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Speciation of phytate ion in aqueous solution. Protonation constants and copper(II) interactions in $NaNO_{3aq}$ at different ionic strengths ☆

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Abstract

The acid base behavior of phytate has been studied (at $t=25$ °C by potentiometry, ISE-H^+ glass electrode) in $NaNO_{3aq}$ at different ionic strengths ($0.1 \leq I/\text{mol L}^{-1} \leq 1.0$). The interactions with copper(II) were investigated in the same experimental conditions in different metal to ligand (Phy) ratios ($1{:}1 \leq Cu^{2+}{:}Phy \leq 4{:}1$), by using both ISE-$H^+$ and ISE-Cu^{2+} electrodes. Phytate acid base behavior in sodium nitrate is very similar to that in sodium chloride, previously investigated. In the experimental conditions adopted, the formation of three $Cu_iH_jPhy^{(12-2i-j)-}$ species is observed: the mononuclear CuH_4Phy^{6-} and CuH_5Phy^{5-}, and the dinuclear $Cu_2H_5Phy^{3-}$. Analysis of complex formation constants at different ionic strengths reveals that both ISE-H^+ and ISE-Cu^{2+} electrodes gave, within the experimental error, analogous values. Dependence of complex formation constants on ionic strength was modeled by EDH (Extended Debye–Hückel) and SIT (Specific ion Interaction Theory) equations. The sequestering ability of phytate toward copper(II) has been evaluated by the calculation of pL_{50} (the total ligand concentration, as $-\log C_L$, able to bind 50% of metal cation), an empirical parameter already proposed for an objective "quantification" of this ability. A thorough analysis of literature data on phytate–copper(II) complexes has been performed.

Keywords: Phytate; Copper(II); Protonation and complex formation constants; Dependence on ionic strength; Ion selective electrodes; Sequestration

1. Introduction

Many scientists and research groups have been studying phytic acid and its derivatives, paying particular attention to biological and environmental aspects (see, for example, references reported in previous contributions [1–10] and in reviews [11–15]). Due to the fact that most of its properties are strictly correlated to its behavior in aqueous solution, during last years our group modeled its acid base properties and its binding ability toward many metal and organometal cations in various aqueous media, with the aim of studying the chemical speciation of phytic acid under different experimental conditions (this is of fundamental importance for a thorough comprehension of all processes involving this molecule) [1–10]. These studies are particularly interesting also from a mere chemico-physical point of view, because the determination of accurate protonation and stability constants for so complex molecules is always a challenging task (just remember, *e.g.*, the theoretical dodecanionic form of the free anion or the various conformations that phytate can assume in solution). At the same time, these difficulties gave us the possibility to emphasize once again that potentiometry is one of the best instrumental techniques for speciation studies (as recommended, *e.g.*, by IUPAC), even for complex systems [16].

In the present paper, the acid base behavior of phytate has been studied (at $t=25$ °C by potentiometry, ISE-H^+ glass electrode) in $NaNO_{3aq}$ at different ionic strengths ($0.1 \leq I/\text{mol L}^{-1} \leq 1.0$). Successively, its interactions with copper(II) were investigated in the same experimental conditions, by using both ISE-H^+ and ISE-Cu^{2+} electrodes. We chose copper(II) because of the importance that this cation has in many areas [copper(II) is involved in many processes of environmental and biological relevance, see, *e.g.*, Refs. in [17]] and as a further step toward a better definition of the network of interactions of phytate ion with the main metal and

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☆ Previous contributions to this series are cited along the manuscript.

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organometal cations. Moreover, among commercial ion selective electrodes, ISE-Cu^{2+} gives quite good performances in terms of reproducibility, repeatability, and accuracy: comparisons among data obtained by ISE-Cu^{2+} and ISE-H^+ electrodes on the same Cu^{2+}–phytate system allowed us to demonstrate again how different techniques, if properly used, yield to the same results.

2. Experimental section

2.1. Chemicals

Nitric acid and sodium hydroxide solutions were prepared by diluting concentrated ampoules (Riedel-deHaën) and were standardized against sodium carbonate and potassium hydrogen phthalate, respectively. $NaNO_3$ solutions were prepared by weighing pure salt (Fluka) dried in an oven at 110 °C. Phytic acid solutions were prepared by weighing Aldrich dipotassium salt ($K_2H_{10}Phy$), and passing it over a strong cationic exchange resin (Dowex 50 W×8 from Fluka). Concentration was checked potentiometrically by alkalimetric titrations, and the absence of potassium was established by flame emission spectrometry. Copper(II) was used in the form of nitrate salt (Fluka) without further purification, its solutions were standardized spectrophotometrically and its purity was always ≥99.5%. All solutions were prepared with analytical grade water (R=18 MΩ cm^{-1}) using grade A glassware.

2.2. Apparatus

Concerning ISE-H^+ measurements, in order to minimize systematic errors potentiometric titrations were carried out (at t=25.0±0.1 °C) using two different apparatuses: the first one 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 burette; the second one was a Crison micro-pH 2002 potentiometer, with the same kind of equipment of the first apparatus. Estimated accuracy was ±0.15 mV and ±0.003 mL for e.m.f. and titrant volume readings, respectively, and was the same for both systems. They were connected to a PC, and automatic titrations were performed using a suitable computer program to control titrant delivery, data acquisition and to check for e.m.f. stability. Some measurements were also carried out using a Metrohm model 809 Titrando apparatus controlled by Metrohm TiAMO 1.0 software for the automatic data acquisition.

For ISE-Cu^{2+} measurements, potentiometric titrations were carried out by using the same kind of apparatuses and equipment, except for the use of an ISE-Cu^{2+} electrode (type 9429SC, from Orion) instead of the half cell glass electrode. During these titrations, free hydrogen ion concentration was simultaneously measured by the above-mentioned potentiometric system.

2.3. Procedure for ISE-H^+ measurements

Potentiometric titrations were carried out in thermostatted cells under magnetic stirring and bubbling purified presaturated N_2 through the solution in order to exclude O_2 and CO_2 inside. The titrand solution consisted of different amounts of phytic acid (2–5 mmol L^{-1}), nitric acid (3–7.5 mmol L^{-1}), copper(II) nitrate (2–4 mmol L^{-1}, not added during measurements for the determination of phytate protonation constants), and the background salt in order to obtain pre-established ionic strength values ($0.1 \leq I$ mol $L^{-1} \leq 1.0$). Potentiometric measurements were carried out by titrating 25 mL of the titrand solution with standard NaOH solutions up to pH ~10.5 (for titration of solutions with no copper) or until the formation of precipitate occurs. For each experiment, independent titrations of strong acid solution 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 electrode potential (E^0) and acidic junction potential ($E_j = j_a[H^+]$). In this way, the pH scale used was the total scale, $pH \equiv -\log [H^+]$, where $[H^+]$ is the free proton concentration (not activity). Experimental conditions are reported in Table 1 for phytate–copper(II) measurements. In this table, pH values at which the formation of scarcely soluble species occurs are also reported for Phy–Cu(II) systems.

For each titration, 80–100 points were collected, and the equilibrium state during titrations was checked adopting some usual precautions. These included checking the time necessary to reach equilibrium and performing back titrations. For measurements performed at low ionic strengths, the contribution of the ligand has to be considered: in the most critical conditions (*i.e.*

Table 1
Experimental conditions for potentiometric measurements at t=25 °C[a]

C_{Cu}[a]	C_{Phy}[a]	C_{NaNO3}[a]	pH range	C_{NaOH}[a]
ISE-H^+				
0.002	0.002	0.096	2.27– 4.19	0.2033[b]
0.003	0.002	0.096	2.29– 3.82	0.2033[b]
0.004	0.002	0.096	2.26– 3.54	0.2033[b]
0.002	0.002	0.253	2.27– 4.18	0.2033[b]
0.003	0.002	0.253	2.19– 3.77	0.2033[b]
0.004	0.002	0.253	2.23– 3.99	0.2033[b]
0.002	0.002	0.507	2.22– 3.98	0.2033[b]
0.003	0.002	0.507	2.21– 3.83	0.2033[b]
0.004	0.002	0.507	2.22– 3.80	0.2033[b]
0.002	0.002	0.748	2.20– 4.18	0.2033[b]
0.003	0.002	0.748	2.19– 4.00	0.2033[b]
0.004	0.002	0.748	2.17– 3.82	0.2033[b]
0.002	0.002	0.997	2.17– 4.18	0.2033[b]
0.003	0.002	0.997	2.16– 3.99	0.2033[b]
0.004	0.002	0.997	2.18– 3.82	0.2033[b]
ISE-Cu^{2+}				
0.01324[b]	0.001	0.103	4.29– 3.70	0.006
0.01324[b]	0.002	0.103	4.13– 3.89	0.012
0.01324[b]	0.001	0.247	4.09– 3.73	0.006
0.01324[b]	0.002	0.247	3.96– 3.67	0.012
0.01324[b]	0.001	0.497	4.05– 3.80	0.006
0.01324[b]	0.002	0.497	3.97– 3.74	0.012
0.01324[b]	0.001	0.748	3.93– 3.79	0.006
0.01324[b]	0.002	0.748	3.76– 3.65	0.012
0.01324[b]	0.001	0.938	3.80– 3.70	0.006
0.01324[b]	0.002	0.938	3.79– 3.70	0.012

[a] Total concentrations expressed in mol L^{-1}, 2–3 titrations for each experimental condition.

[b] As titrant.

I=0.1 mol L^{-1}), this contribution to ionic strength is ~7–8%, which introduces a not dramatic error in calculation. However, this error was taken into account by giving appropriate weights to the results obtained at low ionic strengths in fitting different functions.

2.4. Procedure for ISE-Cu^{2+} measurements

Also in this case, potentiometric titrations were carried out in thermostatted cells under magnetic stirring, bubbling purified presaturated N_2 through the solution, collecting 80–100 points for each titration, and checking for the equilibrium state. The titrand solution consisted of different amounts of phytic acid (1–2 mmol L^{-1}), the background salt in order to obtain pre-established ionic strength values ($0.1 \leq I$ mol $L^{-1} \leq 1.0$), and, eventually, sodium hydroxide to reach an initial pH value of pH ~4. Potentiometric measurements were carried out by titrating 25 mL of the titrand solution with $Cu(NO_3)_2$ solutions until copper concentration was twice that of phytate. Copper electrode calibration was performed by adding known amounts of $Cu(NO_3)_2$ to $NaNO_3$ solutions at different ionic strengths. All experimental conditions are reported in the second part of Table 1.

2.5. Calculations

The non-linear least squares computer program ESAB2M [18] was used for the refinement of all the parameters of the acid–base titration (E^0, K_w, liquid junction potential coefficient, j_a, analytical concentration of reagents). The BSTAC [19] and STACO [20] computer programs were used in the calculation of complex formation constants. Both programs can deal with measurements at different ionic strengths. The ES4ECI [19] program was used to draw speciation diagrams and to calculate species formation percentages. The LIANA [21] program was used to fit different equations.

Protonation and complex formation constants are given according to the equilibrium

$$i\mathrm{Cu}^{2+} + j\mathrm{H}^{+} + \mathrm{Phy}^{12-} = \mathrm{Cu}_i\mathrm{H}_j\mathrm{Phy}^{(12-2i-j)-} \qquad \beta_{ij} \tag{1}$$

or

$$i\mathrm{Cu}^{2+} + \mathrm{H}_j\mathrm{Phy}^{(12-j)-} = \mathrm{Cu}_i\mathrm{H}_j\mathrm{Phy}^{(12-2i-j)-} \qquad K_{ij}. \tag{2}$$

Dependence on ionic strength of phytate protonation constants in $NaNO_{3aq}$ was taken into account by an equation already proposed for the same modeling in alkali metal chlorides [1,2]:

$$\log K_j^{\mathrm{H}} = \log {}^{\mathrm{T}}K_j^{\mathrm{H}} + C_j I - \log(1 + 10^{D_j} I) \tag{3}$$

where K_j^{H} is the protonation constant, ${}^{\mathrm{T}}K_j^{\mathrm{H}}$ is the protonation constant at infinite dilution, C_j and D_j are empirical parameters. The parameter D_j can be considered as a measure of the interaction between Na^+ and $H_jPhy^{(12-j)-}$, as already described [1,2].

Dependence on ionic strength of complex formation constants for $Cu_iH_jPhy^{(12-2i-j)-}$ species expressed in the molar (mol L^{-1}) concentration scale was taken into account by a Debye–Hückel type equation:

$$\log K_{ij} = \log {}^{\mathrm{T}}K_{ij} + DH + CI \tag{4}$$

where C is an empirical parameter, and DH is the Debye–Hückel term

$$DH = -z^{*} 0.51 I^{1/2} / (1 + 1.5 I^{1/2}) \tag{5}$$

with

$$z^{*} = \Sigma(\mathrm{charges})^2_{\mathrm{reactants}} - \Sigma(\mathrm{charges})^2_{\mathrm{products}}. \tag{6}$$

By using appropriate density values, molar to molal [m, mol $kg^{-1}(H_2O)$] scale conversions of I and K_{ij} were performed. When these are expressed in the molal concentration scale, Eq. (4) becomes the classical and widely used SIT (Specific ion Interaction Theory) equation [22–24], where C represents $\Delta\varepsilon$

$$\Delta\varepsilon = \Sigma_p \varepsilon(p, q) \tag{7}$$

and where $\varepsilon(p,q)$ is the SIT interaction coefficient of the p-th species (involved in the equilibrium represented by the formation constant K_{ij}) with the q-th component (of opposite charge). $\Delta\varepsilon$ parameters were determined too. Both STACO and BSTAC computer programs can refine the empirical parameters for Eq. (4).

3. Results and discussion

3.1. Phytate protonation constants and their dependence on ionic strength

Acid base behavior of phytic acid in Na^+ media has been extensively investigated, mainly in perchlorate or chloride salts

Table 2
Experimental phytate protonation constants[a] in $NaNO_{3aq}$ at different ionic strengths and at t= 25 °C

I/mol L^{-1} [b]	log K_1^{H}	log K_2^{H}	log K_3^{H}	log K_4^{H}	log K_5^{H}	log K_6^{H}	log K_7^{H}
0.117	9.54±0.10	9.86±0.09	9.44±0.05	8.15±0.05	6.48±0.03	5.30±0.06	2.95±0.03
0.165	9.46±0.10	9.70±0.09	9.36±0.04	8.07±0.04	6.29±0.03	5.08±0.06	2.81±0.03
0.338	8.40±0.10	9.25±0.07	9.18±0.04	7.62±0.03	6.02±0.02	4.64±0.04	2.80±0.02
0.339	8.41±0.07	10.17±0.07	8.83±0.04	7.62±0.03	5.98±0.02	4.51±0.04	2.69±0.02
0.562	8.60±0.06	9.50±0.06	8.74±0.03	7.56±0.03	5.87±0.02	4.55±0.02	2.61±0.02
1.039	8.46±0.12	9.13±0.12	8.50±0.05	7.33±0.04	5.65±0.02	4.44±0.08	2.50±0.06

[a] K_j^{H} refers to the equilibrium: $\mathrm{H}^{+} + \mathrm{H}_{j-1}\mathrm{Phy}^{(12-j+1)-} = \mathrm{H}_j\mathrm{Phy}^{(12-j)-}$; ±standard deviation.

[b] Mean ionic strength value during titration.

(see, *e.g.* [1] and refs. therein). Although, in this kind of studies, different anions of background salts do not dramatically influence phytate protonation constants, we opted for the determination of these values in sodium nitrate aqueous solutions at different ionic strengths, in order to obtain more accurate data to use in the estimation of stability constants for phytate–copper species in $NaNO_{3aq}$. From potentiometric measurements performed in the experimental conditions reported in Table 1, we obtained log K_j^H values shown in Table 2. Dependence on ionic strength of protonation constants reported in this Table was modeled by Eq. (3), in analogy with the procedure followed for data in $NaCl_{aq}$ [1]. The fitting of log K_j^H values to this equation gave a standard deviation on the whole fit of $\sigma = 0.10$, and yielded to refined empirical parameters reported in Table 3 for each protonation step. In the same Table, empirical parameters for the dependence of phytate protonation constants on ionic strength in $NaCl_{aq}$ [1] are reported in parenthesis. From a rapid evaluation of the same parameters in $NaCl_{aq}$ and $NaNO_{3aq}$, it can be noted that phytate acid base behavior in sodium nitrate is very similar to that in sodium chloride: within the experimental error, C_j and D_j parameters in the two media are comparable, and this is better evidenced from the analysis of Table 4, where phytate protonation constants, calculated by Eq. (3) using the empirical parameters of Table 3, are shown at different ionic strengths for the two sodium salts. This is a further evidence that, below $I = 1$ mol L^{-1}, phytate acid–base properties are scarcely affected by the nature of background salt anions.

3.2. Stability of $Cu_iH_jPhy^{(12-2i-j)-}$ species

Phytate protonation constants in $NaNO_{3aq}$ were used in calculations of stability constants for $Cu_iH_jPhy^{(12-2i-j)-}$ species. Concerning copper(II) hydrolysis, we took into account in our calculations the species and values taken from Ref. [25] that, although they were obtained in sodium perchlorate media, can be easily applied to nitrate solutions (both perchlorate and nitrate anions can be considered as weakly interacting with Cu^{2+}). The formation of a further $Cu_3(OH)_4^{2+}$ species was initially considered, as previously proposed (see, *e.g.*, Refs. in [26]), but preliminary calculations demonstrated that its presence/absence in the model did not affect the stability of copper–phytate complexes in these experimental conditions.

Potentiometric data by ISE-H^+ measurements evidenced, in the experimental conditions of Table 1, the formation of three $Cu_iH_jPhy^{(12-2i-j)-}$ species: the mononuclear CuH_4Phy^{6-} and CuH_5Phy^{5-}, and the dinuclear $Cu_2H_5Phy^{3-}$. Values of complex formation constants at different ionic strengths are reported in Table 5, both in molar and molal concentration scales (molar to molal conversions were performed by using appropriate density values). In the same Table, stability constants of pentaprotonated CuH_5Phy^{5-} and $Cu_2H_5Phy^{3-}$ species, obtained by ISE-Cu^{2+} electrode, are also shown. The narrow pH range explored

Table 4
Phytate protonation constants [a] calculated by Eq. (3) [b] in $NaNO_{3aq}$ and $NaCl_{aq}$ [c] at different ionic strengths and at $t = 25$ °C

I/mol L^{-1}	log K_1^H	log K_2^H	log K_3^H	log K_4^H	log K_5^H	log K_6^H	log K_7^H
0.10	9.48± 0.11 (9.58)	9.98± 0.09 (9.84)	9.53± 0.05 (9.50)	8.20± 0.05 (8.14)	6.49± 0.03 (6.50)	5.17± 0.06 (5.25)	3.02± 0.03 (2.88)
0.25	9.07± 0.08 (9.20)	9.62± 0.08 (9.46)	9.13± 0.04 (9.11)	7.83± 0.04 (7.76)	6.13± 0.02 (6.15)	4.84± 0.05 (4.90)	2.77± 0.02 (2.59)
0.50	8.73± 0.06 (8.93)	9.39± 0.06 (9.19)	8.82± 0.03 (8.83)	7.57± 0.03 (7.48)	5.88± 0.02 (5.88)	4.59± 0.02 (4.65)	2.60± 0.02 (2.37)
0.75	8.52± 0.07 (8.78)	9.28± 0.08 (9.04)	8.64± 0.03 (8.67)	7.43± 0.03 (7.32)	5.75± 0.01 (5.74)	4.46± 0.04 (4.51)	2.54± 0.04 (2.27)
1.00	8.36± 0.11 (8.69)	9.22± 0.11 (8.95)	8.51± 0.04 (8.56)	7.34± 0.04 (7.21)	5.66± 0.01 (5.65)	4.39± 0.07 (4.42)	2.52± 0.06 (2.22)

[a] K_j^H refers to the equilibrium: $H^+ + H_{j-1}Phy^{(12-j+1)} = H_jPhy^{(12-j)-}$; ±standard deviation.
[b] log $K_j^H = \log {}^TK_j^H + C_jI - \log(1 + 10^{D_j}I)$.
[c] Values in parenthesis, from Ref. [1].

Table 3
Empirical parameters of Eq. (3) [a] for the dependence of phytate protonation constants on ionic strength in $NaNO_{3aq}$, at $t = 25$°C

j	log ${}^TK_j^{H\,b}$	C_j^c	D_j^c
1	18.17	−0.13±0.20 (0.13)	9.67±0.12 (9.59)
2	15.32	0.26±0.18 (0.12)	6.37±0.10 (6.50)
3	12.76	−0.02±0.07 (0.06)	4.23±0.05 (4.27)
4	10.05	0.14±0.07 (0.08)	2.86±0.05 (2.91)
5	7.59	0.15±0.04 (0.13)	2.08±0.03 (2.06)
6	6.13	0.19±0.15 (0.14)	1.92±0.09 (1.83)
7	3.52	0.41±0.10 (0.27)	1.39±0.05 (1.56)

[a] log $K_j^H = \log {}^TK_j^H + C_jI - \log(1 + 10^{D_j}I)$.
[b] From Ref. [1].
[c] ±standard deviation, values in parenthesis are referred to $NaCl_{aq}$ (Ref. [1]).

Table 5
Experimental complex formation constants [a] for copper–proton–phytate species in $NaNO_{3aq}$ at different ionic strengths, at $t = 25$ °C

I^b	I^c	log K_{14}		log K_{15}		log K_{25}	
ISE-H^+							
0.134	0.135	–	–	5.04±0.07 [b, d]	5.04 [c]	7.40±0.08 [b, d]	7.38 [c]
0.291	0.294	5.83±0.09 [b, d]	5.81 [c]	3.95±0.06	3.93	6.52±0.06	6.50
0.538	0.549	5.47±0.07	5.46	3.61±0.06	3.60	6.00±0.06	5.97
0.770	0.791	5.35±0.07	5.33	3.38±0.07	3.37	6.58±0.09	6.55
1.019	1.055	5.30±0.11	5.28	3.52±0.11	3.51	6.94±0.12	6.92
ISE-Cu^{2+}							
0.103	0.104	–	–	4.75±0.08	4.75	7.55±0.08	7.53
0.247	0.250	–	–	3.95±0.06	3.95	6.65±0.07	6.63
0.497	0.506	–	–	3.47±0.05	3.46	6.17±0.06	6.14
0.748	0.768	–	–	3.30±0.07	3.29	6.36±0.08	6.33
0.938	0.968	–	–	3.47±0.10	3.46	7.07±0.11	7.05

[a] K_{ij} refers to the equilibrium: $iCu^{2+} + H_jPhy^{(12-j)-} = Cu_iH_jPhy^{(12-2i-j)-}$.
[b] In the molar concentration scale, mol L^{-1}.
[c] In the molal concentration scale, mol kg^{-1} (H_2O).
[d] ±standard deviation.

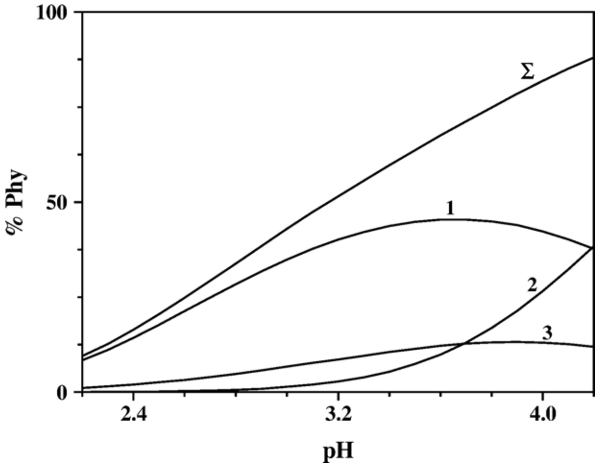

Fig. 1. Distribution diagram of $Cu_iH_jPhy^{(12-2i-j)-}$ species *vs.* pH at I=0.75 mol L^{-1} and t=25 °C. Experimental conditions: C_{Phy}=0.002 mol L^{-1}; C_{Cu}=0.004 mol L^{-1}. Species: 1. $Cu_2H_5Phy^{3-}$; 2. CuH_4Phy^{6-}; 3. CuH_5Phy^{5-}; Σ = percentage of complexed phytate.

during these last measurements (respect to that by ISE-H^+, see Table 1) did not allow the determination of the CuH_4Phy^{6-} species, which is formed at higher pH values than those investigated during ISE-Cu^{2+} measurements (as shown in Fig. 1, where a speciation diagram of phytate in the presence of Cu^{2+} is reported for example). However, the analysis of complex formation constants reported in Table 5 for CuH_5Phy^{5-} and $Cu_2H_5Phy^{3-}$ species at different ionic strengths reveals that both ISE-H^+ and ISE-Cu^{2+} electrodes gave, within the experimental error, analogous values: as already stated in the Introduction, this demonstrate how different techniques, if properly used, yield to the same results.

3.3. Dependence of stability constants of $Cu_iH_jPhy^{(12-2i-j)-}$ species on ionic strength

Complex formation constants reported in Table 5 proved to be fairly dependent on ionic strength. By applying Eq. (4) to stability constants in both molar and molal concentration scales we were able to calculate corresponding infinite dilution values, reported in Table 6 together with refined C and Δε parameters for each set of constants. According to Eq. (7), Δε in Table 6 correspond to the algebraic sum of all SIT interaction coefficients involved in the formation reaction of the $Cu_iH_jPhy^{(12-2i-j)-}$ species and, specifically, to

$$\begin{aligned}\Delta\varepsilon[\mathrm{Cu_iH_jPhy}^{(12-2i-j)-}] = {} & i\varepsilon(\mathrm{Cu}^{2+},\mathrm{NO}_3^-)\\ & + \varepsilon[\mathrm{H}_j\mathrm{Phy}^{(12-j)-},\mathrm{Na}^+]\\ & - \varepsilon[\mathrm{Cu}_i\mathrm{H}_j\mathrm{Phy}^{(12-2i-j)-},\mathrm{Na}^+]. \qquad (8)\end{aligned}$$

Table 6
Empirical parameters of Eq. (4)[a] for the dependence of complex formation constants of $Cu_iH_jPhy^{(12-2i-j)-}$ species[b] on ionic strength in the molar and molal concentration scales in $NaNO_{3aq}$, at t=25°C

Species	z^*	$\log {}^TK_{ij}$ [b]	C [c]	m.d. [d]	Δε [e]	m.d. [d]
$CuPhyH_4^{6-}$	32	10.28±0.14 [f]	1.58±0.20 [f]	0.08	1.56±0.20 [f]	0.09
$CuPhyH_5^{5-}$	28	7.79±0.09	1.36±0.16	0.22	1.35±0.16	0.22
$Cu_2PhyH_5^{3-}$	48	12.53±0.09	4.20±0.17	0.22	4.13±0.17	0.23

[a] $\log K_{ij}=\log {}^TK_{ij}+DH+CI$.
[b] K_{ij} refers to the equilibrium: $iCu^{2+}+H_jPhy^{(12-j)-}=Cu_iH_jPhy^{(12-2i-j)-}$.
[c] To be used for stability constants expressed in the molar concentration scale.
[d] Mean deviation of fit.
[e] To be used for stability constants expressed in the molal concentration scale.
[f] ±standard deviation.

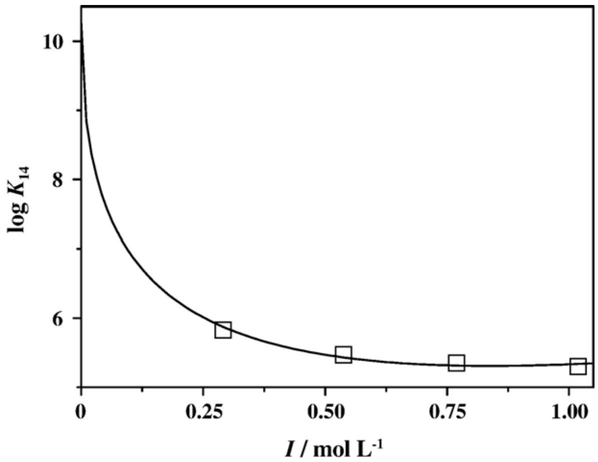

Fig. 2. Dependence of complex formation constants for CuH_4Phy^{6-} species on ionic strength (in mol L^{-1}), in $NaNO_{3aq}$ and at t=25 °C.

The goodness of fits can be easily appreciated by looking at Figs. 2–4, where log K_{ij} values of $Cu_iH_jPhy^{(12-2i-j)-}$ species are plotted *vs.* ionic strength in the molar scale. These figures also show the very good agreement between values obtained by ISE-H^+ and ISE-Cu^{2+}. The application of Eq. (4) with parameters reported in Table 6 allows us to calculate, with fairly good accuracy, complex formation constants at other ionic strength values than those experimentally investigated: some of these values are reported in Table 7.

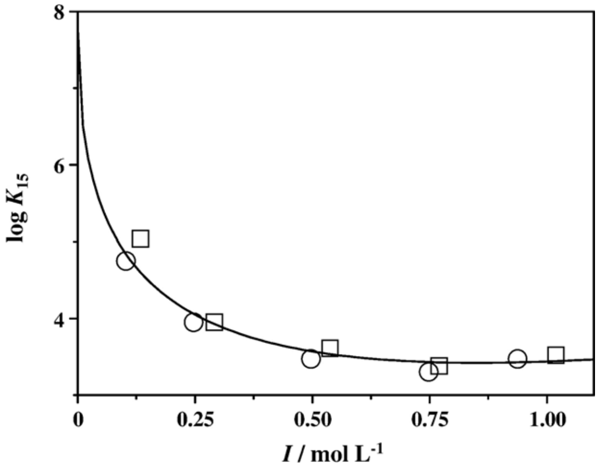

Fig. 3. Dependence of complex formation constants for CuH_5Phy^{5-} species on ionic strength (in mol L^{-1}), in $NaNO_{3aq}$ and at t=25 °C. Squares: values obtained by ISE-H^+ measurements. Circles: values obtained by ISE-Cu^{2+} measurements.

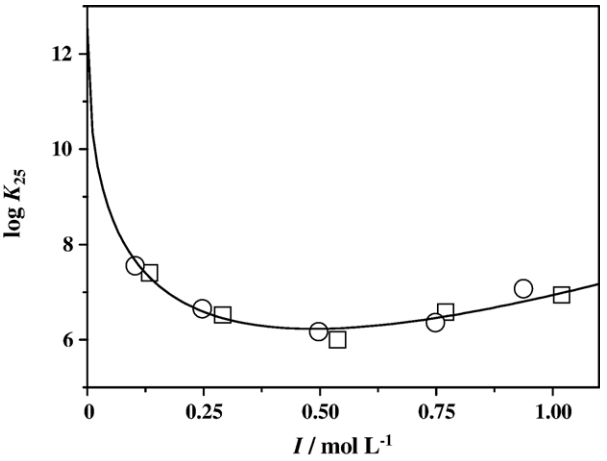

Fig. 4. Dependence of complex formation constants for $Cu_2H_5Phy^{3-}$ species on ionic strength (in mol L^{-1}), in $NaNO_{3aq}$ and at t=25 °C. Squares: values obtained by ISE-H^+ measurements. Circles: values obtained by ISE-Cu^{2+} measurements.

3.4. Sequestering ability of phytate toward copper(II)

One of the most important features of phytate ion is its fairly strong binding ability toward several metal and organometal cations, with the formation of very stable complexes and/or scarcely soluble species. Of course, this characteristic modifies the speciation and the bioavailability of these cations in the presence of phytate and, for direct applications, these changes must be accurately quantified. For this reason, we already proposed the use of a Boltzman type equation for the determination of the sequestering ability of phytate (and other ligands) toward a given cation, once the experimental conditions are fixed [9]. By using any computer program able to calculate free concentrations of various species in solution (*i.e.*, speciation diagrams obtained by, *e.g.*, ES4ECI), the percentage of cation not complexed by a ligand (phytate in our case) can be plotted as a function of total ligand concentration (as $-\log C_L$) at a fixed pH value, ionic strength, temperature and total metal concentration. Curve so obtained can be fitted to the equation

$$y = \frac{A1 - A2}{1 + e^{(x - x0)/S}} + A2 \tag{9}$$

where y represents the total percentage of not-complexed metal (Cu^{2+} in our case). By considering A_1=0 and A_2=100 (the free cation percentage at minimum and maximum $-\log C_L$ values, respectively) refined x_0 represents the value of $-\log C_L$ at 50% of not-complexed cation, while S is the curve slope in that point. X_0 value refined in this way, which we defined for opportunity as pL_{50}, is a very useful parameter because it can give an objective representation of the binding ability of a ligand (L) toward a given cation, once the experimental conditions are fixed. For example, for a given ligand, different authors may report different speciation schemes: especially for very complex systems, this is very frequent and it does not always mean that one of them is not correct, but it may be due to several reasons. In this case, calculation of pL_{50} values may overcome this difficulty, making comparisons easier and faster than other methods commonly used to express the binding ability. The same considerations can also be made to compare the binding ability of different ligands: obstacles related to the different nature and number of species formed may be surmounted by the determination of pL_{50}. Between these other methods, widely used are those where the average number ($\overline{n}$) of metal ions bound by a ligand are expressed as a function of pH or pM (=−log [M], M = metal cation). In the case of copper sequestration by phytate, as results one may obtain curves like that reported in Fig. 5, where $\overline{n}$ is plotted *vs.* pCu at I=0.10 mol L^{-1}, at t=25 °C, pH=5.5 and $C_{Phy}=10^{-3}$ mol L^{-1}. $\overline{n}$ is given by the ratio between the sum of concentrations of copper species (each multiplied by the number of copper ions forming the complexes) and the total phytate concentration, namely the average number of copper ions bound to phytate

$$\overline{n} = \frac{\sum_i i[Cu_iH_jPhy^{(12-2i-j)-}]}{C_{Phy}}. \tag{10}$$

At constant pH, Eq. (10) becomes

$$\overline{n} = \frac{\sum_i i\beta_i'[Cu^{2+}]^i}{1 + \sum_i \beta_i'[Cu^{2+}]^i} \tag{11}$$

where β_i' are conditional complex formation constants that, in the experimental conditions of diagram in Fig. 5, are β_1'=5.87 and β_2'=7.50. However, with respect to these methods, sequestration curves built for the calculation of pL_{50} allow to rapidly evaluate the minimal total ligand concentration able to bind a significant amount of cation. For example, in Fig. 6 the copper(II) sequestration curve in the presence of phytate is reported in Phy/Cu(II) system at the same temperature, pH, medium and ionic strength conditions as in Fig. 5, and at a total concentration of Cu^{2+}, $C_{Cu}=10^{-6}$ mol L^{-1}. In these conditions, pL_{50}=5.73, indicating a quite good sequestering ability of phytate toward copper(II): in fact, this value means that a total ligand concentration of $C_{Phy}=1.8\ 10^{-6}$ mol L^{-1} is already sufficient to bind 50% of Cu^{2+} in solution. Practically, this figure describes better than Fig. 5 how phytate behave toward copper(II): its rapid analysis also suggests the possibility of using phytate as sequestering agent for copper(II) and supports future studies both on the use of plants that naturally synthesize it for phytoremediation purposes or on its direct application in remediation techniques, especially if one takes into account that this ligand is non-toxic and naturally bio-synthesized often in large amounts by many vegetal species (*e.g.*, cereals, legumes potatoes, *etc.*) [11–14]. However, at the end of this discussion on the use of different models for the interpretation of the sequestering ability of a ligand toward a cation, it is important to stress that, whatever the method is, reliable data are essential, and this paper provides all elements necessary for the calculation of all those parameters required for the application of several different models to the determination of phytate sequestering ability toward copper(II) (from protonation and

Table 7
Complex formation constants for copper–proton–phytate species in $NaNO_{3aq}$ at different ionic strengths, at t=25°C, calculated by Eq. (4)[a]

I[b]	$\log K_{14}$[c]		$\log K_{15}$[c]		$\log K_{25}$[c]	
0.10	6.94±0.12[d]	6.93±0.12[e]	4.87±0.08[d]	4.87±0.07[e]	7.70±0.08[d]	7.69±0.08[e]
0.15	6.51±0.11	6.51±0.11	4.50±0.07	4.50±0.07	7.16±0.07	7.15±0.07
0.25	6.01±0.10	6.01±0.09	4.05±0.06	4.05±0.06	6.59±0.07	6.57±0.07
0.50	5.47±0.07	5.46±0.07	3.57±0.05	3.57±0.05	6.23±0.06	6.19±0.06
0.75	5.31±0.07	5.30±0.07	3.43±0.07	3.42±0.07	6.46±0.08	6.40±0.08
1.00	5.33±0.10	5.31±0.10	3.44±0.10	3.43±0.10	6.94±0.12	6.86±0.12

[a] $\log K_{ij} = \log {}^{T}K_{ij} + DH + CI$.
[b] In both molar mol L^{-1}, or molal mol kg^{-1} (H_2O) concentration scales.
[c] K_{ij} refers to the equilibrium: $i Cu^{2+} + H_j Phy^{(12-j)-} = Cu_i H_j Phy^{(12-2i-j)-}$, ±standard deviation.
[d] In the molar concentration scale, mol L^{-1}.
[e] In the molal concentration scale, mol kg^{-1} (H_2O).

complex formation constants one can calculate free concentration of ions, formation percentages of species, $\bar{n}$, pL_{50} and others).

Finally, concerning diagrams in Figs. 5, 6, it is also worth to mention the fact that speciation of Phy/Cu(II) system at pH >4.5 was not experimentally investigated in the present work. However, the low total metal concentration let us suppose that, in the conditions of the sequestration diagram, pH=5.5 or higher can be experimentally achieved without precipitation phenomena. Nevertheless, increasing pH values may yield to the formation of other less-protonated $Cu_i H_j Phy^{(12-2i-j)-}$ species than those experimentally determined: this aspect was taken into account in the calculation of free copper(II) concentrations, including in the speciation model the formation of the triprotonated CuH_3Phy^{7-} species. Concerning its stability, we hypothesized $\log {}^{T}K_{13} \sim 12.8$ and $\log K_{13} \sim 9.0$ at I=0.1 mol L^{-1}. These values come from a linear extrapolation of $\log K_{14}$ and $\log K_{15}$ values at infinite dilution and at different ionic strengths, justified by the fact that other cation/phytate systems already evidenced several regularities in the stability of their complexes, allowing the formulation of many predictive equations that correlate this stability to the ligand protonation step and/or to the number of metal cations bound to phytate (see, *e.g.*, Refs. [2,6,7,9]). However, this rough extrapolation did not significantly alter results shown in Figs. 5 and 6 because in the most critical conditions of the diagrams, changes of ~1 log units in the stability of CuH_3Phy^{7-} species do not sensibly affect phytate sequestering ability. At the same time, the presence in the model of this species better describes phytate and copper(II) speciation in that experimental conditions and cannot be neglected.

3.5. Literature comparisons

In our previous contributions [1–10] we stressed several times that, in spite of the wide number of papers dealing with metal–phytate interactions (with particular emphasis given to the bioavailability of the metal in the presence of the ligand) that can be found in literature, few papers report thermodynamic parameters (such as stability constants) of metal/phytate complexes. In this specific case, to our knowledge and at present time, just six papers deal directly with copper(II)–phytate system [27–32] (two further papers are concerned with "interactions" [33] between phytate and copper and "binding differences" of phytate toward copper(II) [34], but only from a

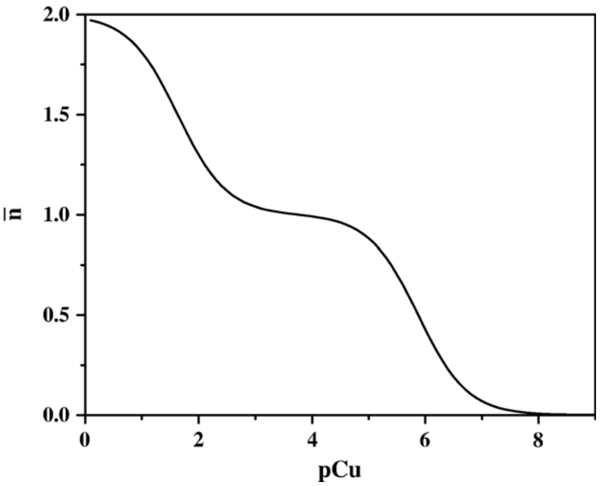

Fig. 5. Average number of copper(II) ion bound to phytate as a function of free copper(II) concentration (as $pCu = -\log[Cu^{2+}]$) in Phy/Cu^{2+} system at I=0.10 mol L^{-1} ionic strength in $NaNO_{3aq}$ at t=25 °C. Conditions: pH=5.5, total phytate concentration, C_{Phy}=0.001 mol L^{-1}.

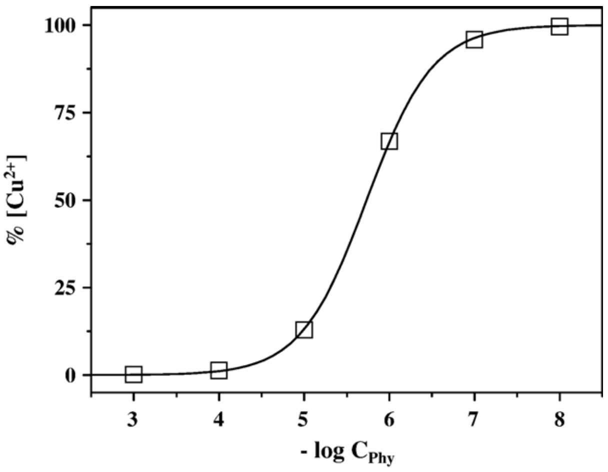

Fig. 6. Copper(II) sequestration diagram in the presence of phytate. Percentage of copper(II) not complexed by phytate as a function of total ligand concentration (as $-\log C_{Phy}$) in Phy/Cu^{2+} system at I=0.10 mol L^{-1} ionic strength in $NaNO_{3aq}$ at t=25 °C. Conditions: pH=5.5, total concentration of copper(II), $C_{Cu}=10^{-6}$ mol L^{-1}.

qualitative and medical point of view). In two of them, by Martin and Evans [27,28], authors evaluated the binding ability of phytate toward copper(II) by potentiometric, calorimetric and spectrophotometric studies at $t=25$ °C in $\mathrm{KNO_3}$ 0.2 mol $\mathrm{L^{-1}}$ aqueous solution. In these papers the authors infer about the amount of metal bound to the ligand as a function of pH and of metal to ligand ratio and make hypothesis about the structure of complexes, but, unfortunately, they do not report any thermodynamic parameter for the formation of copper(II)–phytate complexes, except for an "apparent heat of binding and precipitation" of $\mathrm{Cu^{2+}}$ to phytate of ~22 kcal $\mathrm{mol^{-1}}$, indicating that complexation reaction is endothermic in nature.

With the aim of studying the solubility of some metal ions under pH conditions that may be encountered in the duodenum, Persson et al. [29] studied the interaction between $\mathrm{Cu^{2+}}$ (and $\mathrm{Zn^{2+}}$ and $\mathrm{Cd^{2+}}$) and phytate (and other isolated fractions of inositol phosphates with 6, 5, 4 and 3 phosphate groups) by potentiometry, at $t=25$ °C and in $\mathrm{NaClO_4+NaCl}$ at $I=0.1$ mol $\mathrm{L^{-1}}$ (NaCl = 10 mmol $\mathrm{L^{-1}}$ + $\mathrm{NaClO_4}$ = 90 mmol $\mathrm{L^{-1}}$), by considering total metal and ligand concentrations to be constant ($C_{\mathrm{Cu}}=$ 3.22 mmol $\mathrm{L^{-1}}$ and $C_{\mathrm{Phy}}=10.0$ mmol $\mathrm{L^{-1}}$) and varying pH ($3 \leq \mathrm{pH} \leq 7$). These authors do not report any thermodynamic data, but some important consideration can be made. In the above conditions, they show graphically that phytate binds ~50% of total copper(II) at pH ~5.5. This is in agreement with what we can calculate from our $\mathrm{pL_{50}}$ value at $I=0.1$ mol $\mathrm{L^{-1}}$ and pH = 5.5 (Fig. 5): $\mathrm{pL_{50}} \sim 5.7$, indicating that 50% of copper(II) is bound to phytate when its total concentration is $C_{\mathrm{Phy}} \sim 2 \cdot 10^{-6}$ mol $\mathrm{L^{-1}}$, corresponding to a ligand to metal ratio of L:M ~ 2:1, *i.e.* in very similar ratios to those utilized in Ref. [29] (L/M ~ 3:1). However, we have some problems in comparing our results to those by Persson et al., because the real conditions of ionic strength and ligand to metal ratio are not uniformly defined (differences between data reported in figures and those along the text).

Quantitative data on phytate–copper(II) complexes were reported for the first time by Bebot-Brigaud et al. [30], using ^{31}P-NMR spectroscopy, potentiometry and spectrophotometry, in tetraethylammonium perchlorate ($I=0.17$ mol $\mathrm{L^{-1}}$) at $t=20$ °C. Before this paper, as also stated by the same authors, there was "no published work concerning the nature and stability of the species appearing in aqueous solution, and the sites of phytic acid which are involved in complex formation". Unfortunately, the particular experimental conditions of temperature and ionic medium adopted by these authors make comparisons fairly difficult to do. Further problems are also represented by the fact that authors considered just the formation of 1:1 species, owing to the fact that they worked with metal to ligand ratios that favor the second one, avoiding the formation of polynuclear species and favoring the investigation of wider pH ranges with the absence of precipitation phenomena, whilst the opposite choice was made in the present study, where we worked with a metal excess. However, as stated in previous paragraph, a comparison among $\mathrm{pL_{50}}$ value calculated by the protonation and stability constants by Bebot–Brigaud et al. and that calculated by our dataset in the most similar conditions may be performed and could be very helpful. From their data, at pH = 5.5 and $C_{\mathrm{Cu}}=10^{-6}$ mol $\mathrm{L^{-1}}$, $\mathrm{pL_{50}}=6.35$, whilst we obtained $\mathrm{pL_{50}}=5.55$ at $I=0.15$ mol $\mathrm{L^{-1}}$ from our stability constants in $\mathrm{NaNO_{3aq}}$. This difference, *i.e.* $\Delta\mathrm{pL_{50}}=0.8$, should be ascribed to sodium complexation. In fact, at fixed experimental conditions (pH, ionic strength, and temperature), difference between $\mathrm{pL_{50}}$ values calculated by complex formation constants in one ionic medium considered as scarcely interacting with a ligand (*e.g.*, $\mathrm{Cu}_i\mathrm{H}_j\mathrm{Phy}^{(12-2i-j)-}$ complexes in tetraalkylammonium salts) and in an interacting medium (*e.g.*, $\mathrm{Cu}_i\mathrm{H}_j\mathrm{Phy}^{(12-2i-j)-}$ complexes in alkali metal cation salts), respectively, should be equal to $\mathrm{pL_{50}}$ value determined for the sequestration of cations of interacting medium by the ligand (*e.g.*, $\mathrm{Na}_i\mathrm{H}_j\mathrm{Phy}^{(12-i-j)-}$ complexes in tetraethylammonium media [2]). By this procedure, calculations performed at pH = 5.5 and $I=0.15$ mol $\mathrm{L^{-1}}$by sodium complex formation constants already determined [2] gave a calculated $\mathrm{pL_{50}}$ of ~1.1 units, which is in fairly good agreement with $\Delta\mathrm{pL_{50}}$: residual differences may be ascribed to the slightly different temperature.

Concerning the last two, and most recent works on copper (II)/phytate complexes [31,32], the experimental conditions (of temperature, medium and ionic strength) make comparisons not significant (in the paper by Vasca et al. [31], authors worked in $\mathrm{NaClO_4}$ at $I=3$ mol $\mathrm{L^{-1}}$ and $t=25$ °C, while we reached a maximum of $I=1$ mol $\mathrm{L^{-1}}$; Torres et al. [32] worked at $t=37$ °C).

Acknowledgements

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References

[1] C. De Stefano, D. Milea, S. Sammartano, Speciation of phytate ion in aqueous solution. Protonation constants in tetraethylammonium iodide and sodium chloride, J. Chem. Eng. Data 48 (2003) 114–119.

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

[3] C. De Stefano, O. Giuffrè, D. Milea, C. Rigano, S. Sammartano, Speciation of phytate ion in aqueous solution. Non covalent interactions with biogenic polyamines, Chem. Speciat. Bioavailab. 15 (2002) 29–36.

[4] F. Crea, P. Crea, A. De Robertis, S. Sammartano, Speciation of phytate ion in aqueous solution. Characterisation of Ca–phytate sparingly soluble species, Chem. Speciat. Bioavailab. 16 (2004) 53–60.

[5] C. De Stefano, D. Milea, S. Sammartano, Speciation of phytate ion in aqueous solution. Thermodynamic parameters for protonation in NaCl, Thermochim. Acta 423 (2004) 63–69.

[6] C. De Stefano, D. Milea, S. Sammartano, Speciation of phytate ion in aqueous solution. Dimethyltin(IV) interactions in NaClaq at different ionic strengths, Biophys. Chemist. 116 (2005) 111–120.

[7] C. De Stefano, D. Milea, N. Porcino, S. Sammartano, Speciation of phytate ion in aqueous solution. Sequestering ability towards mercury(II) cation in NaClaq at different ionic strengths, J. Agric. Food Chem. 54 (2006) 459–1466.

[8] P. Crea, A. De Robertis, C. De Stefano, S. Sammartano, Speciation of phytate ion in aqueous solution. Sequestration of magnesium and calcium by phytate at different temperatures and ionic strengths, in NaClaq, Biophys. Chemist. 124 (2006) 18–26.

[9] C. De Stefano, D. Milea, N. Porcino, S. Sammartano, Speciation of phytate ion in aqueous solution. Cadmium(II) interactions in NaClaq at different ionic strengths, Anal. Bioanal. Chem. 386 (2006) 346–356.

[10] F. Crea, P. Crea, D. Milea, N. Porcino, S. Sammartano, Speciation of phytate ion in aqueous solution. Trimethyltin(IV) interactions in self medium, Ann. Chim. (in press) (Rome).

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

[12] B.F. Harland, E.R. Morris, Phytate: a good or a bad food component, Nutr. Res. 15 (1995) 733–754.

[13] B.F. Harland, G. Narula, Food phytate and its hydrolysis products, Nutr. Res. 19 (1999) 947–961.

[14] L. Oatway, T. Vasanthan, J.H. Helm, Phytic acid, Food Rev. Int. 17 (2001) 419–431.

[15] V. Raboy, myo-Inositol-1,2,3,4,5,6-hexakisphosphate, Phytochemistry 64 (2003) 1033–1043.

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

[17] H. Scheinberg, in: E. Merian, Copper (Eds.), Metals and Their Compounds in the Environment: Occurrence, Analysis, and Biological Relevance, VCH, Weinheim, Germany, 1991, pp. 893–908.

[18] C. De Stefano, P. Princi, C. Rigano, S. Sammartano, Computer analysis of equilibrium data in solution. ESAB2M: an improved version of the ESAB program, Ann. Chim (Rome) 77 (1987) 643–675.

[19] C. De Stefano, P. Mineo, C. Rigano, S. Sammartano, Ionic strength dependence of formation constants. XVII. The calculation of equilibrium concentrations and formation constants, Ann. Chim. (Rome) 83 (1993) 243–277.

[20] C. De Stefano, C. Foti, O. Giuffrè, P. Mineo, C. Rigano, S. Sammartano, Binding of tripolyphosphate by aliphatic amines: formation, stability and calculation problems, Ann. Chim. (Rome) 86 (1996) 257–280.

[21] C. De Stefano, S. Sammartano, P. Mineo, C. Rigano, Marine chemistry — an environmental analytical chemistry approach, in: A. Gianguzza, E. Pelizzetti, S. Sammartano (Eds.), Computer Tools for the Speciation of Natural Fluids, Kluwer Academic Publishers, Amsterdam, 1997, pp. 71–83.

[22] G. Biederman, in: Dahlem Workshop on the Nature of Seawater, ed. Ionic Media, (Dahlem Konferenzen, Berlin, 1975) pp.339–362.

[23] G. Biederman, in: E.A. Jenne, E. Rizzarelli, V. Romano, S. Sammartano (Eds.), Metal Complexes in Solution, Introduction to the specific interaction theory with emphasis on chemical equilibria, Piccin, Padua, Italy, 1986, pp. 303–314.

[24] I. Grenthe, I. Puigdomenech, Modelling in Aquatic Chemistry, OECD, Paris, 1997.

[25] A. De Robertis, C. De Stefano, C. Foti, G. Signorino, Thermodynamic parameters for the formation of dimeric hydrolytic species of copper (II) in aqueous NaClO4 solution at different ionic strengths, Talanta 44 (1997) 1839–1846.

[26] F. Crea, D. Milea, S. Sammartano, Enhancement of hydrolysis through the formation of mixed hetero-metal species, Talanta 65 (2005) 229–238.

[27] C.J. Martin, W.J. Evans, Phytic acid: divalent cation interactions. III. A calorimetric and titrimetric study of the pH dependence of copper(II) binding, J. Inorg. Biochem. 28 (1986) 39–55.

[28] C.J. Martin, W.J. Evans, Phytic acid: divalent cation interactions. IV. A spectroscopic determination of Co(II) and Cu(II) binding, J. Inorg. Biochem. 29 (1987) 241–248.

[29] H. Persson, M. Türk, M. Nyman, A.S. Sandberg, Binding of Cu^{2+}, Zn^{2+}, and Cd^{2+} to inositol tri-, tetra-, penta-, and hexaphosphates, J. Agric. Food Chem. 46 (1998) 3194–3200.

[30] A. Bebot-Brigaud, C. Dange, N. Fauconnier, C. Gérard, ^{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. 75 (1999) 71–78.

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

[32] J. Torres, S. Dominguez, M.F. Cerda, G. Obal, A. Mederos, R.F. Irvine, A. Diaz, C. Kremer, 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. 99 (2005) 828–840.

[33] E.T. Champagne, M.S. Fisher, Binding differences of Zn(II) and Copper (II) ions with phytate, J. Inorg. Biochem. 38 (1990) 217–223.

[34] B. Lonnerdal, Phytic acid–trace element (Zn, Cu, Mn) interactions, Int. J. Food Sci. Technol. 37 (2002) 749–758.