, and Hexaphosphates. *J. Agric. Food Chem.* **1998**, *46*, 3194–3200.

(17) Torres, J.; Dominguez, S.; Cerda, M. F.; Obal, G.; Mederos, A.; Irvine, R. F.; Diaz, A.; Kremer, C. Solution behaviour of myo-inositol hexakisphosphate in the presence of multivalent cations. Prediction of a neutral pentamagnesium species under cytosolic/nuclear conditions. *J. Inorg. Biochem.* **2005**, *99*, 828–840.

(18) Vasca, E.; Materazzi, S.; Caruso, T.; Milano, O.; Fontanella, C.; Manfredi, C. Complex formation between phytic acid and divalent metal ions: a solution equilibria and solid state investigation. *Anal. Bioanal. Chem.* **2002**, *374*, 173–178.

(19) Crea, F.; De Stefano, C.; Milea, D.; Sammartano, S. Speciation of phytate ion in aqueous solution. Thermodynamic parameters for zinc(II) sequestration at different ionic strengths and temperatures. *J. Solution Chem.* **2009**, *38* (1), 115–134.

(20) Crea, P.; De Stefano, C.; Milea, D.; Porcino, N.; Sammartano, S. Speciation of phytate ion in aqueous solution. Protonation constants and copper(II) interactions in $NaNO_{3aq}$ at different ionic strengths. *Biophys. Chem.* **2007**, *128* (2−3), 176–184.

(21) Flaschka, H. A. *EDTA Titration*; Pergamon: London, 1959.

(22) Braibanti, A.; Ostacoli, G.; Paoletti, P.; Pettit, L. D.; Sammartano, S. Recommended Procedure for Testing the Potentiometric Apparatus and Technique for the pH-metric Measurement of Metal-Complex Equilibrium Constants. *Pure Appl. Chem.* **1987**, *59*, 1721–1728.

(23) Brainina, K. Z.; Fedorova, N. D. The use of phytic acid in the stripping voltammetric determination [analysis] of rare earth metals. In *Phytic Acid [Symp. "Appl. Phytic Acid,"]*; Graf, E., Ed.; Pilatus Press: Minneapolis, 1986; pp 329−338.

(24) De Stefano, C.; Sammartano, S.; Mineo, P.; Rigano, C. Computer Tools for the Speciation of Natural Fluids. In *Marine Chemistry - An Environmental Analytical Chemistry Approach*; Gianguzza, A., Pelizzetti, E., Sammartano, S., Eds.; Kluwer Academic Publishers: Amsterdam, 1997; pp 71−83.

(25) Cigala, R. M.; Crea, F.; Lando, G.; Milea, D.; Sammartano, S. Solubility and acid-base properties of concentrated phytate in self-medium and in $NaCl_{aq}$ at $t = 25$ °C. *J. Chem. Thermodyn.* **2010**, *42*, 1393–1399.

(26) De Stefano, C.; Milea, D.; Porcino, N.; Sammartano, S. Speciation of phytate ion in aqueous solution. Cadmium(II) interactions in $NaCl_{aq}$ at different ionic strengths. *Anal. Bioanal. Chem.* **2006**, *386* (2), 346–356.

(27) Cukrowski, I.; Adsetts, M. Experimental and calculated complex formation curves for a labile metal-ligand system. A differential pulse polarographic study of the Pb(II)-(*N*,*N*,*N*′,*N*′-tetramethylethylenediamine)-OH system at fixed ligand to metal ratio and varied pH. *J. Electroanal. Chem.* **1997**, *429*, 129–137.

(28) Cukrowski, I.; Hancock, R. D.; Luckay, R. C. Formation constant calculation for non-labile complexes based on a labile part of the metal-ligand system. A differential pulse polarographic study at fixed ligand to metal ratio and varied pH: application to polarographically inactive complexes. *Anal. Chim. Acta* **1996**, *319*, 39–48.

(29) Weast, R. C. *Handbook of Chemistry and Physics*, 66th ed.; CRC Press, Inc.: Boca Raton, FL, 1985−1986.

(30) Crea, F.; De Robertis, A.; Sammartano, S. Medium and alkyl chain effects on the protonation of dicarboxylates in $NaCl_{(aq)}$ and $Et_4NI_{(aq)}$ at 25 °C. *J. Solution Chem.* **2004**, *33*, 497–526.

(31) Biederman, G. Ionic Media. In *Dahlem Workshop on the Nature of Seawater*, Dahlem Konferenzen, Berlin, 1975; pp 339−362.

(32) Setschenow, J. Z. Uber Die Konstitution Der Salzlosungenauf Grund Ihres Verhaltens Zu Kohlensaure. *Z. Phys. Chem.* **1889**, *4*, 117–125.

(33) Bretti, C.; Foti, C.; Sammartano, S. A new approach in the use of SIT in determining the dependence on ionic strength of activity coefficients. Application to some chloride salts of interest in the speciation of natural fluids. *Chem. Spec. Bioavail.* **2004**, *16* (3), 105–110.

(34) De Stefano, C.; Milea, D.; Porcino, N.; Sammartano, S. Speciation of phytate ion in aqueous solution. Sequestering ability towards mercury(II) cation in $NaCl_{aq}$ at different ionic strengths. *J. Agric. Food Chem.* **2006**, *54* (4), 1459–1466.

(35) Baes, C. F.; Mesmer, R. E. *The Hydrolysis of Cations*; Wiley: New York, 1976.

(36) Li, N.; Wahlberg, O.; Puigdomenech, I. Equilibrium Studies of Phytate Ions - Metal Ion Phytate Complexes Formed in Aqueous Solution; Methods and Characterization of the Phytate Ligand. *Chem. Scr.* **1989**, *29*, 91–95.

(37) De Stefano, C.; Milea, D.; Pettignano, A.; Sammartano, S. Speciation of phytate ion in aqueous solution. Alkali metal complex formation in different ionic media. *Anal. Bioanal. Chem.* **2003**, *376* (7), 1030–1040.

(38) Bretti, C.; Foti, C.; Porcino, N.; Sammartano, S. SIT parameters for 1:1 electrolytes and correlation with Pitzer coefficients. *J. Solution Chem.* **2006**, *35* (10), 1401–1415.

(39) Grenthe, I.; Puigdomenech, I. *Modelling in Aquatic Chemistry*; Nuclear Energy Agency: Paris, 1997.

(40) Gamsjager, H.; Bugajski, J.; Gadja, T.; Lemire, R. J.; Preis, W. *Chemical Thermodynamics of Nickel*; Elsevier: Amsterdam, 2005.

(41) Crea, P.; De Robertis, A.; De Stefano, C.; Sammartano, S. Speciation of phytate ion in aqueous solution. Sequestration of magnesium and calcium by phytate at different temperatures and ionic strengths, in $NaCl_{aq}$. *Biophys. Chem.* **2006**, *124*, 18–26.

(42) Daniele, P. G.; Foti, C.; Gianguzza, A.; Prenesti, E.; Sammartano, S. Weak alkali and alkaline earth metal complexes of low molecular weight ligands in aqueous solution. *Coord. Chem. Rev.* **2008**, *252*, 1093–1107.

(43) Martin, C. J.; Evans, W. J., III. A calorimetric and titrimetric study of the pH dependence of copper(II) binding. *J. Inorg. Biochem.* **1986**, *28*, 39–55.

(44) Martin, C. J.; Evans, W. J. Phytic acid: divalent cation interactions. IV. a spectroscopic determination of Co(II) and Cu(II) binding. *J. Inorg. Biochem.* **1987**, *29*, 241–248.

(45) Martin, C. J.; Evans, W. J. Phytic acid-zinc ion interactions: a calorimetric and titrimetric study. *J. Inorg. Biochem.* **1986**, *26* (3), 169–183.

(46) De Stefano, C.; Milea, D.; Sammartano, S. Speciation of phytate ion in aqueous solution. Dimethyltin(IV) interactions in $NaCl_{aq}$ at different ionic strengths. *Biophys. Chem.* **2005**, *116*, 111–120.

Received for review April 20, 2010. Accepted July 17, 2010. We thank the University of Messina for financial support.

JE100384F