

Sequestering Ability of Phytate toward Biologically and Environmentally Relevant Trivalent Metal Cations[†]

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ABSTRACT: Quantitative parameters for the interactions between phytate (Phy) and Al^{3+} , Fe^{3+} , and Cr^{3+} were determined potentiometrically in NaNO_3 aqueous solutions at $I = 0.10 \text{ mol L}^{-1}$ and $T = 298.15 \text{ K}$. Different complex species were found in a wide pH range. The various species are partially protonated, depending on the pH in which they are formed, and are indicated with the general formula MH_qPhy (with $0 \leq q \leq 6$). In all cases, the stability of the FeH_qPhy species is several log K units higher than that of the analogous AlH_qPhy and CrH_qPhy species. For example, for the MH_2Phy species, the stability trend is $\log K_2 = 15.81, 20.61, \text{ and } 16.70$ for $\text{Al}^{3+}, \text{Fe}^{3+}, \text{ and } \text{Cr}^{3+}$, respectively. The sequestering ability of phytate toward the considered metal cations was evaluated by calculating the $\text{pL}_{0.5}$ values (i.e., the total ligand concentration necessary to bind 50% of the cation present in trace in solution) at different pH values. In general, phytate results in a quite good sequestering agent toward all three cations in the whole investigated pH range, but the order of $\text{pL}_{0.5}$ depends on it. For example, at pH 5.0 it is $\text{pL}_{0.5} = 5.33, 5.44, \text{ and } 5.75$ for $\text{Fe}^{3+}, \text{Cr}^{3+}, \text{ and } \text{Al}^{3+}$, respectively ($\text{Fe}^{3+} < \text{Cr}^{3+} < \text{Al}^{3+}$); at pH 7.4 it is $\text{pL}_{0.5} = 9.94, 9.23, \text{ and } 8.71$ ($\text{Al}^{3+} < \text{Cr}^{3+} < \text{Fe}^{3+}$), whereas at pH 9.0 it is $\text{pL}_{0.5} = 10.42, 10.87, \text{ and } 8.34$ ($\text{Al}^{3+} < \text{Fe}^{3+} < \text{Cr}^{3+}$). All of the $\text{pL}_{0.5}$ values, and therefore the sequestering ability, regularly increase with increasing pH, and the dependence of $\text{pL}_{0.5}$ on pH was modeled using some empirical equations.

KEYWORDS: iron(III), chromium(III), aluminum(III), phytate complexes, sequestering ability, speciation

■ INTRODUCTION

Phytic acid [1,2,3,4,5,6-hexakis(dihydrogen phosphate) myo-inositol] (Phy) and its salts (the phytates, sometimes also denoted phytines²) are widely present in nature, mostly in seeds and grains, but are ubiquitous in all eukaryotic cells,³ where they play several key roles.^{2–18} In the past decades, interest in the study of phytic acid has been growing considerably, from both environmental and biological points of view, as well as from the technological and industrial perspective. New biological functions and technological applications are being continuously discovered, so that any attempts at their description and classification in this context would be limiting and probably outside the aims of this paper: for a comprehensive and exhaustive picture of phytate properties one may refer to some past and recent reviews and books published on these topics.^{2–18} However, it is important to stress here that most of these properties are strictly related to the ability of phytate to form quite strong soluble and sparingly soluble complexes with various proteins, carbohydrates, other organic ligands, and several metal and organometal cations, influencing their (bio)availability and their activity (see, e.g., references in refs 2–18). This means that the chemical speciation (unambiguously defined by IUPAC as the distribution of an element among defined chemical species in a system¹⁹) of both phytate and all of these substances is mutually and deeply affected by their strong interactions. For this reason, several efforts were addressed during the years to the determination of various formation thermodynamic parameters (e.g., activity coefficients, stability constants, entropy and enthalpy changes, solubility) of different phytate complexes in aqueous solution, because they represent the basis

for a correct speciation study of any substances in different natural fluids (e.g., natural waters, soil solutions, biological fluids). Some of these results have been recently reviewed by this research group,²⁰ but new and more accurate investigations on the complexation by phytate of different metal and organometal cations, as well as other organic molecules, are being continuously published (see, e.g., refs 1 and 21–34). In this context, the quantitative studies on the binding ability of phytic acid toward trivalent metal cations are very few (see, e.g., refs 28, 31, and 34–37), although several biological functions, the nutritional properties of some foods, and/or numerous environmentally relevant processes are strictly dependent on the extent of these interactions. In fact, this aspect is particularly relevant from both the agricultural/botanical and the dietary/medical points of view: for example, it is well-known that iron intake via plant food is strongly limited by its chelation by phytate, causing iron deficiency anemia in populations sustained by staple food crops.^{38,39} It is also demonstrated that phytate is deeply involved in the better adaptation of plants to grow in low-P–high-Al acid soils.⁴⁰ In this light, this paper reports the results of a quantitative study on the speciation and the sequestering ability of phytate in the presence of three environmentally and biologically relevant trivalent metal cations, namely, Fe^{3+} , Al^{3+} , and Cr^{3+} . Speciation studies were performed to model the behavior of the M^{3+}/Phy systems in different conditions as a function of pH and, when possible, a

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critical comparison of the thermodynamic results obtained in this paper with literature findings is also reported. The sequestering ability of phytic acid toward the studied metal cations was also quantified by the calculation of the $pL_{0.5}$ values (i.e., the total ligand concentration necessary to bind 50% of the cation present in trace in solution, generally $c_M < 10^{-9}$ mol L^{-1})⁴¹ at different pH values.

MATERIALS AND METHODS

Chemicals. Phytic acid solutions were prepared by weighing the dipotassium salt ($K_2H_{10}Phy$, purity > 95%, main impurity is water) and passing it over a strong cationic exchange resin (Dowex 50W X 8) in H^+ form. The concentration was checked potentiometrically by alkalimetric titrations, and the absence of potassium was established by flame emission spectrometry, always being lower than the LOQ (limit of quantification), $<0.1 \mu g L^{-1}$. Al^{3+} , Fe^{3+} , and Cr^{3+} were used as nitrate salts ($Al(NO_3)_3 \cdot 9H_2O$, $Fe(NO_3)_3 \cdot 9H_2O$, $Cr(NO_3)_3 \cdot 9H_2O$) without further purification. Their solutions were standardized against EDTA,⁴² and their purity was always $\geq 99\%$. In the case of the Fe^{3+} , the spectrophotometric standardization with *o*-phenanthroline⁴³ was also used with comparable results between the two methods. Nitric acid and sodium hydroxide solutions were prepared by diluting concentrated ampules and were standardized against sodium carbonate and potassium hydrogen phthalate, respectively, previously dried in an oven at $T = 383.15$ K for 2 h. $NaNO_3$ aqueous solutions were prepared by weighing the pure salt dried in an oven at $T = 383.15$ K for 2 h. All solutions were prepared with analytical grade water ($R = 18$ M Ω cm^{-1}) using grade A glassware. All of the chemicals were purchased from Sigma-Aldrich (Italy) and its brands at the highest available purity.

Apparatus and Procedure for Potentiometric Measurements. To minimize systematic errors and to check the repeatability of the systems, for all three investigated M^{3+}/Phy systems, various ISE- H^+ potentiometric titrations were performed, at $T = 298.15 \pm 0.1$ K in thermostatted cells, by two operators using two different setups. The first setup consisted of a model 713 Metrohm potentiometer, equipped with a half-cell glass electrode (Ross type 8101, from Thermo-Orion) and a double-junction reference electrode (type 900200, from Thermo-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 Titrand apparatus controlled by Metrohm TiAMO 1.2 software equipped with the same half-cell glass electrode of the first apparatus (Ross type 8101, from Thermo-Orion) 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 . The titrand solution consisted of different amounts of metal cation (0.5–3 mmol L^{-1}), phytate (0.5–5 mmol L^{-1}), a slight excess of nitric acid (1–2 mmol L^{-1}), and $NaNO_3$ to obtain a pre-established ionic strength value ($I = 0.1$ mol L^{-1}). The potentiometric measurements were performed by titrating 25 mL of the titrand solution with NaOH standard solutions up to pH ~ 8.5 –9.0. To reach this pH value, avoiding the formation of scarcely soluble species at lower pH values, ligand-to-metal ratios in favor of the former ($1:1 \leq c_{Phy}/c_M \leq 5:1$) were used in most measurements. However, in all cases when the formation of precipitate was noted, the titrations were stopped at that point. For each experiment, independent titrations of strong acid solutions with a standard base were performed in the same conditions of the systems under investigation, to determine the electrode potential (E^0) and the acidic junction potential ($E_j = j_a [H^+]$). In this way, the pH scale used was the free scale, $pH \equiv -\log [H^+]$, where $[H^+]$ is the free proton concentration (not activity). The reliability of the calibration in the alkaline pH range was checked by calculating the appropriate pK_w values. For each titration, 80–100 data points were collected.

Due to the small dehydration rate constants for trivalent metal cations such as those here considered, the full equilibration of complex species formed may take very long time. That is why particular attention has been paid to this last aspect, verifying the equilibrium state during titrations by some usual precautions⁴⁴ such as checking the time required to reach equilibrium and performing back-titrations.

Finally, for measurements performed at low ionic strengths, the contribution of the ligand to the total ionic strength must be considered. At the highest Phy concentrations, this contribution was taken into account by giving appropriate weights to the results obtained, because the used computer programs can deal with measurements at different ionic strengths and can also determine the ionic strength changes within a single titration.

Ligand Competition and Batch Measurements. In addition to the above-cited potentiometric titrations, further measurements were also performed by different procedures, to verify the reliability of the results obtained. In fact, two difficulties arise when dealing with the systems investigated in the present work: (i) the above-mentioned slow complexation kinetics of some M^{3+} species and (ii) the high stability of phytate/ $M(III)$ species. The first problem has been already discussed. Concerning the last aspect, it must be emphasized that it seriously limits the use of ISE- H^+ potentiometric titrations for the determination of these species, as well detailed, for example, by Delgado et al.:⁴⁵ “The determination of very high values of stability constants has led usually to critical problems, because in most cases the pH-potentiometric techniques (titrations using a strong base to neutralize the protons released by the formation of the complex and followed by a couple of glass/reference electrodes) cannot be applied, as the complex is already formed at very low pH values” and protons are already displaced from the ligand. This generally occurs in the case of relatively stable species, characterized by formation constants $>10^{20}$ (as in the case of the Fe^{3+}/Phy and Al^{3+}/Phy systems), where we cannot observe an equilibrium of the proton during the titration, because the reaction in eq 1



is almost completely shifted toward the formation of the products. To overcome this problem, other procedures may be adopted.⁴⁵ In this paper, the so-called “ligand competition” method was used for the Fe^{3+}/Phy and Al^{3+}/Phy systems, in addition to the classical (above-described) titrations. It consists of potentiometric titrations in the presence of another ligand; its stability constants with the metal cation under investigation are known and are comparable to those of the ligand to be studied. In this way, the competition between the two ligands for the metal cation hinders the complete proton displacement from the investigated ligand, making reliable its potentiometric titration by ISE- H^+ . In our case, we selected ethylenediaminetetraacetic acid (EDTA) as competing ligand.

In the case of the Cr^{3+}/Phy system, considering the greater kinetic inertness of the chromium aquo-ion $[Cr(H_2O)_6]^{3+}$ at room temperature, further “batch experiments” were performed: various solutions with different amounts of $Cr(NO_3)_3$ and phytic acid were initially prepared at the desired ionic strength in different flasks. Each solution was then divided into 35–50 aliquots of 25 mL, and known amounts of NaOH standard solution were added to each aliquot in new flasks. The solutions prepared in this way were fluxed with purified presaturated N_2 , then capped, heated at $T = 353.15$ K for at least 1 day, and then maintained under stirring in a dark thermostatted room (at $T = 298.15 \pm 0.1$ K). The pH was measured, using a calibrated (as above-described) microelectrode purchased from Metrohm (model 6.0224.100), as a function of time, until the electrode potential remained constant (± 0.15 mV, usually 1 week was sufficient). For each initial solution, the potential reading of various flasks was then reported as a function of the added NaOH, simulating a classical titration.

Calculations. The calculation programs were reviewed in ref 46. The nonlinear least-squares computer program ESAB2M was used to refine all of the parameters of the acid–base titrations (E^0 , K_w , liquid junction potential coefficient, j_a , and analytical concentration of

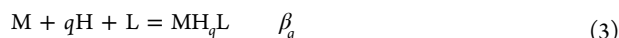
reagents). The BSTAC and STACO computer programs were used in the calculation of complex formation constants.

The charges of all the species are omitted for simplicity.

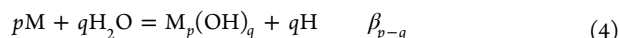
All equilibria are expressed by the following equations:



or



Cation hydrolysis is expressed by



Uncertainties throughout the paper are given as $\pm 95\%$ confidence interval (CI).

RESULTS AND DISCUSSION

Acid–Base Properties of Ligands and Metal Cations.

For the analysis of results, the acid–base properties of both the ligands and the metal cations must be known in the same conditions of the investigated systems.⁴⁷ This aspect is particularly relevant in the case of phytate, because its acid–base behavior is deeply affected by both the nature of the ionic medium and the ionic strength (see, e.g., references in refs 20, 30, and 48). For example, the conditional protonation constants determined in weakly interacting media (e.g., tetralkylammonium salts) are generally 2–3 log units higher than the corresponding values in aqueous solutions of alkali metal cations.^{21,48,49} This lowering is the result of the strong interactions between phytate and these cations, which influences most of its solution properties.²⁰ For example, in its completely deprotonated form, phytate should be regarded as a dodeca-anion, whereas it has been observed that in the presence of aqueous solutions of alkali metal cations it is never present in this form and has a variable effective charge ranging from $z = 5^-$ to $z = 7^-$.^{29,30,50,51} This is also why, in this paper, the charges of various phytate species have been omitted for simplicity. In light of the above considerations, the conditional protonation constants of phytate at $T = 298.15$ K and at $I = 0.1$ mol L⁻¹ in NaNO_{3aq} were taken from our previous paper,⁵² whereas the hydrolysis constants of Al³⁺ and Cr³⁺ were calculated in these conditions from the data of Cigala et al.⁵³ and Baes and Mesmer.⁵⁴ Concerning the Fe³⁺ hydrolysis, both the speciation models and the constants reported by the different authors^{35,54–57} show large discrepancies. During calculations, several models were considered and, finally, the one adopted consists of the mononuclear Fe(OH), Fe(OH)₂, Fe(OH)₃, and Fe(OH)₄ species and the polynuclear Fe₂(OH)₂ and Fe₃(OH)₄ species. For the ligand competition experiments, the protonation constants of EDTA and its stability constants with Al³⁺ and Fe³⁺ were taken from ref 56.

Formation and Stability of Metal(III)–Proton–Phytate Species. The analysis of experimental data evidenced the formation of different MH_qPhy species, the conditional stability constants of which are reported in Table 1. For the three investigated systems, five common species, namely, MPhy, MHPhy, MH₂Phy, MH₃Phy, and MH₄Phy, are formed. In addition, the MH₅Phy and MH₆Phy species were determined for the Al³⁺/Phy and Cr³⁺/Phy systems, whereas the M(OH)-Phy species was observed in the case of Fe³⁺/Phy. However, this last species can be considered as minor. For example, in the ligand and metal concentrations of the speciation diagram shown in Figure 1, it is not formed at all. However, its formation, observed during measurements in other conditions,

Table 1. Conditional Formation Constants of the MH_qPhy Species Determined in This Work, in NaNO_{3aq} at $I = 0.10$ mol L⁻¹ and $T = 298.15$ K

	Al ³⁺	Fe ³⁺	Cr ³⁺
log K ₋₁ ^a	10.7 ± 0.1 ^b		
log K ₀	23.1 ± 0.2 ^b	28.5 ± 0.2	22.6 ± 0.2 ^b
log K ₁	19.17 ± 0.06	24.78 ± 0.15	18.78 ± 0.16
log K ₂	15.81 ± 0.05	20.61 ± 0.08	16.70 ± 0.14
log K ₃	12.29 ± 0.04	15.42 ± 0.08	14.73 ± 0.10
log K ₄	9.29 ± 0.03	10.23 ± 0.06	9.78 ± 0.11
log K ₅	6.56 ± 0.03		6.17 ± 0.13
log K ₆	4.12 ± 0.10		3.0 ± 0.4

^alog K_q refers to equilibrium: M + H_qL = MH_qL; charges omitted for simplicity. ^b±95% CI.

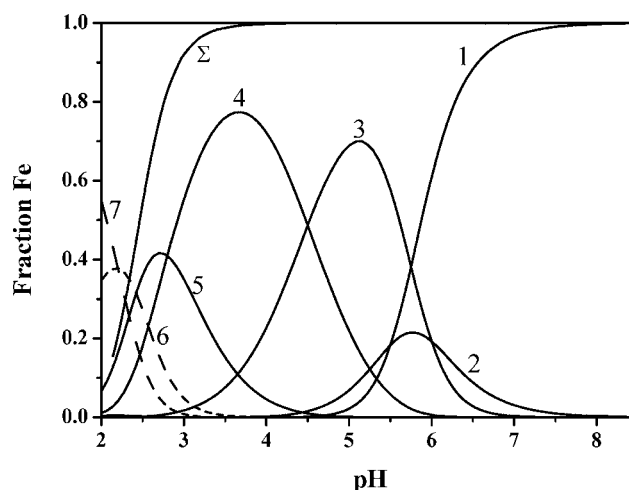
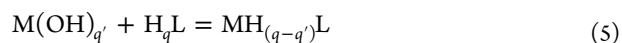


Figure 1. Speciation diagram of Fe³⁺/Phy system; fraction of Fe versus pH. Experimental conditions: $c_{\text{phy}} = 3$ mmol L⁻¹; $c_{\text{Fe}} = 1$ mmol L⁻¹; $I = 0.10$ mol L⁻¹ in NaNO_{3aq}, $T = 298.15$ K. Species: 1, FePhy; 2, FeHPhy; 3, FeH₂Phy; 4, FeH₃Phy; 5, FeH₄Phy; 6, Fe(OH); 7, Fe(OH)₂ (charges omitted for simplicity); Σ , sum of all FeH_qPhy species.

can be ascribed to the greater tendency of Fe³⁺ to undergo hydrolysis than Al³⁺ and Cr³⁺ cations. For this reason, the less protonated species are favored in the former case than in the latter, whereas the opposite is observed for those species with more protons. In fact, as already detailed for the formation of phytate complexes with other cations undergoing strong hydrolysis,⁵⁸ it is important to stress that the stability constants expressed by the equilibria in eqs 2 and 3 are formally correct, although the possibility that mixed protonated hydrolytic species of the kind



may be formed must be considered. For example, the formation equilibrium for the FePhy species may be written as



or as



During calculations, both to determine formation constants and to draw speciation profiles, it is unimportant how the equilibrium is expressed. Nevertheless, it is important to determine the effective nature of the equilibria involved in the species formation. Although potentiometric data alone do not

permit this kind of inference, the pH range in which the complexes are formed is a reasonably accurate indicator of the involvement of the correct equilibrium. As a consequence, as happens for Fe^{3+} , the stronger tendency of this cation to undergo hydrolysis favors the formation of the above-cited less protonated complexes, according to eq 5.

The speciation schemes reported in Table 1 are in good agreement with literature findings on the same systems,^{28,31,34–37} although the conditional stability constants can hardly be compared, due to the different experimental conditions adopted. Concerning the comparisons with other systems, the stability of the MH_qPhy species is generally higher for trivalent metal cations than the divalent, except for Sn^{2+} and Hg^{2+} ,⁴⁷ which show comparable values. Within the M^{3+}/Phy systems, the stability of their complexes (Table 1) follows the trend $\text{Fe}^{3+} > \text{Cr}^{3+} > \text{Al}^{3+}$. As an example, for the MH_2Phy species it is $\log K_2 = 20.61, 16.70,$ and 15.51 for $\text{Fe}^{3+}, \text{Cr}^{3+},$ and Al^{3+} , respectively.

In Figure 2, the speciation diagram of the $\text{Cr}^{3+}/\text{Phy}$ system is reported. In these conditions ($c_{\text{Cr}} = 1 \text{ mmol L}^{-1}$ and $c_{\text{Phy}} = 3$

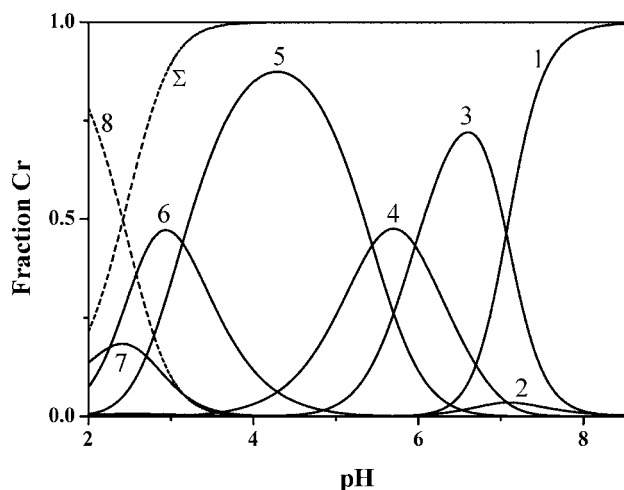


Figure 2. Speciation diagram of the $\text{Cr}^{3+}/\text{Phy}$ system; fraction of Cr versus pH. Experimental conditions: $c_{\text{Phy}} = 3 \text{ mmol L}^{-1}$; $c_{\text{Cr}} = 1 \text{ mmol L}^{-1}$; $I = 0.10 \text{ mol L}^{-1}$ in $\text{NaNO}_{3\text{aq}}$; $T = 298.15 \text{ K}$. Species: 1, CrPhy ; 2, CrHPhy ; 3, CrH_2Phy ; 4, CrH_3Phy ; 5, CrH_4Phy ; 6, CrH_5Phy ; 7, CrH_6Phy ; 8, Cr (charges omitted for simplicity); Σ , sum of all CrH_qPhy species.

mmol L^{-1}), all of the CrH_qPhy species show high formation percentages, dominating chromium speciation. For example, the CrPhy , CrH_2Phy , and CrH_4Phy species represent ~ 95 , ~ 75 , and $\sim 80\%$ of total chromium (at $\text{pH} > 8$, ~ 6.5 , and ~ 4 , respectively). On the contrary, the CrHPhy and the CrH_6Phy species reach ~ 5 and $\sim 20\%$ at $\text{pH} \sim 7$ and ~ 2.5 , respectively.

Quite different trends are observed for the formation of the AlH_qPhy species in the same conditions, as shown in the speciation diagram reported in Figure 3. In this case, slight differences occur both in the species formation percentages and in the pH range over which they are formed. For example, it has already been shown that the CrH_4Phy species reaches a maximum of 95% at $\text{pH} \sim 4.0\text{--}4.5$, whereas the analogue AlH_4Phy reaches a maximum of $\sim 70\%$ at $\text{pH} \sim 3.0\text{--}3.5$. The same holds for CrHPhy , formed in negligible amounts at $\text{pH} \sim 7.0$, whereas the AlHPhy at $\text{pH} > 6.0$ is already $> 50\%$.

All of the speciation diagrams reported are plotted up to $\text{pH} \sim 8.5$. However, as already stated, lower (in the cases in which

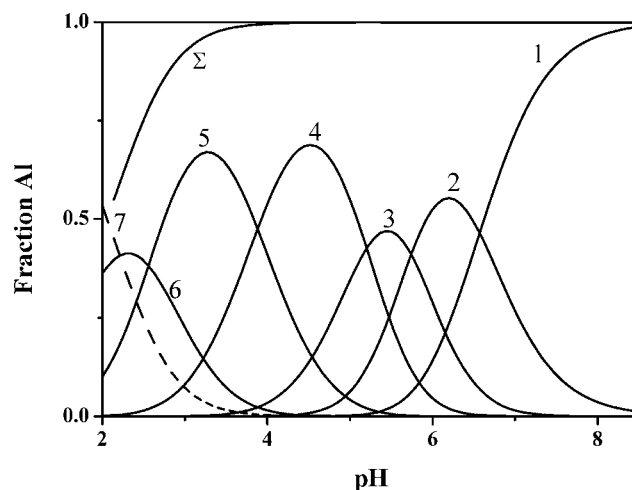


Figure 3. Speciation diagram of the $\text{Al}^{3+}/\text{Phy}$ system; fraction of Al versus pH. Experimental conditions: $c_{\text{Phy}} = 3 \text{ mmol L}^{-1}$; $c_{\text{Al}} = 1 \text{ mmol L}^{-1}$; $I = 0.10 \text{ mol L}^{-1}$ in $\text{NaNO}_{3\text{aq}}$; $T = 298.15 \text{ K}$. Species: 1, AlPhy ; 2, AlHPhy ; 3, AlH_2Phy ; 4, AlH_3Phy ; 5, AlH_4Phy ; 6, AlH_5Phy ; 7, Al (charges omitted for simplicity); Σ , sum of all AlH_qPhy species.

the formation of sparingly soluble species was observed) and higher pH values were also reached during the measurements, depending on the different experimental conditions. In particular, the latter (higher pH) was also the case of the ligand competition measurements, in which the addition of another strong ligand such as EDTA hampered the formation of precipitate, allowing the investigation of a wider pH range. For example, the usual pH values reached in the simple M^{3+}/Phy measurements at low M^{3+}/Phy ratios were $\text{pH} \sim 6.0$ and ~ 8.0 for Fe^{3+} and Al^{3+} , respectively, whereas $\text{pH} \sim 9.0$ and ~ 10.0 were reached in the $\text{Fe}^{3+}/\text{Phy}/\text{EDTA}$ and $\text{Al}^{3+}/\text{Phy}/\text{EDTA}$ systems. A speciation diagram of the $\text{Fe}^{3+}/\text{Phy}/\text{EDTA}$ system is reported in Figure 4 in the following experimental conditions: $c_{\text{Phy}} = 2 \text{ mmol L}^{-1}$, $c_{\text{EDTA}} = 1 \text{ mmol L}^{-1}$, $c_{\text{Fe}} = 2 \text{ mmol L}^{-1}$. Although EDTA is present in lower concentration than phytate, the $\text{Fe}(\text{EDTA})$ and $\text{FeOH}(\text{EDTA})$ species are

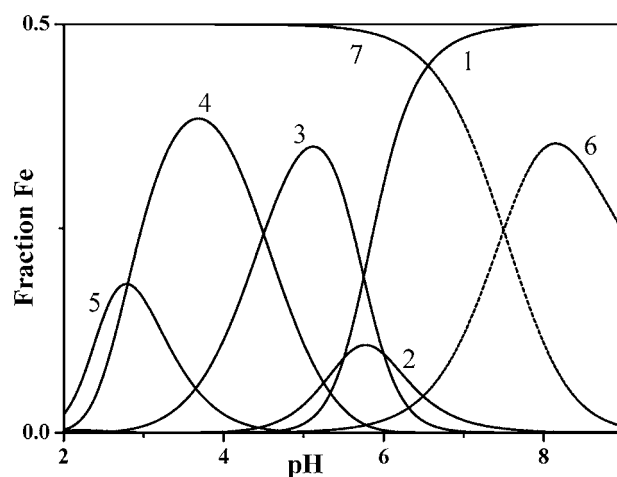


Figure 4. Speciation diagram of the $\text{Fe}^{3+}/\text{Phy}/\text{EDTA}$ system; fraction of Fe versus pH. Experimental conditions: $c_{\text{Phy}} = 2 \text{ mmol L}^{-1}$; $c_{\text{EDTA}} = 1 \text{ mmol L}^{-1}$; $c_{\text{Fe}} = 2 \text{ mmol L}^{-1}$; $I = 0.10 \text{ mol L}^{-1}$ in $\text{NaNO}_{3\text{aq}}$; $T = 298.15 \text{ K}$. Species: 1, FePhy ; 2, FeHPhy ; 3, FeH_2Phy ; 4, FeH_3Phy ; 5, FeH_4Phy ; 6, $\text{FeOH}(\text{EDTA})$; 7, $\text{Fe}(\text{EDTA})$ (charges omitted for simplicity).

both formed in comparable formation percentages: ~50% Fe^{3+} is present as $\text{Fe}(\text{EDTA})$ at $\text{pH} < 7$ and ~40% as $\text{FeOH}(\text{EDTA})$ $\text{pH} > 8$, whereas the FeH_qPhy species, formed in all the investigated pH range, reach ~50% as FePhy at $\text{pH} > 7.0$ and ~40% as FeH_2Phy and FeH_3Phy $\text{pH} \sim 5.0$ and ~ 3.5 , respectively. Lower formation percentages are observed for the FeH_4Phy (~20% at $\text{pH} \sim 2.5$) and the FeHPhy species (~15% at $\text{pH} \sim 6.0$), whereas the formation of the $\text{Fe}(\text{OH})\text{Phy}$ species is not observed in those experimental conditions. The speciation diagram of Figure 4 is also useful to assess the distribution of Fe^{3+} in conditions that may be frequently observed in foods or soils. In fact, chlorosis in plants and other problems caused by iron deficiency are frequently treated by amending soils with FeEDTA or other iron chelates,^{59,60} and NaFeEDTA has been recommended as a food additive for iron fortification, particularly in the cases of high-phytate diets.⁶¹ As a consequence, iron availability may be modified by the simultaneous presence of phytate and EDTA.

The speciation diagrams in Figures 1–4, obtained by the ES4ECI program using the conditional stability constants reported in Table 1 (with the appropriate protonation and hydrolysis constants), are just four examples of the possible conditions in which the investigated metal cations can be present with phytate. Of course, the stability constants provided can be used to draw the speciation diagrams in other possible systems with different phytate and metal concentrations.

Sequestering Ability of Phytate and Dependence on pH. The above-cited examples are just two of the numerous cases where speciation studies are useful. In fact, one of its most important applications is the estimation of the sequestering ability of a given ligand toward one or more metal cations or of one or more ligands toward a given cation. This is a key information, for example, in remediation studies (assisted or not) of polluted sites or in the prediction of the availability of some components in real systems. To this aim, the simple analysis of single sets of stability constants of metal/ligand systems is not always sufficient to assess the global binding (sequestering) ability of a ligand toward a given cation in real conditions, owing to the difficulties regarding, for example, the different number and/or nature of complexes formed by single ligands. Moreover, other factors influence the formation yields of the species, such as the solution conditions, the acid–base properties of the cation and the ligand (because both hydrolysis and protonation reactions are competitive with respect to the formation reaction), and the competition between other metals and ligands simultaneously present in natural systems. Because of this, two metal–ligand systems may show the same formation percentages (in given conditions), even with different formation constants. This problem can be overcome by the calculation of $\text{pL}_{0.5}$ (also called pL_{50}), an empirical parameter (proposed by our group in recent years) that, once the experimental conditions (ionic strength, ionic medium, temperature, pH) are fixed, can give an objective representation of the sequestering ability of a ligand (L) toward a metal ion (M). A detailed description of the method is given, for example, in ref 41. In practice, using any computer program able to calculate the free concentrations of various species in solution (i.e., speciation diagrams obtained by, e.g., ES4ECI), the mole fractions (x) (or the percentages) of a cation complexed by a ligand (phytate in our case) can be determined at various total ligand concentrations (as $-\log c_L$) and can be plotted as a function of it at fixed pH, ionic strength, and temperature values. The result is a diagram (sequestration diagram)

representing a sigmoid curve (similar to a dose–response curve) with asymptotes of 1 (or 100 if expressed in percentage) for $\text{pL} \rightarrow -\infty$ and 0 for $\text{pL} \rightarrow +\infty$

$$x = \left[\frac{1}{1 + 10^{(\text{pL} - \text{pL}_{0.5})}} \right] \quad (8)$$

where the parameter $\text{pL}_{0.5}$ represents the total ligand concentration necessary to sequester 50% of the metal ion present in trace in solution. Therefore, the higher the $\text{pL}_{0.5}$ is, the stronger is the sequestering ability toward a given cation. In general, when a sequestration diagram is drawn, x values are dependent on the total metal concentration c_M . However, when c_M is low enough (generally $c_M < 10^{-9}$ mol L^{-1}), x and the relative sequestration diagrams become independent of it, as well as the corresponding $\text{pL}_{0.5}$. Of course, several other parameters have been proposed for the same purpose,⁶² but here it is important to stress that, different from most other methods, all of the side interactions occurring in the system (metal hydrolysis, ligand protonation, interactions with other components) are taken into account in the speciation model used in the calculation of $\text{pL}_{0.5}$, but are excluded from its estimation and do not give any contribution. In this way, the $\text{pL}_{0.5}$ value quantifies the sequestering power of a ligand, “cleaned” from all competitive reactions, simplifying comparisons. In fact, this conditional parameter is particularly useful to make comparisons among systems in different conditions or with different speciation (e.g., ligands and metals with different acid–base properties or with a different number of metal complexes formed, as well as the cases where polynuclear species are formed). In these cases, the simple analysis of the stability constants is not sufficient to give an immediate idea of the sequestration of a cation by different ligands. For example, concerning the cases cited in the previous paragraph, where it is important to establish which ligand among EDTA and phytate is a stronger chelant toward Fe^{3+} , from the simple comparison of the stability constants of the FeL species it could be expected that the former is weaker than the latter ($\log K = 25.1$ and 28.5 , for $\text{Fe}(\text{EDTA})$ and FePhy , respectively). Nevertheless, by looking at the sequestering ability, the conclusion is different and supports the observation that Fe-EDTA is successfully employed as iron supplier also in the cases where phytate is present. In fact, in all of the investigated pH range, EDTA shows a greater sequestering ability than Phy : at $\text{pH} 7.4$, for example, it is $\text{pL}_{0.5} = 13.5$ and 9.9 , for EDTA and Phy , respectively. These values indicate that both ligands are good sequestering agents toward Fe^{3+} , but their difference (~3.5 log units) allows Fe^{3+} to be preferentially bound to EDTA than phytate. This depends, of course, on the different acid–base properties of the two ligands, which deprotonate at different pH values and for which information is not immediately accessible from the simple analysis of the $\log K_{ML}$ values. For this reason, the analysis of the $\text{pL}_{0.5}$ is a very interesting tool, also for nonchemists, to make fast comparisons.

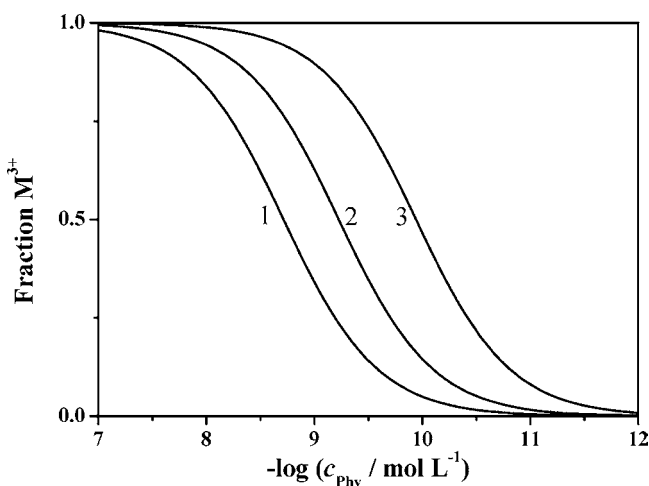
Analogously, from the simple analysis of the stability constants of the three investigated systems, it is not possible to make inferences about the greater sequestering ability of phytate toward Al^{3+} , Fe^{3+} , or Cr^{3+} , especially in different pH conditions. In fact, the $\text{pL}_{0.5}$ values calculated at different pH values (reported in Table 2) show that phytic acid is a quite good sequestering agent toward these cations over the entire pH range. However, phytate sequesters preferentially one cation or another, depending on the pH. For example, in the

Table 2. $pL_{0.5}$ Values^a for M^{3+} /Phy Systems Calculated at Different pH Values

pH	$pL_{0.5}$		
	Al^{3+}	Fe^{3+}	Cr^{3+}
4.0	5.75	5.33	5.44
5.0	7.21	6.73	6.82
6.0	7.41	8.10	7.80
7.4	8.71	9.94	9.23
8.1	8.91	10.44	10.32
9.0	8.34	10.42	10.87

^a ± 0.1 (95% CI).

pH range ~ 6.0 – 8.0 , the $pL_{0.5}$ values of the Fe^{3+} /Phy system are higher than the Cr^{3+} /Phy, in turn higher than the Al^{3+} /Phy system, whereas the trend Al^{3+} /Phy > Cr^{3+} /Phy > Fe^{3+} /Phy is observed at pH < 6.0. Finally, at pH > 9.0, when the competition of hydrolytic species is more relevant for both Al^{3+} and Fe^{3+} than Cr^{3+} , the sequestering ability of phytate is greater toward the latter than the former. As an example, the comparison of the sequestration diagrams for the three M^{3+} /Phy systems is reported at pH 7.4 in Figure 5. However, the different behavior

**Figure 5.** Sequestration diagram of phytate toward different trivalent metal cations at pH 7.4, $I = 0.10 \text{ mol L}^{-1}$ in $NaNO_{3aq}$, $T = 298.15 \text{ K}$. Molar fraction of the complexed metal versus $-\log^1(c_{phy}/\text{mol L}^{-1})$. Curves: 1, Al^{3+} ; 2, Cr^{3+} ; 3, Fe^{3+} .

observed at various pH values is an indication of the possibility for phytate to selectively sequester one of these cations in the presence of the others, by simply varying the acidity conditions of the system. This “selective sequestration” is one of the various aspects in support of the use of phytate as a commercial sequestering agent. In addition, it shows at least three other great advantages with respect to other classical chelating agents: (1) it is essentially nontoxic and already present in nature in great amounts; (2) it has a very low cost (because it can be extracted from a wide number of vegetables in high percentages); and, thanks to its acid base properties, (3) it shows an almost constant high sequestering ability over a very wide pH range, so that it could be employed in very different conditions.

Empirical (Predictive) Relationships. Another aspect that makes the $pL_{0.5}$ interesting is that, although its values are dependent on the selected conditions, they very often show regular trends that may be exploited for the formulation of

some empirical relationships to be used with predictive purposes. Simple equations have been proposed for the calculation of $pL_{0.5}$ as a function of pH, ionic strength, temperature, and the concentration of other competing ligands (see, e.g., refs 1, 29, 33, and 41). For example, $pL_{0.5}$ values in Table 2 generally increase with increasing pH and, in the case of Cr^{3+} , the function is

$$pL_{0.5}(\pm 0.1) = 1.21 + 1.09(\text{pH})$$

(with a correlation coefficient, $r = 0.996$) in the pH range $4.0 \leq \text{pH} \leq 9.0$. For the other two systems, we calculated, at $4.0 \leq \text{pH} \leq 8.0$

$$pL_{0.5}(\pm 0.1) = 0.35 + 1.27(\text{pH}) \quad (r = 0.996)$$

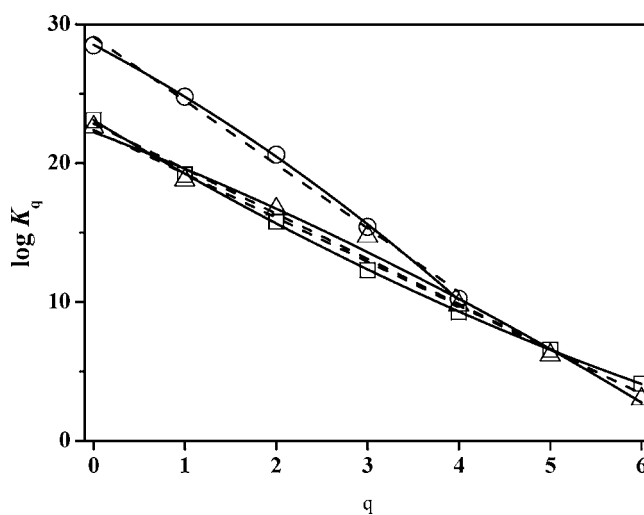
and

$$pL_{0.5}(\pm 0.2) = 3.07 + 0.75(\text{pH}) \quad (r = 0.966)$$

for Fe^{3+} and Al^{3+} , respectively.

This kind of empirical relationship makes the $pL_{0.5}$ a very helpful tool, especially when the information about stabilities is incomplete: these equations make it possible to estimate with good approximation the sequestering ability of a ligand toward a metal cation in conditions different from the experimental.

As already evidenced for other M^{z+} /Phy systems,^{29,47,58,63} also the formation constants of phytate metal complexes usually show some regular trends, which can be used to estimate the stability of other species not experimentally determined, but which can be formed in other contexts. In Figure 6, the

**Figure 6.** $\log K_q$ values versus q : dependence of the conditional stability constants ($\log K_q$) of $MH_q\text{Phy}$ complexes on the number of protons (q) of the species for the Al^{3+} /Phy (squares), Fe^{3+} /Phy (circles), and Cr^{3+} /Phy (triangles) systems.

dependence of the stability constants ($\log K_q$) of different $MH_q\text{Phy}$ species on the number of protons (q) of the complex is reported for all three investigated cations. The $\log K_q$ values were fitted to a first-degree (linear, dashed line) and a second-degree (straight line) polynomial curve, obtaining quite high correlation coefficients in all cases. The refined parameters for the three systems and the two fits are reported in Table 3 together with the corresponding correlation coefficients. As is obvious, the polynomial fit is better than the linear and can be used for the estimation of unknown stability constants.

Table 3. Empirical Parameters of the Equations for the Dependence of the Conditional Stability Constants^a of MH_qPhy Complexes on the Number of Protons of the Species, in NaNO_{3aq} at I = 0.10 mol L⁻¹ and T = 298.15 K

M ³⁺	a	b	c	r ^b
log K _q = a + bq				
Al	22.4	-3.17		0.996
Fe	29.1	-4.59		0.996
Cr	22.9	-3.25		0.992
log K _q = a + bq + cq ²				
Al	23.1	-4.00	0.14	0.999
Fe	28.5	-3.46	-0.28	0.999
Cr	22.2	-2.51	-0.12	0.993

^alog K_q refers to equilibrium: M + H_qL = MH_qL, charges omitted for simplicity. ^bCorrelation coefficient.

Nevertheless, the linear fit is more reliable if the order of magnitude of the experimental errors made during the determination of the stability constants is taken into account.

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Author Contributions

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REFERENCES

- Cigala, R. M.; Crea, F.; De Stefano, C.; Lando, G.; Milea, D.; Sammartano, S. Thermodynamics of binary and ternary interactions in the tin(II)/phytate system in aqueous solutions, in the presence of Cl⁻ or F⁻. *J. Chem. Thermodyn.* **2012**, *51*, 88–96.
- Maga, J. A. Phytate: its chemistry, occurrence, food interactions, nutritional significance, and methods of analysis. *J. Agric. Food Chem.* **1982**, *30* (1), 1–9.
- Raboy, V. myo-inositol-1,2,3,4,5,6-hexakisphosphate. *Phytochemistry* **2003**, *64*, 1033–1043.
- Graf, E. Applications of phytic acid. *J. Am. Oil Chem. Soc.* **1983**, *60* (11), 1861–1867.
- Reddy, N. R.; Pierson, M. D.; Sathe, S. K.; Salunkhe, D. K. *Phytates in Cereals and Legumes*; CRC Press: Boca Raton, FL, 1989.
- Graf, E.; Eaton, J. W. Antioxidant functions of phytic acid. *Free Radical Biol. Med.* **1990**, *8* (1), 61–69.
- Harland, B. F.; Morris, E. R. Phytate: a good or a bad food component? *Nutr. Res. (N.Y.)* **1995**, *15* (5), 733–754.
- Harland, B. F.; Narula, G. Food phytate and its hydrolysis products. *Nutr. Res. (N.Y.)* **1999**, *19* (6), 947–961.
- Urbano, G.; Lopez-Jurado, M.; Vidal-Valverde, C.; Tenorio, E.; Porres, J. The role of phytic acid in legumes: antinutrient or beneficial function? *J. Physiol. Biochem.* **2000**, *56* (3), 283–294.
- Oatway, L.; Vasanthan, T.; Helm, J. H. Phytic acid. *Food Rev. Int.* **2001**, *17* (4), 419–431.
- Shears, S. B. Assessing the omnipotence of inositol hexakisphosphate. *Cell. Signal.* **2001**, *13*, 151–158.

(12) Fox, C. H.; Eberl, M. Phytic acid (IP6), novel broad spectrum anti-neoplastic agent: a systematic review. *Complement. Ther. Med.* **2002**, *10* (4), 229–234.

(13) Lopez, H. W.; Leenhardt, F.; Coudray, C.; Remesy, C. Minerals and phytic acid interactions: is it a real problem for human nutrition? *Int. J. Food Sci. Technol.* **2002**, *37* (7), 727–739.

(14) Reddy, N. R.; Sathe, S. K. *Food Phytates*; CRC Press: Boca Raton, FL, 2002.

(15) Shamsuddin, A. M. Anti-cancer function of phytic acid. *Int. J. Food Sci. Technol.* **2002**, *37* (7), 769–782.

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

(17) Kumar, V.; Sinha, A. K.; Makkar, H. P. S.; Becker, K. Dietary roles of phytate and phytase in human nutrition: a review. *Food Chem.* **2010**, *120* (4), 945–959.

(18) Ali, M.; Shuja, M. N.; Zahoor, M.; Qadri, I. Phytic acid: how far have we come? *Afr. J. Biotechnol.* **2010**, *9* (11), 1551–1554.

(19) Templeton, D. M.; Ariese, F.; Cornelis, R.; Danielsson, L. G.; Muntau, H.; van Leeuwen, H. P.; Lobinski, R. Guidelines for terms related to chemical speciation and fractionation of elements. Definitions, structural aspects, and methodological approaches. *Pure Appl. Chem.* **2000**, *72* (8), 1453–1470.

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

(21) Crea, F.; Crea, P.; De Stefano, C.; Milea, D.; Sammartano, S. Speciation of phytate ion in aqueous solution. Protonation in CsCl_{aq} at different ionic strengths and mixing effects in LiCl_{aq} + CsCl_{aq}. *J. Mol. Liq.* **2008**, *138*, 76–83.

(22) Crea, F.; De Stefano, C.; Porcino, N.; Sammartano, S. Sequestering ability of phytate towards protonated BPEI and other polyammonium cations in aqueous solution. *Biophys. Chem.* **2008**, *136*, 108–114.

(23) Crea, P.; De Stefano, C.; Milea, D.; Sammartano, S. Formation and stability of mixed Mg²⁺/Ca²⁺/phytate species in seawater media. Consequences on ligand speciation. *Mar. Chem.* **2008**, *112* (3–4), 142–148.

(24) Heighton, L.; Schmidt, W. F.; Siefert, R. L. Kinetic and equilibrium constants of phytic acid and ferric and ferrous phytate derived from nuclear magnetic resonance spectroscopy. *J. Agric. Food Chem.* **2008**, *56* (20), 9543–9547.

(25) Torres, J.; Veiga, N.; Gancheff, J. S.; Dominguez, S.; Mederos, A.; Sundberg, M.; Sanchez, A.; Castiglioni, J.; Diaz, A.; Kremer, C. Interaction of myo-inositol hexakisphosphate with alkali and alkaline earth metal ions: spectroscopic, potentiometric and theoretical studies. *J. Mol. Struct.* **2008**, *874* (1–3), 77–88.

(26) 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.

(27) De Carli, L.; Schnitzler, E.; Ionashiro, M.; Szpoganicz, B.; Rosso, N. D. Equilibrium, thermoanalytical and spectroscopic studies to characterize phytic acid complexes with Mn(II) and Co(II). *J. Braz. Chem. Soc.* **2009**, *20*, 1515–1522.

(28) Quirrenbach, H. R.; Kanumfre, F.; Rosso, N. D.; Carvalho, M. A. Behaviour of phytic acid in the presence of iron(II) and iron(III). *Cienc. Tecnol. Aliment.* **2009**, *29* (1), 24–32.

(29) Cigala, R. M.; Crea, F.; De Stefano, C.; Lando, G.; Milea, D.; Sammartano, S. Electrochemical study on the stability of phytate complexes with Cu²⁺, Pb²⁺, Zn²⁺, and Ni²⁺: a comparison of different techniques. *J. Chem. Eng. Data* **2010**, *55*, 4757–4767.

(30) 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 = 298.15 K. *J. Chem. Thermodyn.* **2010**, *42*, 1393–1399.

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

- (32) 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* (2), 179–195.
- (33) Gianguzza, A.; Milea, D.; Pettignano, A.; Sammartano, S. Palladium(II) sequestration by phytate in aqueous solution. Speciation analysis and ionic medium effects. *Environ. Chem.* **2010**, *7*, 259–267.
- (34) Sala, M.; Makuc, D.; Kolar, J.; Plavec, J.; Pihlar, B. Potentiometric and ^{31}P NMR studies on inositol phosphates and their interaction with iron(III) ions. *Carbohydr. Res.* **2011**, *346* (4), 488–494.
- (35) 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.
- (36) Evans, W. J.; Martin, C. J. Heat of complex formation of Al(III) and Cd(II) with phytic acid. IX. *J. Inorg. Biochem.* **1988**, *34*, 11–18.
- (37) Evans, W. J.; Martin, C. J. The interaction of inositol hexaphosphate with Fe(III) and Cr(III). A calorimetric investigation. XV. *J. Inorg. Biochem.* **1991**, *41*, 245–255.
- (38) Aluru, M. R.; Rodermeil, S. R.; Reddy, M. B. Genetic modification of low phytic acid 1-1 maize to enhance iron content and bioavailability. *J. Agric. Food Chem.* **2011**, *59* (24), 12954–12962.
- (39) Petry, N.; Egli, I.; Zeder, C.; Walczyk, T.; Hurrell, R. Polyphenols and phytic acid contribute to the low iron bioavailability from common beans in young women. *J. Nutr.* **2010**, *140* (11), 1977–1982.
- (40) Du, Y.-M.; Tian, J.; Liao, H.; Bai, C.-J.; Yan, X.-L.; Liu, G.-D. Aluminium tolerance and high phosphorus efficiency helps *Stylosanthes* better adapt to low-P acid soils. *Ann. Bot.* **2009**, *103* (8), 1239–1247.
- (41) Gianguzza, A.; Giuffrè, O.; Piazzese, D.; Sammartano, S. Aqueous solution chemistry of alkytlin(IV) compounds for speciation studies in biological fluids and natural waters. *Coord. Chem. Rev.* **2012**, *256*, 222–239.
- (42) Flaschka, H. A. *EDTA Titration*; Pergamon: London, UK, 1959.
- (43) Mehlig, J. P.; Hulett, H. R. Spectrophotometric determination of iron with o-phenanthroline and with nitro-o-phenanthroline. *Ind. Eng. Chem. Anal. Ed.* **1942**, *14* (11), 869–871.
- (44) 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.
- (45) Delgado, R.; do Carmo Figueira, M.; Quintino, S. Redox method for the determination of stability constants of some trivalent metal complexes. *Talanta* **1997**, *45* (2), 451–462.
- (46) 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, The Netherlands, 1997; pp 71–83.
- (47) 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.
- (48) De Stefano, C.; Milea, D.; Sammartano, S. Speciation of phytate ion in aqueous solution. Protonation constants in tetraethylammonium iodide and sodium chloride. *J. Chem. Eng. Data* **2003**, *48*, 114–119.
- (49) 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.
- (50) Li, N.; Wahlberg, O. Equilibrium studies of phytate ions. 2. Equilibria between phytate ions, sodium ions and protons in sodium perchlorate media. *Acta Chem. Scand.* **1989**, *43*, 401–406.
- (51) 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.
- (52) 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 $\text{NaNO}_{3\text{aq}}$ at different ionic strengths. *Biophys. Chem.* **2007**, *128* (2–3), 176–184.
- (53) Cigala, R. M.; De Stefano, C.; Giacalone, A.; Gianguzza, A. Speciation of Al^{3+} in fairly concentrated solutions (20–200 mmol L^{-1}) at $I = 1 \text{ mol L}^{-1}$ (NaNO_3), in the acidic pH range, at different temperatures. *Chem. Spec. Bioavail.* **2011**, *23* (11), 33–37.
- (54) Baes, C. F.; Mesmer, R. E. *The Hydrolysis of Cations*; Wiley: New York, 1976.
- (55) Daniele, P. G.; Rigano, C.; Sammartano, S.; Zelano, V. Ionic strength dependence of formation constants. XVIII. The hydrolysis of iron(III) in aqueous KNO_3 solutions. *Talanta* **1994**, *41*, 1577–1582.
- (56) Martell, A. E.; Smith, R. M.; Motekaitis, R. J. *NIST Critically Selected Stability Constants of Metal Complexes Database*, 8.0; National Institute of Standard and Technology: Gaithersburg, MD, 2004.
- (57) Millero, F. J.; Yao, W.; Aicher, J. The speciation of Fe(II) and Fe(III) in natural waters. *Mar. Chem.* **1995**, *50*, 21–39.
- (58) 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.
- (59) Cantera, R. G.; Zamarreno, A. M.; Garcia-Mina, J. M. Characterization of commercial iron chelates and their behavior in an alkaline and calcareous soil. *J. Agric. Food Chem.* **2002**, *50* (26), 7609–7615.
- (60) Hasegawa, H.; Rahman, M. A.; Saitou, K.; Kobayashi, M.; Okumura, C. Influence of chelating ligands on bioavailability and mobility of iron in plant growth media and their effect on radish growth. *Environ. Exp. Bot.* **2011**, *71* (3), 345–351.
- (61) Yang, Z.; Siekmann, J.; Schofield, D. Fortifying complementary foods with NaFeEDTA – considerations for developing countries. *Matern. Child Nutr.* **2011**, *7*, 123–128.
- (62) Bazzicalupi, C.; Bianchi, A.; Giorgi, C.; Clares, M. P.; Garcia-Espana, E. Addressing selectivity criteria in binding equilibria. *Coord. Chem. Rev.* **2012**, *256*, 13–27.
- (63) 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.