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Review

Formation and stability of phytate complexes in solution

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Contents

Abstract

1,2,3,4,5,6 hexakis (di-hydrogen phosphate) myo-inositol, best known as phytic acid, is a very important molecule from a biological, environmental and technological point of view. For a thorough understanding of phytate properties and the mechanisms involving this ligand, a careful study of its acid–base behavior and of the formation and stability of its complexes in solution is necessary. Unfortunately, regarding the thermodynamic data on phytate complexes in solution, some are lacking, while some others exhibit large discrepancies between different authors. This motivated a detailed evaluation of the literature on this topic, aimed at identifying the most accurate data on phytate coordination chemistry in solution. This review presents the results of this, reporting and analyzing the most significant thermodynamic parameters published for both phytate protonation and complex formation with several metal and organometal cations, as well as polyammonium ligands.

Keywords: Phytate; Protonation; Metal complexes; Organometal complexes; Polyammonium complexes; Thermodynamic parameters

1. Introduction

1,2,3,4,5,6 hexakis (di-hydrogen phosphate) myo-inositol (Fig. 1), best known as phytic acid, was first identified in 1855 [1]. Phytates, its salts (the completely deprotonated form is denoted in this work by Phy^{12-}), are widely present in nature (plants, animals and soils [2]), mainly as calcium, magnesium

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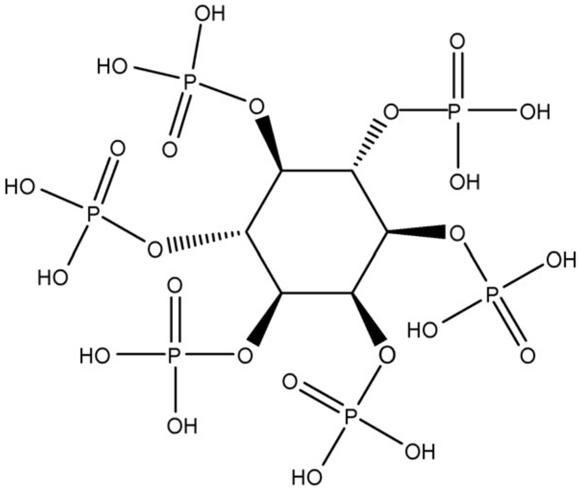

Fig. 1. Basic structure of phytic acid.

and potassium mixed salts (also called phytines) [3]. The existence of this compound in seeds was first reported in 1903 [4] and it is now accepted as being ubiquitous among plant seeds and grains, comprising 0.5–5% (w/w) [2]. On the other hand, although the first report that phytate is present in animal cells appeared in 1941 (which turned out to be a case of mistaken identity) [5], it only became clear in the late 1980s and 1990s that it is also always present in eukaryotic species (typically being the most abundant inositol phosphate in cells) [6]. As a result of this widespread occurrence in nature, its important biological activity and the high number of applications, over the last century it has been the subject of investigation for many scientists in different fields, amply demonstrated by the huge number of papers, reviews and books that have been published (see, for example, references in some recent and past books and reviews [1–3,5–12]). New functions, properties and applications of phytate are being regularly discovered and reported in literature (see, e.g., some papers published in 2007 [13–17]). Here it suffices to say that it modifies the bioavailability of several metal ions (such as calcium, iron, zinc, copper: see, e.g. [18–22] and references therein); it is regarded as the primary storage form of both phosphate and inositol in plant seeds and grains (see, also [23,24] and references therein); it has been reported to be an antioxidant (see also [8,25–29]); it shows marked anticarcinogenic/antineoplastic properties (e.g. [28,30–35]); it may reduce and prevent kidney stone formation [36,37] and it plays key roles in a number of crucial physiological activities, other than in the treatment of different pathologies (thoroughly reviewed, e.g., in Refs. [2,5,6,11]). Among its industrial and/or technological applications (some of them reviewed in [1]), of particular note is the use of phytates in environmental remediation problems, such as the immobilization and in situ treatment of soils contaminated by many metals (including heavy metals and radionuclides, see, e.g. [38–45]).

Most of these phytate properties and applications are significantly influenced by its particular behavior in solution (primarily aqueous media, such as biological fluids, natural and waste waters, “soil solutions”), where it strongly interacts with many metal and non-metal ions, proteins and starch, mainly by electrostatic interactions [1,2,5,11]. Moreover, the peculiar characteristics of its structure allow this ligand to assume two different conformations. While the hexaorthophosphate structure of phytic acid proposed by Anderson [46] has been accepted since the late 1960s [47], many studies have been carried out during the last decades to understand better which phytate conformational states predominate in solution (e.g. [4,48–52]). It transpires that, depending on the experimental conditions, this ligand may exist in solution either in the so-called equatorial conformation in which one phosphate group is oriented in the axial position and five are in the equatorial (1-ax/5-eq), or in the inverted axial conformation (5-ax/1-eq). Various techniques, including potentiometry [49], Raman spectroscopy [48], ^{1}H NMR [49–52], ^{13}C NMR [48,49] and ^{31}P NMR spectroscopy [4,48,49,52], and molecular modeling [51] have been used to probe phytate conformational preferences. These works have demonstrated that the conformation in aqueous solutions depends on many parameters, such as pH, ionic strength, ionic medium (mainly due to the nature of the cation in the supporting electrolyte). According to one of the most recent of these papers [51], in which the effect of alkali metal ions on the conformation adopted by phytic acid was also investigated, this ligand exists at $\mathrm{pH} < 9$ in the sterically unhindered 1ax/5eq form, and at $\mathrm{pH} > 9.5$ in the sterically hindered 5ax/1eq form. Moreover, these authors showed that at pH 5.0, phytic acid adopts the 1ax/5eq form regardless of the alkali metal cation present as counter ion. On the contrary, at pH 11, the exclusive presence of the 5ax/1eq form was observed with Na^+, K^+, Rb^+, Cs^+, whilst with Li^+ ions the two conformations are simultaneously present.

For all the above-cited reasons, to gain a thorough understanding of phytate properties and the mechanisms involving this ligand, a careful study of its acid base behavior and of the formation and stability of its complexes in solution is necessary. After some years of studies on this direction, we realized that, regarding the thermodynamic data on phytate complexes in solution, some are lacking, while some others exhibit large discrepancies between different authors. This motivated a detailed evaluation of the literature on this topic, aimed at identifying the most accurate data on phytate coordination chemistry in solution. This review presents the results of this, reporting and analyzing the most significant thermodynamic parameters published for both phytate protonation and complex formation with several metal and organometal cations (some protonation and complex formation constants are reported in Tables 1 and 2, respectively), as well as other organic ligands. Potentiometry and multinuclear NMR have been the main instrumental techniques used for the determination of stability constants, as recommended by IUPAC [53], while calorimetry has been applied in the determination of protonation and complex formation enthalpies.

2. Acid–base properties

Compared to the large number of papers published on phytate properties and applications, relatively few report quantitative data on the acid–base behavior of this ligand [4,18,22,48,49,54–68], despite being well known that acid–base

Table 1
Selected phytate protonation constants[a] in different aqueous media and ionic strengths, at $t = 25\,^{\circ}\mathrm{C}$

I[b]	Medium	$\log K_1$[c]	$\log K_2$[c]	$\log K_3$	$\log K_4$	$\log K_5$	$\log K_6$	$\log K_7$	$\log K_8$	$\log K_9$	$\log K_{10}$	$\log K_{11}$	$\log K_{12}$	Reference
0.10	$(C_2H_5)_4NI$	*16.70*	*14.40*	12.21	9.92	7.53	6.11	3.53						[63]
0.10	$(n\text{-}C_4H_9)_4NBr$	>12	>12	>12	11.50	7.97	6.41	3.93	2.73	2.00	<1.50	<1.50	<1.50	[57]
0.10	LiCl	9.71	9.46	8.63	7.60	6.27	5.00	2.63						[64]
0.10	$NaNO_3$	9.48	9.98	9.53	8.20	6.49	5.17	3.02						[67]
0.10	NaCl	9.58	9.84	9.50	8.14	6.50	5.25	2.88						[63]
0.10	KCl	10.24	9.50	9.93	8.37	6.62	5.35	2.93						[64]
0.10	CsCl	10.42	10.33	10.11	8.62	6.53	5.16	3.18						[68]
0.15	$(CH_3)_4NCl$	10.8	10.5	10.33	8.79	6.90	5.72	3.10	1.9	1.9	–	–	–	[22]
0.15	$NaClO_4$	8.59	10.53	9.02	7.82	6.13	4.88	2.49	1.98	–	–	–	–	[60]
0.17	$(C_2H_5)_4NClO_4$	>13	>13	12.3	9.92	7.42	6.13	3.59	2.0	2.4	1.0	<1	<1	[18]
0.20	KCl	9.53	9.53	9.19	7.98	6.25	5.20	3.16	2.38	2.38	1.92	1.92	1.92	[56]
0.50	$(C_2H_5)_4NI$	*14.91*	*13.29*	11.55	9.79	7.50	6.12	3.61						[63]
0.50	LiCl	9.06	8.81	7.96	6.93	5.63	4.39	2.08						[64]
0.50	$NaNO_3$	8.73	9.39	8.82	7.57	5.88	4.59	2.60						[67]
0.50	NaCl	8.93	9.19	8.83	7.48	5.88	4.65	2.37						[63]
0.50	KCl	9.59	8.85	9.26	7.71	6.01	4.77	2.43						[64]
0.50	CsCl	9.79	9.54	9.51	7.93	5.78	4.51	2.49						[68]
1.00	$(C_2H_5)_4NI$	*13.60*	*12.48*	11.06	9.71	7.50	6.16	3.72						[63]
1.00	LiCl	8.83	8.57	7.69	6.67	5.40	4.15	1.92						[64]
1.00	$NaNO_3$	8.36	9.22	8.51	7.34	5.66	4.39	2.52						[67]
1.00	NaCl	8.69	8.95	8.56	7.21	5.65	4.42	2.22						[63]
1.00	$NaClO_4$	8.41	9.19	8.29	7.03	5.38	4.14	1.77	1.80	–	–	–	–	[60]
1.00	KCl	9.35	8.61	8.99	7.45	5.77	4.54	2.28						[64]
1.00	CsCl	9.82	9.38	9.41	7.77	5.57	4.34	2.33						[68]
3.00	LiCl	8.60	8.34	7.34	6.35	5.18	3.95	2.00						[64]
3.00	NaCl	8.47	8.71	8.21	6.89	5.43	4.22	2.30						[63]
3.00	$NaClO_4$	8.29	8.62	8.01	6.61	5.07	3.86	1.52	1.63	–	–	–	–	[60]
3.00	KCl	9.13	8.38	8.64	7.13	5.56	4.34	2.36						[64]
5.00	NaCl	8.50	8.74	8.12	6.83	5.47	4.27	2.63						[63]

[a] $\log K_i$ refers to equilibrium: $H^+ + H_{i-1}Phy^{(12-i+1)-} = H_iPhy^{(12-i)-}$.

[b] In $\mathrm{mol\,L^{-1}}$.

[c] Predicted values in italics.

Table 2
Selected stability constants[a,b] of some phytate metal complexes

Cation	I[c]	Medium	t (°C)	log K_{13}	log K_{14}	log K_{15}	log K_{16}	Other i:j species	Reference
Mg^{2+}	0		10	7.93	6.49	5.47		2:3, 2:4, 2:5, 3:2, 3:3, 3:4, 3:5	[88]
	0		25	7.82	6.66	6.03			[88]
	0.15	$NaClO_4$	37	10.5	9.76	8.76	7.25	1:2	[22]
Ca^{2+}	0		10	7.67	6.34	5.31		2:3, 2:4, 2:5, 3:2, 3:3, 3:4, 3:5	[88]
	0		25	7.64	5.82	5.41			[88]
	0.15	$NaClO_4$	37		8.3	8.4	7.4		[22]
Cd^{2+}	0.15	NaCl	25		5.25	4.71	4.42	1:7, 2:4, 2:5, 2:6, 2:7, 3:4	[104]
	0.15	$NaClO_4$	37	9.7	8.76	7.53	6.92	1:2	[22]
Cu^{2+}	0		25		10.28	7.79		2:5	[67]
	0.15	$NaClO_4$	37	13.5	12.15	9.07	5.73		[22]
Zn^{2+}	0.15	$NaClO_4$	37	11.3	10.3	8.54	6.94		[22]
Ni^{2+}	0.15	$NaClO_4$	37		8.78	8.44	7.20		[22]
	0.10	KCl	36	7.27	5.96	5.18	5.05	1:0, 1:1, 1:2, 1:7	[66]
Co^{2+}	0.15	$NaClO_4$	37	9.1	7.90	6.96	6.26	1:2, 1:7	[22]
Hg^{2+}	0.15	NaCl	25	15.64	15.94	16.26	16.45	1:0, 1:1, 1:2, 1:7, 2:0, 2:1, 2:2	[111]
	1.0	NaCl	25	14.74	15.17	15.54	15.70		[111]
Mn^{2+}	0.15	$NaClO_4$	37		8.78	8.44	7.20		[22]
Fe^{2+}	0.15	$NaClO_4$	37	10.52	8.99	7.71	5.94	1:2	[22]
Fe^{3+}	0.15	$NaClO_4$	37	18.20	12.7	8.89		1:2	[22]
Al^{3+}	0.15	$NaClO_4$	37	20.1	16.4	12.2	8.48	1:2	[22]
$(CH_3)_2Sn^{2+}$	0		25	14.01	11.64	9.16	6.59	1:0, 1:1, 1:2, 1:7, 2:0–2:5, 3:0–3:5	[113]
$(CH_3)_3Sn^+$	∼0.050		25			2.45		2:5, 3:4, 3:5, 4:6, 5:1	[114]
	∼0.075		25			3.25			[114]

[a] K_{ij} refers to the equilibrium: $i\,M^{n+} + H_jPhy^{(12-j)-} = M_iH_jPhy^{(12-in-j)-}$.

[b] Complex formation constants by Bebot-Brigaud et al. [18] were not reported because they were expressed by formation equilibria different from those reported in this table [see footnote a].

[c] In $mol\,L^{-1}$.

equilibria affect phenomena such as solubility and/or interactions with other ions and ligands. An accurate knowledge of phytate acid–base properties (as well as the corresponding stability constants) is therefore essential for a thorough understanding of its reactions in solution. However, large discrepancies among thermodynamic data on phytate protonation (mainly stability constants) are very frequent. These differences can be predominantly ascribed to the striking effect of medium and ionic strength on the stability of various phytate protonated species, but difficulties related to the peculiar characteristics of this ligand are also important. As far as the protonation/deprotonation processes are concerned, it must be remembered that these occur over a very wide pH range and that the protonated species are sometimes of very similar stability, making their accurate determination challenging. A clear example is given by one of the first papers published on phytate protonation, where for phytate the authors reported three average protonation constants only [54]. Few papers describe protonation constant values obtained in different media [22,57,58,64,65,68], many deal with only one medium (mainly tetra-alkylammonium [4,18,49], sodium [48,54,59–62,67] or potassium [55,56,66] salts). This makes analysis of results difficult because the cation of ionic medium has a pronounced effect: at given ionic strengths, log K values determined in alkali metal cation ionic media are ∼2–3 log units lower than corresponding values in tetra-alkylammonium solutions, no doubt as a result of strong phytate–alkali metal cation interactions that affect the whole phytate acid–base behavior. This cation effect is even evident at low ionic strength values, as shown in Fig. 2, where the average number of protons bound to phytate is reported versus pH in three different ionic media [$(C_2H_5)_4NI$ [63], LiCl [64] and CsCl [68]] at $I = 0.1\ mol\,L^{-1}$ and $t = 25\ °C$. On the other hand, recent studies have demonstrated

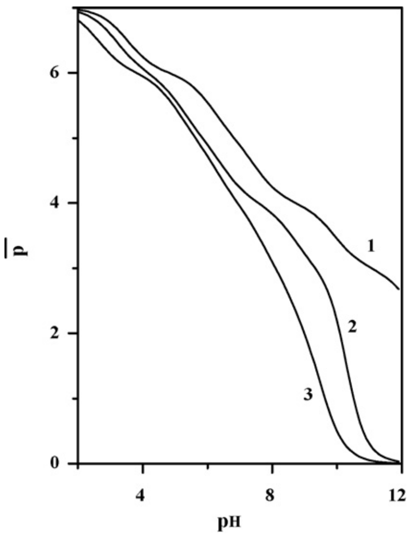

Fig. 2. Average number of protons bound to phytate vs. pH at $I = 0.1\ mol\,L^{-1}$ in three different aqueous ionic media at $t = 25\ °C$. Total phytate concentration, $C_{Phy} = 0.001\ mol\,L^{-1}$. Curves: (1) $(C_2H_5)_4NI_{aq}$; (2) $LiCl_{aq}$; (3) $CsCl_{aq}$.

that the anionic nature of the supporting electrolyte does not greatly influence phytate protonation constant values [67]. Similar issues arise in the evaluation of the effect of ionic strength on phytate protonation: few papers [60,61,63–65,67,68] report data at different ionic strengths. Even amongst them, equations for the modeling of the dependence of phytate protonation constants on I are often missing [60,61]; only in Refs. [63–65,67,68] different equations are proposed to model this dependence. As concerns phytate acid base properties, it should also be mentioned that owing to the similarities between the stability of first three protonated species, in alkali metal media, a peculiar effect occurs: whereas in LiCl a normal stability trend is observed for first three phytate conditional protonation constants ($\log K_1 > \log K_2 > \log K_3$), in NaCl $\log K_2 > \log K_1 > \log K_3$ is found, and in KCl and CsCl it is $\log K_1 > \log K_3 > \log K_2$. This behavior has also been reported in $NaClO_4$ by other authors [59,60], and can be better observed in Table 1, where some phytate protonation constants obtained at different ionic strengths in various ionic media are reported.

Worthy of mention are also the attempts made by many research groups [4,18,48–52,59,60] to define the correct phytate protonation sequence, especially in relation to the above-cited conformational inversions that occur when varying pH. Unfortunately, results reported by these authors are not all in agreement, probably because, as previously stated for the phytate conformational preferences, they are influenced by different experimental conditions adopted in various experiments. A careful evaluation of these processes lies outside the scope of this review. However, we can strongly recommend, for a deeper analysis of each single paper on this topic, to pay particular attention to the experimental conditions reported by various authors.

Phytate protonation enthalpies and entropies have been reported in NaCl at different ionic strengths [65] and in KCl at $I = 0.2\ \mathrm{mol\,L^{-1}}$ [55,69]. Despite the different supporting electrolytes and ionic strengths involved, some comparisons can be made. Marini et al. [55] and Martin et al. [69] determined ionization enthalpy values for all twelve ionization steps grouping the first five and the last three or four steps together. In contrast, data for each individual protonation step from the first to the seventh are reported by De Stefano et al. [65]. Despite these differences, it is clear that: (i) the stepwise protonation enthalpies follow the same trend in both cases, i.e. they increase slightly with increasing protonation step; (ii) in both cases $T\Delta S$ is significantly higher than the corresponding ΔH, which is close to $0\ \mathrm{kJ\,mol^{-1}}$; (iii) where direct comparisons are possible (for protonation steps $5 \le i \le 7$), protonation enthalpies and entropies are similar. For example, with the seventh protonation step at $I = 0.252\ \mathrm{mol\,kg^{-1}}$ in NaCl De Stefano et al. [65] obtained $\Delta H = 4.2$ and $T\Delta S = 19\ \mathrm{kJ\,mol^{-1}}$, whereas Marini et al. [55] report (in KCl $0.2\ \mathrm{mol\,kg^{-1}}$) $\Delta H \sim 4.7$ and $T\Delta S \sim 24\ \mathrm{kJ\,mol^{-1}}$, respectively.

3. Alkali metal complexes

As clearly shown in Fig. 2, the average number of protons bound to the phytate anion has a markedly different profile in various ionic media; these differences significantly exceed what might be expected from different activity coefficients, and should be ascribed to the formation of phytate alkali metal complexes, whose stability constants have been determined by different authors, mainly by potentiometric technique. However, Isbrandt and Oertel [48] also reported Raman spectroscopic observations on the interactions of alkali and alkaline earth cations with phytate that support the potentiometric results and also differentiate between the conformer preference for the different metal ions. In particular, the Raman spectrum of solid $Na_{12}Phy$ is characteristic of the 5ax/1eq conformer, in contrast to the 1ax/5eq structure of the Ca_6Phy salt. Li and Wahlberg [60], analyzing protonation data in $NaClO_4$ at $0.15 \le I\ (\mathrm{mol\,L^{-1}}) \le 3.0$, found the following sodium species Na_7Phy^{5-}, Na_7HPhy^{4-}, $Na_5H_2Phy^{5-}$, $Na_4H_3Phy^{5-}$, $Na_3H_4Phy^{5-}$, $Na_2H_5Phy^{5-}$ and $Na_4H_6Phy^{2-}$. At $I = 1\ \mathrm{mol\,L^{-1}}$ they found that in the range $4 \le \mathrm{pH} \le 10$ more than 90% of phytate is complexed by sodium cation. Bieth et al. [58] reported protonation data in Li^+, Na^+, K^+ and Cs^+ salts, and the $\Delta\log K$ values (differences between protonation constants in alkali metal and in tetra-*n*-butylammonium salts) were related to the binding ability of phytate towards these cations. From potentiometric measurements in $(C_2H_5)_4NI$, LiCl, NaCl, KCl and CsCl [63,64,68], the formation of the species M_6Phy^{6-}, M_5HPhy^{6-}, $M_4H_2Phy^{6-}$, $M_3H_3Phy^{6-}$, $M_2H_4Phy^{6-}$, MH_5Phy^{6-}, MH_6Phy^{5-}, $M_2H_5Phy^{5-}$, $M_3H_4Phy^{5-}$, $M_4H_3Phy^{5-}$, $M_5H_2Phy^{5-}$, M_6HPhy^{5-} and M_7Phy^{5-} was found, whose stability follows the trend $Li^+ > Na^+ > K^+ \ge Cs^+$. The relative formation constants were obtained, in the range $0 < I\ (\mathrm{mol\,L^{-1}}) \le 1$, by comparing the protonation constants in alkali metal chlorides with those measured in tetraethylammonium iodide (considered to be a non-interacting medium) [70–72]. Torres et al. [22], using an analogous method (comparison of protonation constants in $(CH_3)_4NCl$ and in $NaClO_4$, at $I = 0.15\ \mathrm{mol\,L^{-1}}$), found the formation of the sodium species $Na_2H_5Phy^{5-}$, $Na_3H_4Phy^{5-}$, $Na_4H_3Phy^{5-}$, $Na_5H_2Phy^{5-}$ and Na_6Phy^{6-}. The data reported by De Stefano et al. [64] on Na^+ complexes show a linear dependence of stability on the charge product $i\cdot|12-j| = \zeta$ for the reaction

$$i\mathrm{Na}^+ + \mathrm{H}_j\mathrm{Phy}^{(12-j)-} = \mathrm{Na}_i\mathrm{H}_j\mathrm{Phy}^{(12-i-j)-} \qquad K_{ij} \qquad (1)$$

The $\log K_{ij}/\zeta$ ratio is reasonably constant with a mean value of 0.40 ± 0.02 at infinite dilution, and 0.31 ± 0.05 at $I = 0.1\ \mathrm{mol\,L^{-1}}$. Formation constants for Na^+ complexes reported by Torres et al. [22] show a satisfactory constant value $\log K_{ij}/\zeta = 0.18 \pm 0.03$, at $I = 0.15\ \mathrm{mol\,L^{-1}}$ and $t = 37\ ^\circ\mathrm{C}$. The difference between the latter and the former values are due in part (i) to the different temperature and ionic strength, and mainly (ii) to the different reference supporting electrolyte, $(C_2H_5)_4NI$ in Ref. [64] and $(CH_3)_4NCl$ in Ref. [22]. Tetraethylammonium salts make a better reference supporting electrolyte for studying weak complexes than tetramethylammonium salts which are known to interact with highly charged polyanions [73]. The use of $(CH_3)_4NCl$ as a reference supporting electrolyte in the calculations by Torres et al. [22] is noteworthy, as is the use in their experiments of Na_6H_6Phy over a $0.5 \le C_{\mathrm{Phy}}\ (\mathrm{mmol\,L^{-1}}) \le 10$

total concentration range. This means that even in the measurements with $(CH_3)_4NCl$ they always had Na^+ in solution, with a total concentration range $3.0 \leq C_{Na}$ ($\mathrm{mmol\,L^{-1}}$) ≤ 60, and a Na^+:Phy ratio of 6:1. This presence significantly reduced the protonation constant values in $(CH_3)_4NCl$ (so that they are lower than analogous values usually obtained in tetra-alkylammonium media, e.g. in Refs. [18,57]), and would have affected the stability constant values for $Na_iH_jPhy^{(12-i-j)-}$ complexes.

Data reported by Li and Wahlberg [60] are expressed in terms of proton displacement according to the equilibrium

$$\begin{aligned} &Na_qH_pPhy^{(12-p-q)-} + Na^+ \\ &\quad = Na_{q+1}H_{p-1}Phy^{(12-p-q)-} + H^+ \end{aligned} \tag{2}$$

and, as an example, for $(p,q) = (7,0)$, (3,4) and (2,5) they reported, at $I = 1\,\mathrm{mol\,L^{-1}}$, $\log K = -1.6$, -7.1 and -8.3, respectively. From Ref. [64] for the same equilibria, at the same ionic strength, we calculate $\log K = -1.6$, -8.2 and -8.6. Given the different media used (chloride and perchlorate) and the different speciation schemes adopted, this agreement is satisfactory. Owing to the high negative charge, it is likely that phytate forms mixed alkali metal complexes in mixed electrolyte solutions, as shown in an investigation in Li^+/Cs^+ chloride solutions [68]. Since these mixed complexes are not very strong, a stability quite close to that statistically predicted [74,75] is expected.

4. Alkaline earth metal complexes

Due to its high density of negatively charged phosphate groups, phytate strongly interacts with many cations which are assumed to play an important role in mineral storage. The interactions of phytate with divalent cations, such as alkaline earth metals [58,76–78] and transition metal cations [69,78–82] have been considered, providing useful information on the nature of the complexes and their solubility in various conditions. However, the results differ surprisingly between different authors, presumably depending on the experimental conditions used. The interaction of phytic acid with alkaline earth metals, but also with other cations, has been studied by potentiometric [ISE-H^+ and ISE-Ca^{2+}] and calorimetric titrations [55,83], as well as Raman spectroscopy [48]. Several studies have been carried out changing phytate to metal ion(s) concentration ratios at different pH values [3,25,76,84–86]. Worth mentioning is the paper proposed by Graf [76] in which the Ca^{2+}–phytate formation constants were reported at different ionic strengths and temperatures. The apparent formation constants are also strongly influenced by both the concentration of phytic acid (due both to the high charge density of phytate) and temperature. At pH 4.8 only three Ca^{2+}–phytate soluble species were identified (the stoichiometry is not clearly defined), whose formation entropy changes are positive (108.8, 79.5 and 79.5 $\mathrm{kJ\,mol^{-1}\,K^{-1}}$, for K_1, K_2 and K_3, respectively), presumably as a result of the partial disruption of the oriented layer of water around the highly charged phytate ion. The corresponding enthalpy changes are 12.5, 8.4 and 10.1 $\mathrm{kJ\,mol^{-1}}$ for K_1, K_2 and K_3, respectively. At $4.8 \leq \mathrm{pH} \leq 10.4$, a several thousand-fold increase of the strength of Ca^{2+}–phytate interaction is observed, and in this case more than three binding sites are available for the complexation with alkaline earth metals. Phytate forms soluble complexes with Ca^{2+} at low pH values with $1{:}1 \leq Ca^{2+}{:}Phy \leq 2{:}1$ ratios, whereas other Ca^{2+}–phytate complexes with higher Ca^{2+}:Phy ratios precipitate even at low pH values [76]. The higher stability is reached at $Ca^{2+}{:}Phy = 6{:}1$, where at $8 \leq \mathrm{pH} \leq 10.5$, 4.5–4.8 moles of Ca^{2+} are bound per phytate mole, as reported by Evans et al. [87] and Marini et al. [83] who used elemental analysis measurements.

Since five protons dissociate from phytic acid at pH < 2.5 and Ca^{2+} binding does not significantly occur at pH < 5, it could be supposed that binding might only take place when the second hydrogen from an oxo-anion (appropriately oriented) had been removed. This is supported by ^{31}P NMR measurements [54]. At pH > 6 further protons are lost (the last two proton dissociate at pH 10.5) allowing the formation of Ca^{2+}–phytate complexes with a high Ca^{2+}:Phy mole ratio. Assuming that Ca^{2+} is bound by coordinate bonds through reaction with paired oxo-dianions of the phosphoryl groups, and that binding occurs to the axial form, at least two different models containing six metal ions are feasible [77]. From calorimetric and potentiometric studies, Marini et al. [83] observed that the binding of Ca^{2+} to phytate at $2.0 \leq \mathrm{pH} \leq 12.0$ is a potentiometrically and thermally reversible system. The binding of calcium by phytic acid is endothermic in the investigated pH range ($\Delta H = 94.1\,\mathrm{kJ\,mol^{-1}}$). There was no hysteresis, presumably indicating that the complex forms simultaneously with the removal of proton. Few papers report stability constants for alkaline earth–phytate complexes [22,88]. Torres et al. [22] carried out measurements at $t = 37\,°C$ in $NaClO_4$ and Me_4NCl at $I = 0.15\,\mathrm{mol\,L^{-1}}$. From these potentiometric studies they determined the formation constants in $1{:}1 \leq Ca^{2+}{:}Phy \leq 1{:}5$ and $1{:}1 \leq Mg^{2+}{:}Phy \leq 1{:}3$ ratios. In the presence of a phytate excess, formation of soluble complexes predominates; whilst, when the metal ion is the component in excess, sparingly soluble species tend to be formed. Moreover the stability constants of the soluble species are strongly influenced by ionic strength, apparently as a result of the formation of stable complexes with monovalent cations (Na^+) [88]. Dependence of these stability constants on ionic strength was also modeled by Crea et al. [88] using a Debye–Hückel type equation. From the distribution diagrams reported in Ref. [88], at pH 7.4 CaH_5Phy^{5-} and CaH_4Phy^{6-} are the predominant complexes. The stability constant values reported by Torres et al. [22] show that under these experimental conditions (i.e. of ligand excess) only species with 1:1 stoichiometry are detected over the pH range investigated. Free Ca^{2+} can only be detected at pH < 4. Small variations in the stability data were observed with respect to different cations (i.e., Mg^{2+}); generally those with small ionic radii have higher stability constants and therefore give rise to more highly deprotonated complexes at any given pH. This suggests that interactions are dominated by electrostatic phenomena [89]. An accurate study was carried out by Crea et al. [88] using ISE-H^+ and ISE-Ca^{2+} potentiometric measurements in NaCl aqueous solutions at different ionic strengths $0.1 \leq I$ ($\mathrm{mol\,L^{-1}}$) ≤ 0.75 and at $t = 10$ and 25 °C, at $1{:}1 \leq M^{2+}{:}Phy \leq 3{:}1$ ratios. Under the investigated experimental conditions, both Ca^{2+}–phytate and Mg^{2+}–phytate systems show the same speciation; the formation of mono- and polynuclear metal species with general formula $M_iH_jPhy^{(12-2i-j)-}$ ($M=Ca^{2+}$ or Mg^{2+}) with $1 \le i \le 3$ and $3 \le j \le 5$, and the $M_3H_2Phy^{4-}$ species, were detected both at $t = 10$ and 25 °C. The data obtained for the Ca^{2+}–phytate system by both ISE-H^+ and ISE-Ca^{2+} measurements are reasonably consistent. Similar results were obtained by Odani et al. [90] who carried out pH titrations at $I = 0.1$ mol L^{-1} in tetraethylammoniun perchlorate at $t = 25$ °C. In this paper they reported for the interaction of phytate with alkali metals (Na^+ and K^+):$M_iH_jPhy^{(12-i-j)-}$ complexes with $1 \le i \le 3$ and $0 \le j \le 6$; and for the interaction with alkaline earth metal cations, that the stability order was: $Mg^{2+} > Ca^{2+}$ for the mononuclear $MH_jPhy^{(12-2i-j)-}$ complexes, and $Ca^{2+} > Mg^{2+}$ for the polynuclear $M_iH_jPhy^{(12-2i-j)-}$ ($i = 2$–3) complexes. This reversal of trend was explained by ^{31}P NMR spectra, which revealed how Ca^{2+} forms stable bridge structures with phosphates, better than Mg^{2+}. Stability constants obtained at different ionic strengths allowed Crea et al. [88] to calculate formation constants at infinite dilution. In the calculations relative to the ionic strength dependence of formation constants, the total formal charge of phytate was taken into account, i.e. $z = -12$, although, owing to strong interference of sodium ion, effective charge may be significantly lower, as reported by Li et al. [59,60,91], who proposed $z = -5$. This can substantially change the values of formation constants at infinite dilution. Distribution diagrams reported by Crea et al. [88] show that, in accordance with Torres et al. [22], the MH_5Phy^{5-} species is predominant at pH ~ 5 for both Ca^{2+} and Mg^{2+} phytate systems but, in agreement with Odani et al. [90], an opposite trend in the formation percentages of other species is observed between Ca^{2+} and Mg^{2+} for the polynuclear complexes at other pH values. As an example, in Ref. [88] the trend $M_3H_3Phy^{3-} > M_2H_4Phy^{4-} > MH_5Phy^{5-}$ was observed for Ca^{2+}, and the opposite was for Mg^{2+}. Nevertheless, a comparison between the data reported by Crea et al. [88] and by Torres et al. [22] reveals that the stability constants of the latter authors are very high (by several orders of magnitude) relative to the stabilities of other metal ion complexes. Data reported at different pH, temperatures and ionic strengths also allowed Crea et al. [88] to obtain an empirical equation for the dependence of alkaline earth–phytate complexes on both proton and metal ions bound to ligand

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

where P_1–P_4 are empirical parameters. The $\log K_{ij}$ values refer to the equilibrium

$$iM^{n+} + H_jPhy^{(12-j)-} = M_iH_jPhy^{(12-in-j)-} \qquad K_{ij} \qquad (4)$$

and decrease monotonically as the j index (the proton) increases; a reverse trend is observed for $\log K_{ij}$ versus i (the metal ion). This equation is useful since it allows to predict the stability of species formed outside the investigated experimental conditions.

The formation of sparingly soluble metal–phytate species may occur from the interaction of phytate with alkaline earth metals. Many papers in literature deal with the problem of metal–phytate precipitation and the results reported are sometimes unreliable. Analysis of literature data demonstrates that stoichiometry of precipitate depends on C_M:C_{Phy} (C_x = analytical concentration of components) ratios, on pH and on ionic strength of solutions containing interacting cations, such as Na^+, which allows the formation of mixed $Na_iCa_kH_jPhy^{(12-i-2k-j)-}$ complexes. Some authors [76,77,86,87] reported the formation of a precipitate with formula Ca_5H_2Phy; Martin and Evans [77] assumed that precipitation takes place only if one phosphate group is transformed into the oxo-dianion form. More recently Nash et al. [42] reported the formation of $Ca_{4.6}(H,Na)_{2.8}Phy$ species at $6.0 \le pH \le 7.5$ and $1{:}1 \le C_{Ca}{:}C_{Phy} \le 6{:}1$, and Gryspan and Cheryan [86] and Crea et al. [92] investigated the formation of precipitate as a function of pH and metal to ligand ratio. These authors reported that lower pH values promote the formation of protonated species and that at the same pH values, high Ca^{2+} concentration promotes the formation of less protonated species. The formation of a Ca_6Phy species is possible if both high pH (~10.5) values and Ca^{2+}:Phy ratios are used [76,92,93]; moreover in these last conditions, if large amounts of Na^+ are also present in solution, it is possible to observe the formation of a solid with Ca_5Na_2Phy stoichiometry, characterized by thermal analysis, ICP and SEM [92]. Crea et al. [92] also reported the values of solubility products and formation enthalpies for these complexes at pH 10.5: $pK_{s0} = 58.3$ and $\Delta H = 7.6$ kJ mol^{-1} for Ca_6Phy and $pK_{s0} = 75.7$ and $\Delta H = 3.7$ kJ mol^{-1} for Ca_5Na_2Phy. A number of solid metal phytate complexes have been studied by other authors: $Ca_2Na_8Phy \cdot 3H_2O$, $Na_{12}Phy \cdot 3H_2O$ [94], Na_2Mg_5Phy [95], $Ca_5K_2Phy \cdot xH_2O$ [96]. Torres et al. [22] and Veiga et al. [97] reported the formation of a $Mg_5H_2Phy \cdot 22H_2O$ and a $Ca_5H_2Phy \cdot 16H_2O$ complex. Evans and Pierce Jr. [98] isolated, at pH ~6 and an initial phosphorus to metal ratio P:Mg^{2+} = 1:1, a solid powdered product with non-stoichiometric atomic ratio ($1.31{:}1 \le P{:}Mg^{2+} \le 1.37{:}1$). Binding ability of phytate toward metal ions was widely used for exchange processes; Nash et al. [42] report that in presence of macroscopic concentrations of $UO_2(NO_3)_2$, there is a quantitative exchange of Ca^{2+} by $UO_2{}^{2+}$ from Ca_iPhy precipitates, indicating that they can be used for the removal of actinides from groundwater.

5. Divalent inorganic cation complexes

Phytic acid, in its various deprotonated forms, has the ability to bind or chelate multivalent cations. As with the alkaline earth cations, the solubility of phytate–metal complexes is pH dependent and this is a limiting factor in the study of these complexes in solution [99]. Tamim and Angel [99] report that many papers have been published, in which the stability order of some metal–phytate complexes was found to be: $Cu^{2+} > Zn^{2+} > Co^{2+} > Mn^{2+} > Fe^{2+} > Ca^{2+}$. Other researchers [100] reported the order of mineral potency as inhibitors of phytate hydrolysis at neutral pH to be $Zn^{2+} \gg Fe^{2+} > Mn^{2+} > Fe^{3+} > Ca^{2+} > Mg^{2+}$. Study in animals and humans subjects showed that high concentrations of phytic acid in their diet might cause metal deficiency and that phytate content is negatively correlated to metal adsorption, as in the case of zinc [19]. In order to simplify matters, thermodynamic data on various phytate metal complexes are discussed in separate paragraphs, each referred to single cations and ordered by importance (mainly by the amount of data and the number of published papers).

5.1. Cd^{2+} complexes

Many papers deal with phytate–cadmium interactions in literature, but only a few authors have reported thermodynamic parameters. Interactions between Cd^{2+} and phytate have been studied by potentiometric, spectrophotometric and ^{31}P NMR techniques, at different metal to ligand ratios, ionic media and ionic strengths [18,22,101–104]. Cadmium is often one of the most biotoxic elements and it is regarded as a priority pollutant especially on account of its widespread anthropogenic sources in the environment. These studies are restricted by the pH value at which the formation of scarcely soluble species occurs, which is again dependent on ionic strength, Cd^{2+}:Phy ratio and on temperature. In this order, different speciation models were reported in literature on the interaction between these two components. In fact, whilst De Stefano et al. [104] report the formation of several polynuclear species ($Cd_iH_jPhy^{(12-2i-j)-}$, with $i = 1$, 2, $4 \leq j \leq 7$, and the $Cd_3H_4Phy^{2-}$ species), Bebot-Brigaud et al. [18] considered just the formation of 1:1 complexes, owing to the fact that they worked with low metal to ligand ratios, namely $1 \leq C_{Phy}:C_{Cd}{}^{2+} \leq 6.7$; a ligand excess that avoids the formation of polynuclear species and allows the study the Cd^{2+}–phytate speciation to higher pH (pH > 10), where the formation of less protonated complexes is possible without the formation of sparingly soluble species. Despite the differences in the experimental conditions adopted in the cited papers, the results from De Stefano et al. [104] and from Bebot-Brigaud et al. [18] are consistent if the formation percentages of similar species proposed by these authors are considered. For example, both authors reported, in the range $6 \leq pH \leq 8$, that the main species are the CdH_4Phy^{6-} and CdH_3Phy^{7-} complexes, even though the two papers report different formation percentages. However, these differences can be explained given that measurements by De Stefano et al. [104] were made in sodium chloride medium and that chloride ion binds cadmium more strongly than perchlorate (used by Bebot-Brigaud et al. [18]). De Stefano et al. [104] also modeled the dependence of stability constants for $Cd_iH_jPhy^{(12-2i-j)-}$ complexes on ionic strength by means of an extended Debye–Hückel type equation and by SIT (Specific ion Interaction Theory) model [75,105,106]. Other results were reported by Vasca et al. [103] ($NaClO_4$ 3.0 mol L^{-1} and $t = 25$ °C) and Torres et al. [22] ($NaClO_4$ 0.15 mol L^{-1} and $t = 37$ °C). The first ones found only the CdH_5Phy^{5-} species with a $\log K_{15} = 3.64$ [according to Eq. (4)], which has the same order of magnitude of the values obtained by De Stefano et al. [104] in 1 mol L^{-1} NaCl aqueous solution at $t = 25$ °C ($\log K_{15} = 3.93$). Attempts to compare the data reported by De Stefano et al. [104], Bebot-Brigaud et al. [18] and Vasca et al. [103] with those reported by Torres et al. [22] are made difficult by the differences in temperatures used ($t = 37$ °C) by this last group. An interesting calorimetric study is reported by Evans and Martin [101] in KNO_3 0.2 mol L^{-1}, $t = 25$ °C, pH ~3.5 and in $1{:}1 \leq Cd^{2+}{:}Phy \leq 6{:}1$ ratios. The formation heats are endothermic ($4.3 \leq \Delta H$ (kJ mol^{-1}) ≤ 14.1), as also found for other phytate–metal ions interactions [79,81]. Their formation enthalpies are the sum of a number of factors that include complex formation heat, the heat for H_2O removal from the hydrated, protonated phytate and from metal ions, the heat of phytate deprotonation and the heat of hydration of protons released by phytate. Other contributing factors might involve the hydrolysis heats of the complexes formed and structural changes induced by metal ion binding. Owing to the fact that the binding of a metal ion by phytic acid involves the water molecules displacement both from metal and phytate, these water molecules are polarized by these components and are under considerable restraint, and thus, give relatively low entropy changes. The neutralization of charge that accompanies chelate formation releases this restraint and produces an increase in entropy. The positive changes in the enthalpies are a reflection of the fact that many solvent–metal ion and solvent–phytate bonds must be broken before the reaction can occur and the energy required for this is greater than any energy liberated in forming the metal ion–phytate bond [101]. In these experiments, Evans and Martin [101] reported that heat of precipitation of cadmium–phytate complexes having 5.4 mol of Cd^{2+} bound to one mole of phytate was 84.5 kJ mol^{-1}. It might also be useful to make comparisons with data reported by Persson et al. [102], obtained by potentiometric investigation (at $I = 0.1$ mol L^{-1} in $NaClO_4$ and $t = 25$ °C) on the phytate binding ability toward cadmium(II). These authors reported that the sequestering ability of phytate versus Cd^{2+} was significant at constant component concentrations between pH ($3 \leq pH \leq 7$) it can reach about 40% of total Cd^{2+} at pH > 4.5. These results are in good agreement with those reported by De Stefano et al. [104] and by Bebot-Brigaud et al. [18].

5.2. Cu^{2+} complexes

Few thermodynamic parameters are reported in literature for interaction of copper(II) with phytate. Some papers report only qualitative data [19,102,107]. For example, Persson et al. [102], studying the interaction of Cu^{2+} toward phytate in $NaClO_4$ 0.1 mol L^{-1}, at $t = 25$ °C at different pH values, demonstrated that at pH ~5.5 phytate binds ~50% of total copper. Other studies were carried out by Martin and Evans [79,81] at $t = 25$ °C in KNO_3 0.2 mol L^{-1}. They calculated the concentration of metal ion bound to ligand as a function of pH at $1{:}1 \leq Cu^{2+}{:}Phy \leq 6{:}1$ ratio, and they determined the apparent binding heat, which indicates that the complexation reaction is endothermic. The first quantitative thermodynamic data on the Cu^{2+}–phytate system were reported by Bebot-Brigaud et al. [18], who used potentiometry and spectrophotometry in tetraethylammonium perchlorate at $I = 0.1$ mol L^{-1} and $t = 20$ °C. Owing to a very low metal to ligand concentration ratio, they reported only the formation of variously protonated 1:1 metal phytate complexes, avoiding the formation of polynuclear species, but favoring the investigation of wider pH ranges. In these experimental conditions, they did not observe formation of insoluble species. In Crea et al. [67] measurements were carried out in $NaNO_3$ aqueous solutions at different ionic strengths ($0.1 \leq I$

(mol L^{-1}) ≤ 1.0) at $t = 25\,°C$ by ISE-H^+ and ISE-Cu^{2+} electrodes at metal to ligand ratios 1:1 ≤ Cu^{2+}:Phy ≤ 4:1. At each investigated ionic strength values they obtained the following species: CuH_5Phy^{5-}, $Cu_2H_5Phy^{3-}$ and CuH_4Phy^{6-}; the last one was not found by ISE-Cu^{2+} measurements because these were carried out a pH ~ 4, whilst its formation occurs at higher pH value. Measurements performed at different ionic strengths allowed to study the dependence of formation constants on I by extended Debye–Hückel and SIT (Specific ion Interaction Theory) equations [75,105,106]. As concerns the papers by Torres et al. [22] and Vasca et al. [103], the experimental conditions adopted do not allow satisfactory comparison (Vasca et al. [103] used 3.0 mol L^{-1} $NaClO_4$ at $t = 25\,°C$ whereas Torres et al. [22] carried out their measurements at $I = 0.15$ mol L^{-1} and $t = 37\,°C$ albeit in the same ionic medium). However, the speciation model proposed by Torres et al. [22] is in agreement with that proposed by Bebot-Brigaud et al. [18] owing to the fact that similar metal/ligand ratios were used. On the other hand, Vasca et al. [103] obtained the species CuH_5Phy^{5-} and $Cu_2H_3Phy^{5-}$ with $\log K_{15} = 2.63$ and $\log K_{23} = 8.97$, respectively, [according to Eq. (4)]. They also characterized the solid species $Cu_6H_2PhyCl_2{\cdot}2.5H_2O$ by thermogravimetric and elemental analysis. The precipitate has a metal/ligand ratio of 6:1, probably due to the fact that this Cu^{2+}–phytate species is formed in the pH range before the formation of solid phase. Evans and Pierce Jr [98] isolated, in the same conditions reported for Mg^{2+}, a solid powdered product with a non-stoichiometric atomic ratio 1.09:1 ≤ P:Cu^{2+} ≤ 1.14:1.

5.3. Zn^{2+} complexes

Despite many important papers on the interaction and absorption of Zn^{2+} towards phytate are reported in literature (e.g. [19,108]), few data deal with thermodynamic parameters such as formation constants and enthalpies. At present time, this information is reported only by a few authors [18,22,107], whilst others [102,109,110] limited their investigations to the characterization of the stoichiometry of the Zn^{2+}–phytate complexes. The stoichiometry of insoluble species can vary with the initial metal–phytate ratio but can reach no more than a ratio of 5:1 [102,109,110]; Rodrigues-Filho et al. [109] also proposed a mixed $Na_4MnZn_4Phy(NO_3)_2{\cdot}8H_2O$ and a $Na_3Zn_5Phy(OH){\cdot}9H_2O$ species, characterized by elemental analysis, FT-IR, NMR, XPS (X-ray photoelectron spectroscopy) and EPR (electron paramagnetic resonance). In these complexes, only one Zn coordination environment is observed, and results indicate a bidentate coordination mode by two different phosphate groups. As regards formation constants, Bebot-Brigaud et al. [18] and Torres et al. [22] carried out speciation studies by using different techniques under different experimental conditions. Bebot-Brigaud et al. [18], using ^{31}P NMR titrations, reported several 1:1 protonated species. They also reported the ZnH_7Phy^{3-} species at low pH values, and at higher pH values a fully deprotonated $ZnPhy^{10-}$ species. ^{31}P NMR titrations also revealed the phytate coordination sites at various pH values. Martin and Evans [69] carried out a calorimetric and titration study on the interaction of phytate with Zn^{2+} in the range 2.5 ≤ pH ≤ 11, at $t = 25\,°C$ in 0.2 mol L^{-1} KCl. The phytate complex formation was obtained at pH < 6, with ~3.5 mol of Zn^{2+} bound per mol of phytate. Using potentiometric titrations, they also determined the stability constants per phosphate group bound by Zn^{2+} at different metal to ligand ratios; whilst the apparent binding heats at four mol ratios were determined from calorimetric titrations carried out in the range 2.6 ≤ pH ≤ 6.1. The mean value obtained for the binding of 3.5 mol of Zn^{2+} per mol of phytate was 104.3 ± 2.1 kJ mol^{-1}. These authors also calculated the solubility product of a Zn^{2+}–phytate insoluble complexes with Zn^{2+}:Phy ~ 4:1. The value they obtained was $pK_{s0} = 30.4$. This compared with a value of $pK_{s0} = 22$ for the Ca^{2+}–phytate, having Ca^{2+}:Phy ~ 5:1.

5.4. Ni^{2+} complexes

The most interesting investigations on Ni^{2+}–phytate complexes, were carried out by Bebot-Brigaud et al. [18], but studies have also been reported by Torres et al. [22] and De Carli et al. [66] in different ionic media, ionic strengths and temperature. Similar speciation models were proposed in all these papers, owing to the low metal to ligand ratios used. Nevertheless a good agreement between the formation constants was found. Investigation carried out up to pH 12 allowed Bebot-Brigaud et al. [18] and De Carli et al. [66] to characterize low protonated species such as $NiHPhy^{9-}$ and NiH_2Phy^{8-}. Other data were reported by Vasca et al. [103] in 3 mol L^{-1} $NaClO_4$ at $t = 25\,°C$; they reported values of $\log K_{15} = 3.78$ and $\log K_{23} = 11.85$ [according to Eq. (4)], owing to the higher metal to ligand ratio used. Vasca et al. [103] characterized a solid compound with general formula: $Ni_6H_4PhyCl{\cdot}2H_2O$ by elemental analysis and ICP spectroscopy, whilst De Carli et al. [66] carried out a thermogravimetric and IR study on a solid Ni^{2+}–Phy compound, without reporting any definite stoichiometry.

5.5. Co^{2+} complexes

The only available data for Co^{2+} complexes were reported by Bebot-Brigaud et al. [18], Vasca et al. [103] and Torres et al. [22] under experimental conditions already cited for the Cd^{2+}, Cu^{2+}, Zn^{2+} and Ni^{2+}–phytate systems. Analysis of thermodynamic data reveals that there is no agreement between the data reported by these authors owing to the different experimental conditions used. Vasca et al. [103] proposed a speciation model similar to the ones reported for the other metal–phytate systems, obtaining the following values: $\log K_{15} = 3.84$ and $\log K_{23} = 11.96$ [according to Eq. (4)]. For the synthesized insoluble species the general formula: $Co_6H_4PhyCl_4{\cdot}2H_2O$ was described. Evans and Pierce Jr [98] isolated, in the conditions already reported for Mg^{2+}, a solid powdered product with a non-stoichiometric atomic ratio 1.05:1 ≤ P:Co^{2+} ≤ 1.09:1. An interesting spectrophotometric study was proposed by Martin and Evans [81]. These authors studied the interaction of phytate with the Co^{2+} aqua ion as a function of pH in 0.2 mol L^{-1} KNO_3. They observed in the range 2.5 ≤ pH ≤ 4.8, a lowering in the molar absorptivity of Co^{2+} solution in presence of phytate due to the binding reaction. At pH > 5 insoluble complexes are formed. From absorptivity data they calculated the mol of Co^{2+} bound per mol of phytate in the range $2.5 \le \mathrm{pH} \le 9$, assuming that binding occurs when fully deprotonated phosphoryl groups (oxo-dianions) are formed. By curve-fitting they calculated formation constants values of 5.33 and 7.48, for five and one groups, respectively.

5.6. *Hg^{2+} complexes*

To our knowledge, the only thermodynamic data on the interaction of phytate with mercury(II) are those reported by De Stefano et al. [111]. Investigations were carried out in NaCl aqueous solution at $I = 0.15$ and $1.0\,\mathrm{mol\,L^{-1}}$, in the range $2.5 \le \mathrm{pH} \le 9.5$. Eleven different complexes were identified and in particular eight mononuclear ($\mathrm{HgH}_j\mathrm{Phy}^{(10-j)-}$ with $0 \le j \le 7$) and three dinuclear ($\mathrm{Hg_2H}_j\mathrm{Phy}^{(8-j)-}$ with $0 \le j \le 2$) species. The formation constants obtained were fairly dependent on ionic strength, phytate protonation step and metal to ligand ratio. The results obtained show that phytate is a very good sequestering agent for Hg^{2+}, indicating its potential for use in the remediation of sites polluted by this cation.

5.7. *Other divalent cation complexes*

The literature contains few data for the interaction of phytate with other divalent cations [19,22,44,45,103,109]. Simsek and Ulusoy [44,45] and Lönnerdal [19] report only on the absorption of some divalent cations such as $UO_2{}^{2+}$, Pb^{2+}, Ra^{2+}, Mn^{2+}, Fe^{2+} by phytate in different substrates. Evans and Pierce Jr [98] isolated also for Mn^{2+} a solid powdered product with non-stoichiometric atomic ratio $1.26{:}1 \le \mathrm{P{:}Mn^{2+}} \le 1.30{:}1$. Torres et al. [22] and Vasca et al. [103] provide some thermodynamic data (formation constants and stoichiometry of some solid species) obtained in different experimental conditions but which are not readily comparable: Vasca et al. [103] found [at $I = 3.0\,\mathrm{mol\,L^{-1}}$ in $NaClO_4$ at $t = 25\,°C$, according to Eq. (4)] $\log K_{15} = 3.85$ and $\log K_{23} = 12.43$ for Mn^{2+}, and $\log K_{15} = 3.87$ and $\log K_{23} = 12.87$ for Pb^{2+} complexes; Torres et al. [22] reported $\log K_{14} = 8.78$, $\log K_{15} = 8.44$ and $\log K_{16} = 7.20$ for Mn^{2+}, and $\log K_{13} = 10.52$, $\log K_{14} = 8.99$, $\log K_{15} = 7.77$ and $\log K_{16} = 5.94$ for Fe^{2+} complexes at $I = 0.15\,\mathrm{mol\,L^{-1}}$ in $NaClO_4$ at $t = 37\,°C$.

6. Trivalent inorganic cation complexes

The thermodynamic parameters reported in literature for phytate complexes with trivalent cations are similar to those with divalent cations. This is due to the fact that the determination of stability constants for complexes with M^{3+} metals generally involves an additional problem, namely the strong hydrolysis reaction of the cations. To our knowledge, the only available data are reported in Refs. [22,62,101], whilst a solid powdered product with non-stoichiometric atomic ratio ($2.51{:}1 \le \mathrm{P{:}Fe^{3+}} \le 2.69{:}1$) was isolated for Fe^{3+} by Evans and Pierce Jr [98] at pH ∼2. The M^{3+}–Phy interaction can be modeled in simple electrostatic terms, in the same way as the divalent cations, despite the complexation of trivalent cations which gives stability constants that are at least five orders of magnitude higher, owing to the higher cation charge [22]. Investigations tend to be performed at low metal to ligand ratios because of the formation of polynuclear insoluble species. Evans and Martin [101] studied the formation of soluble complexes for $1{:}1 \le \mathrm{M^{3+}{:}Phy} \le 4{:}1$, where a theoretical maximum combination of the phytate molecule with Al^{3+} should occur. They observed that the endothermic complexation heats are ∼6–27 times higher than those of the interaction of phytate with divalent cations. Accordingly, at M:Phy = 1:1, at a final pH ∼2.3, Evans and Martin [101] obtained $\Delta H = 29.4\,\mathrm{kJ\,mol^{-1}}$ for Al^{3+}–Phy complexation, whilst the corresponding precipitation heat lies in the range 92.1–$113.0\,\mathrm{kJ\,mol^{-1}}$. These observations are applicable to the other trivalent cations because when they interact with phytic acid there would be a larger displacement of hydrogen ions with a concomitant greater pH drop; the combination with phytic acid induces the release of water molecules, which could result in a higher endothermic reaction heat. From the data reported by Siddiqi et al. [62] in $NaClO_4$ $0.1\,\mathrm{mol\,L^{-1}}$ at different pH values, results that sodium ion concentration may interfere in the complexation of phytic acid with rare earth ions, because phytate preferably complexes with sodium ions, reducing the availability of phytic acid for complexation with lanthanide ions. They also calculated the order of stability constants: $Ho^{3+} > Dy^{3+} > Tb^{3+} > Sm^{3+} > Gd^{3+} > Nd^{3+} > Pr^{3+} > Ce^{3+}$. Two other interesting studies are those reported by Simsek and Ulusoy [44], and Witter et al. [112], despite the few thermodynamic data reported in these papers. The first authors determined the adsorption of Bi^{3+} and Ac^{3+} by some polyacrylamide-zeolite substrates modified with phytic acid, whilst Witter et al. [112] studied the complexing ability of natural seawater toward Fe^{3+} and calculated the relative conditional stability constants as well as the stability constants of nine model ligands, with phytic acid among them. These ligands were chosen to represent Fe-binding organic functional groups present in seawater as a result of cell lysis. The particularly interesting result of this study is that, despite vast differences in the molecular structures and functional groups of the Fe^{3+}-biding model ligands, the conditional stability constants were relatively invariant, regardless of the ligand structures, and they fell within the range $\log K_{\mathrm{Fe^{3+}-L}} = 18$–$23$ that has been reported for the unknown Fe^{3+}–L complexes in seawater.

7. Organotin(IV) complexes

The literature also contains few data on the interaction of phytate with organotin(IV) compounds [113,114]. In De Stefano et al. [113] the complexes of phytic acid with dimethyltin(IV) (DMT) in NaCl aqueous solution at different ionic strengths were studied, with the dependence of the stability constants on ionic strength modeled by a Debye–Hückel type equation. In this paper, 18 different phytate-DMT species were obtained at each investigated ionic strength ($0.1 \le I\,(\mathrm{mol\,L^{-1}}) \le 1.0$). In particular, eight mononuclear $\mathrm{DMT}_i\mathrm{H}_j\mathrm{Phy}^{(12-2i-j)-}$ complexes with $0 \le j \le 7$, six dinuclear ($0 \le j \le 5$) and four trinuclear ($0 \le j \le 3$) were obtained. As already observed for other phytate–metal systems is evident, that different conditions (i.e., ionic strength and metal to ligand ratios) affect the DMT and phytate speciations: when the metal to ligand ratio is higher (i.e., DMT:Phy = 4:1) complexes containing more than one dimethyltin(IV) cation were formed in significantly large percentage than the mononuclear phytate species. Similar results were obtained by Crea et al. [114] for the interaction with trimethyltin(IV) (TMT). In this case, measurements carried out at high trimethyltin(IV) concentrations allowed the formation of higher polynuclear $\mathrm{TMT}_i\mathrm{H}_j\mathrm{Phy}^{(12-i-j)-}$ complexes with $i \leq 5$. From the formation constants obtained for TMT, it appears that stability of this complexes has the same order of magnitude of Li^+–phytate, Na^+–phytate and K^+–phytate complexes. It is interesting to note how the alkali metal cations form with phytate some species that have similar stoichiometry of those formed with TMT cation.

8. Polyammonium cation complexes

Polyammonium cations form stable complexes with inorganic and organic polyanions [115–117]. A study was reported on the formation and stability of phytate–polyammonium cations [ethylenediamine (en), putrescine (ptr), spermidine (spd) and spermine (sper)] [118]. Several species were formed for each system (nine, eight, eight and six for en, ptr, spd and sper, respectively), with stability depending on the product of charges of reactants for the generic equilibrium

$$p\mathrm{H}_i\mathrm{A}^{i+} + \mathrm{H}_j\mathrm{Phy}^{(12-j)-} = \mathrm{H}_j\mathrm{Phy}(\mathrm{H}_i\mathrm{A})_p{}^{(12-\mathrm{j}-i\cdot p)-} \qquad (5)$$

By considering the parameter $\zeta = |(\sum z_{\mathrm{cation}})z_{\mathrm{anion}}|$ it was found for this equilibrium $\log K = a\cdot\zeta$, with $a = 0.40$, 0.42, 0.30 and 0.34 for en, ptr, spd and sper, respectively, at $I = 0\ \mathrm{mol\,L^{-1}}$ and $t = 25\,^{\circ}\mathrm{C}$. These values of a are very close to that found for sodium complexes ($a = 0.40$). Unpublished data from this laboratory showed that also an ammonium polyelectrolyte (branched polyethyleneimine MW = 750 kDa) forms complexes with phytate, having stability comparable with that of low molecular weight polyammonium cations. There is no other quantitative study on the interaction of phytate with polyammonium cations. Nevertheless, some indirect evidence confirms these interactions. Naik and Srivastava [119] report that phytate can be bound by spermine. Riche and Garling [21] affirm that phytate forms complexes with some proteins through ε-aminogroups.

9. Discussion and conclusions

In this review we have reported and analyzed the most significant thermodynamic parameters published for both phytate protonation and complex formation with a variety of metal and organometallic cations, as well a few other organic ligands. The analysis of these data revealed that phytate forms quite stable complexes and behaves mainly as a hard base [57,62]. This conclusion is evidenced by most of the authors. Nevertheless, the stability of these complexes is usually lower than that expected for such a highly charged anion. When compared with other common chelating agents, such as EDTA [22,111,113], we observe that usually the phytate metal complexes are less stable than analogous complexes with these other ligands: by comparing their stability with that of phytate, generally we find a strong correlation between $\log\beta$ (phytate) versus $\log\beta$ (other ligands). As an example, we fitted $\log\beta$ (M^{2+}, $\mathrm{H_4Phy}^{8-}$), with $\mathrm{M}^{2+} = \mathrm{Mg}^{2+}$, Ca^{2+}, Cu^{2+}, Co^{2+}, Hg^{2+} and $(\mathrm{CH_3})_2\mathrm{Sn}^{2+}$, versus $\log\beta$ (M^{2+}, citrate) and $\log\beta$ (M^{2+}, pyrophosphate) [120–122], with a fairly good correlation coefficient ($r = 0.96$ for Phy-citrate and $r = 0.98$ for Phy-pyrophosphate). Note that also citrate and pyrophosphate form stronger complexes than phytate.

However, comparisons between the binding ability of two or more ligands may frequently represent a challenging task, owing to some difficulties regarding, for example, the different number and/or nature of complexes formed. This problem has been overcome for phytate and other ligands by the calculation of pL_{50}, an empirical parameter that, once the conditions (pH, ionic strength, supporting electrolyte, temperature, metal concentration) are fixed, can give an objective representation of this binding ability. A detailed description of the method is given, e.g., in Ref. [67]. As reported elsewhere [67], pL_{50} represents the total ligand concentration necessary to bind 50% of cation in solution. In other words, the higher the pL_{50} is, the stronger the binding ability of the ligand. Analogously, for a ligand, the higher the pL_{50} is, the stronger the binding ability toward a given cation. Specifically, from calculated pL_{50} for phytate in presence of various cations at pH 5.5, $I = 0.15\ \mathrm{mol\,L^{-1}}$, and $C_\mathrm{M} = 10^{-9}\ \mathrm{mol\,L^{-1}}$ (C_M = analytical concentration of cation in solution), we obtained the following sequestering trend for some phytate divalent cation complexes:

$$\mathrm{Hg}^{2+} \gg \mathrm{Cu}^{2+} > \mathrm{Ni}^{2+} \geq \mathrm{Cd}^{2+} \geq (\mathrm{CH_3})_2\mathrm{Sn}^{2+} \gg \mathrm{Mg}^{2+} > \mathrm{Ca}^{2+}$$

The sequestering trend observed is dependent on several factors, such as the hydrolysis reaction of the cations and the anion interaction of supporting electrolyte with the cations. Previous studies demonstrated that the dependence of pL_{50} on experimental conditions can be modeled by very simple relationships as a function of different variables. For example, at $I = 0.15\ \mathrm{mol\,L^{-1}}$ in NaCl solutions at $t = 25\,^{\circ}\mathrm{C}$, pL_{50} for Cd^{2+} complexation by phytate is a linear function of pH [104]:

$$\mathrm{pL}_{50} = 2.0 + 0.35\,\mathrm{pH} \qquad (6)$$

In addition to the above correlations, several other empirical relationships were proposed to relate different parameters for the determination of phytate thermodynamic data (including protonation and complex formation constants). This is particularly important for the calculation of data that are not experimentally accessible. For example, protonation constants in tetraethylammonium salts are so high (the first two steps) that in particular they can be only obtained by an extrapolation technique [63]. The extrapolation to infinite dilution also requires the use of empirical relationships different from those normally adopted for both protonation and formation constants [63,64,67,68,88,104,111,113]. Predicted complex formation constants were also calculated for pH and concentration conditions not experimentally accessible [67,88,104,111,113]. In particular, the pH value of solutions under investigation is a crucial problem, since very often at pH > 5–6 sparingly soluble species are formed. As another example, predicted formation constants for Mg^{2+} and Ca^{2+} were reported under conditions relevant to a speciation model for natural fluids ($5 < \text{pH} < 8$), where phytate is widely present [88].

This last aspect is especially relevant because this review has shown how sparse are the thermodynamic parameters for phytate coordination chemistry in solution. The parameters available are mainly complex formation constants; there are even fewer data for formation enthalpies and entropies. Only a small number of constants have been reported for solubility data. In spite of this, the use of the predictive relationships combined with an accurate selection of literature data can provide a good picture of phytate speciation in natural systems, as well as other solutions (e.g. wastewaters) where phytate may be present. For these purposes, the modeling of the dependence of thermodynamic parameters on medium and ionic strength is of fundamental importance, because the natural fluids are multielectrolyte solutions (where many different inorganic salts, as well as organic ligands, are present). These solutions cover a very wide range of ionic strengths (from $I \sim 0.01\,\text{mol}\,\text{L}^{-1}$ for freshwaters to $I > 6\,\text{mol}\,\text{L}^{-1}$ for hyper-saline waters). Unfortunately, to our knowledge, many studies of phytate–metal complexes have only been performed by our group.

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References

[1] L. Oatway, T. Vasanthan, J.H. Helm, Food Rev. Int. 17 (2001) 419.
[2] U. Konietzny, K.D. Jany, R. G, J. Ernaehrungsmedizin 8 (2006) 18.
[3] J.A. Maga, J. Agric. Food Chem. 30 (1982) 1.
[4] A.J.R. Costello, T. Glonek, T.C. Myers, Carbohydr. Res. 46 (1976) 159.
[5] S.B. Shears, Cell. Sign. 13 (2001) 151.
[6] V. Raboy, Phytochemistry 64 (2003) 1033.
[7] N.R. Reddy, M.D. Pierson, S.K. Sathe, D.K. Salunkhe, Phytates in Cereals and Legumes, CRC Press, Boca Raton, FL, 1989.
[8] E. Graf, J.W. Eaton, Free Radic. Biol. Med. 8 (1990) 61.
[9] B.F. Harland, E.R. Morris, Nutr. Res. 15 (1995) 733.
[10] B.F. Harland, G. Narula, Nutr. Res. 19 (1999) 947.
[11] G. Urbano, M. Lopez-Jurado, P. Aranda, C. Vidal-Valverde, E. Tenorio, J. Porres, J. Physiol. Biochem. 56 (2000) 283.
[12] N.R. Reddy, S.K. Sathe, Food Phytates, CRC Press, Boca Raton, FL, 2001.
[13] F. Grases, B. Isern, P. Sanchis, J. Perello, J.J. Torres, A. Costa-Bauza, Front. Biosci. 12 (2007) 2580.
[14] J. Kim, H.Y. Paik, H. Joung, L.R. Woodhouse, S. Li, J.C. King, J. Am. Coll. Nutr. 26 (2007) 1.
[15] A.S. Knox, D.I. Kaplan, R.L. Brigmon, Abstracts of Papers, 233rd ACS National Meeting, Chicago, IL, United States, March 25–29, 2007 (NUCL).
[16] G. Parc, J. Rembur, P. Rech, D. Chriqui, Plant Cell Rep. 26 (2007) 145.
[17] R.J. Watts, D.D. Finn, L.M. Cutler, J.T. Schmidt, A.L. Teel, J. Contam. Hydrol. 91 (2007) 312.
[18] A. Bebot-Brigaud, C. Dange, N. Fauconnier, C. Gérard, J. Inorg. Biochem. 75 (1999) 71.
[19] B. Lonnerdal, Int. J. Food Sci. Technol. 37 (2002) 749.
[20] H.W. Lopez, F. Leenhardt, C. Coudray, C. Remesy, Int. J. Food Sci. Technol. 37 (2002) 727.
[21] M. Riche, D.L. Garling, Aquacol. Nutr. 10 (2004) 389.
[22] J. Torres, S. Dominguez, M.F. Cerda, G. Obal, A. Mederos, R.F. Irvine, A. Diaz, C. Kremer, J. Inorg. Biochem. 99 (2005) 828.
[23] J.N.A. Lott, in: D.R. Murray (Ed.), Seed Physiology, Academic Press, New York, 1984, p. 139.
[24] I. Ockenden, J.A. Dorsh, M.M. Reid, L. Lin, L.K. Grant, V. Raboy, J.N.A. Lott, Plant Sci. 167 (2004) 1131.
[25] N.R. Reddy, S.K. Sathe, D.K. Salunkhe, Adv. Food Res. 28 (1982) 1.
[26] E. Graf, K.L. Empson, J.W. Eaton, J. Biol. Chem. 262 (1987) 11647.
[27] P.T. Hawkins, D.R. Poyner, T.R. Jackson, A.J. Letcher, D.A. Lander, R.F. Irvine, Biochem. J. 294 (1993) 929.
[28] K. Midorikawa, M. Murata, S. Oikawa, Y. Hiraku, S. Kawanishi, Biochem. Biophys. Res. Commun. 288 (2001) 552.
[29] H.R. Park, H.J. Ahn, J.H. Kim, H.S. Yook, S. Kim, C.H. Lee, M.W. Byun, J. Agric. Food Chem. 52 (2004) 2572.
[30] E. Graf, J.W. Eaton, Cancer 56 (1985) 717.
[31] E. Graf, J.W. Eaton, Nutr. Cancer 19 (1993) 11.
[32] M. Jenab, L.U. Thompson, Carcinogenesis 19 (1998) 1087.
[33] A.M. Shamsuddin, Int. J. Food Sci. Technol. 37 (2002) 769.
[34] I. Vucenik, A. Passaniti, M.I. Vitolo, K. Tantivejkul, P. Eggleton, A.M. Shamsuddin, Carcinogenesis 25 (2004) 2115.
[35] I. Vucenik, A.M. Shamsuddin, Nutr. Cancer 55 (2006) 109.
[36] J.A. Munoz, M. Valiente, Anal. Chem. 75 (2003) 6374.
[37] J.A. Munoz, M. Valiente, Urol. Res. 33 (2005) 267.
[38] M.P. Jensen, K.L. Nash, L.R. Morss, E.H. Appleman, M.A. Schmidt, In: J.S. Gaffney, N.A. Marley, S.B. Clark (Eds.), Humic and Fulvic Acids—Isolation, Structure, and Environmental Role, ACS Symsium Series 651, Washinghton, 1996, p. 272.
[39] G.T. Tsao, Y. Zheng, J. Lu, C.S. Gong, Appl. Biochem. Biotechnol. 63–65 (1997) 731.
[40] J. Lehrfeld, J. Appl. Polym. Sci. 66 (1997) 491.
[41] A.J. Francis, C.J. Dodge, Environ. Sci. Technol. 32 (1998) 3993.
[42] K.L. Nash, M.P. Jensen, M.A. Schmidt, J. Alloys Compd. 271–273 (1998) 257.
[43] J.C. Seaman, J.M. Hutchison, B.P. Jackson, V.M. Vulava, J. Environ. Qual. 32 (2003) 153.
[44] U. Simsek, U. Ulusoy, J. Radioanal. Nucl. Chem. V261 (2004) 79.
[45] U. Ulusoy, S. Simsek, J. Hazard. Mater. 127 (2005) 163.
[46] R.J. Anderson, J. Biol. Chem. 17 (1914) 171.
[47] L.F. Johnson, M.E. Tate, Can. J. Chem. 47 (1969) 63.
[48] L.R. Isbrandt, R.P. Oertel, J. Am. Chem. Soc. 102 (9) (1980) 3144.
[49] C. Brigando, J.C. Mossoyan, F. Favier, D. Benlian, J. Chem. Soc., Dalton Trans. (1995) 575.
[50] L.G. Barrientos, P.P.N. Murty, Carbohydr. Res. 296 (1996) 39.
[51] A.T. Bauman, G.M. Chateauneuf, B.R. Boyd, R.E. Brown, P.P.N. Murthy, Tetrahedron Lett. 40 (1999) 4489.
[52] G. Paton, M. Noailly, J.C. Mossoyan, J. Phys. Org. Chem. 12 (1999) 401.
[53] D.M. Templeton, F. Ariese, R. Cornelis, L.G. Danielsson, H. Muntau, H.P. van Leeuwen, R. Lobinski, Pure Appl. Chem. 72 (2000) 1453.
[54] R. Barré, J.E. Courtois, G. Wormser, Bull. Soc. Chim. Biol. 36 (1954) 455.
[55] M.A. Marini, W.J. Evans, C.J. Martin, Anal. Lett. 14 (1981) 707.
[56] W.J. Evans, E.J. McCourtney, R.I. Shrager, JAOCS 59 (1982) 189.
[57] H. Bieth, B. Spiess, J. Chem. Soc., Faraday Trans. 82 (1986) 1935.
[58] H. Bieth, P. Jost, B. Spiess, C. Wehrer, Anal. Lett. 22 (1989) 703.
[59] N. Li, O. Wahlberg, I. Puigdomenech, L.O. Ohman, Acta Chem. Scand. 43 (1989) 331.
[60] N. Li, O. Wahlberg, Acta Chem. Scand. 43 (1989) 401.
[61] Z. Balogi, L.S. Làsztity, Acta Chem. Hung. 128 (1991) 393.
[62] K.S. Siddiqi, S.A. Shah, F.M.A.M. Aqra, S. Tabassum, S.A.A. Zaidi, D. Benlian, Ind. J. Chem. 32A (1993) 421.
[63] C. De Stefano, D. Milea, S. Sammartano, J. Chem. Eng. Data 48 (2003) 114.
[64] C. De Stefano, D. Milea, A. Pettignano, S. Sammartano, Anal. Bioanal. Chem. 376 (2003) 1030.
[65] C. De Stefano, D. Milea, S. Sammartano, Thermochim. Acta 423 (2004) 63.
[66] L. De Carli, N.D. Rosso, E. Schnitzler, P.I.B. Carneir, Cienc. Tecnol. Ali. 26 (2006) 19.
[67] P. Crea, C. De Stefano, D. Milea, N. Porcino, S. Sammartano, Biophys. Chem. 128 (2007) 176.

[68] F. Crea, P. Crea, C. De Stefano, D. Milea, S. Sammartano, J. Mol. Liq. 138 (2008) 76.

[69] C.J. Martin, W.J. Evans, J. Inorg. Biochem. 26 (1986) 169.

[70] P.G. Daniele, A. De Robertis, C. De Stefano, S. Sammartano, C. Rigano, J. Chem. Soc., Dalton Trans. (1985) 2353.

[71] P.G. Daniele, C. Rigano, S. Sammartano, Anal. Chem. 57 (1985) 2956.

[72] A. De Robertis, C. De Stefano, S. Sammartano, C. Rigano, Talanta 34 (1987) 933.

[73] C. De Stefano, O. Giuffrè, S. Sammartano, J. Chem. Eng. Data 50 (2005) 1917.

[74] M.T. Beck, I. Nagypàl, Chemistry of Complex Equilibria, Orwood, New York, 1990.

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

[76] E. Graf, J. Agric. Food Chem. 31 (1983) 851.

[77] C.J. Martin, W.J. Evans, J. Inorg. Biochem. 27 (1986) 17.

[78] K.B. Nolan, P.A. Duffin, B.J. McWeeny, J. Sci. Food Agric. 40 (1987) 79.

[79] C.J. Martin, W.J. Evans, J. Inorg. Biochem. 28 (1986) 39.

[80] E.T. Champagne, O. Hinojosa, J. Inorg. Biochem. 30 (1987) 15.

[81] C.J. Martin, W.J. Evans, J. Inorg. Biochem. 29 (1987) 241.

[82] C.J. Martin, J.W. Evans, J. Inorg. Biochem. 30 (1987) 101.

[83] M.A. Marini, W.J. Evans, N.M. Morrist, J. Appl. Biochem. 7 (1985) 180.

[84] J.W. Erdman, JAOCS 56 (1979) 736.

[85] M. Cheryan, Crit. Rev. Food Sci. Nutr. 13 (1980) 297.

[86] F. Grynspan, M. Cheryan, JAOCS 60 (1983) 1761.

[87] W.J. Evans, A.G. Pierce, JAOCS (September) (1981) 850.

[88] P. Crea, A. De Robertis, C. De Stefano, S. Sammartano, Biophys. Chem. 124 (2006) 18.

[89] A.E. Martell, R.D. Hancock, Metal Complexes in Aqueous Solutions, Plenum Press, New York, 1996.

[90] A. Odani, R. Takamido, O. Yamauchi, J. Inorg. Biochem. 67 (1997) 378.

[91] N. Li, O. Wahlberg, I. Puigdomenech, Chem. Scripta 29 (1989) 91.

[92] F. Crea, P. Crea, A. De Robertis, S. Sammartano, Chem. Spec. Bioavail. 16 (2004) 53.

[93] J.G. Hay, Cereal Chem. 19 (1942) 326.

[94] S. Posternak, Helv. Chim. Acta 4 (1921) 150.

[95] B.L. O'Dell, A.J. De Boland, J. Agric. Food. Chem. 24 (1976) 804.

[96] J.W. Evans, M.A. Marini, C.J. Martin, J. Inorg. Biochem. 19 (1983) 129.

[97] N. Veiga, J. Torres, S. Dominguez, A. Mederos, R.F. Irvine, A. Diaz, C. Kremer, J. Inorg. Biochem. 100 (2006) 1800.

[98] J.W. Evans, A.J. Pierce Jr., J. Food Sci. 47 (1982) 1014.

[99] N.M. Tamim, R. Angel, J. Agric. Food Chem. 51 (2003) 4687.

[100] D.D. Maenz, C.M. Engele-Schan, R.W. Newkirk, H.L. Classen, Anim. Feed Sci. Technol. 81 (1999) 177.

[101] W.J. Evans, C.J. Martin, J. Inorg. Biochem. 34 (1988) 11.

[102] H. Persson, M. Türk, M. Nyman, A.S. Sandberg, J. Agric. Food Chem. 46 (1998) 3194.

[103] E. Vasca, S. Materazzi, T. Caruso, O. Milano, C. Fontanella, C. Manfredi, Anal. Bioanal. Chem. 374 (2002) 173.

[104] C. De Stefano, D. Milea, N. Porcino, S. Sammartano, Anal. Bioanal. Chem. 386 (2006) 346.

[105] G. Biederman (Ed.), Dahlem Workshop on the Nature of Seawater, Dahlem Konferenzen, Berlin, 1975, p. 339.

[106] G. Biederman, in: E.A. Jenne, E. Rizzarelli, V. Romano, S. Sammartano (Eds.), Metal Complexes in Solution, Piccin, Padua, Italy, 1986, p. 303.

[107] E.T. Champagne, M.S. Fisher, J. Inorg. Biochem. 38 (1990) 217.

[108] N. Nosworthy, R.A. Caldwell, Sci. Food Agric. 44 (1988) 143.

[109] U.P. Rodrigues-Filho, S. Vaz, M.P. Felicissimo, M. Scarpellini, D.R. Cardoso, R.C.J. Vinhas, R. Landers, J.F. Schneider, B.R. McGarvey, M.L. Andersen, L.H. Skibsted, J. Inorg. Biochem. 99 (2005) 1973.

[110] A.G. Pierce Jr., Inorg. Chim. Acta 106 (1985) L9.

[111] C. De Stefano, D. Milea, N. Porcino, S. Sammartano, J. Agric. Food Chem. 54 (2006) 1459.

[112] A.E. Witter, D.A. Hutchins, A. Butler, G.W. Luther III, Mar. Chem. 69 (2000) 1.

[113] C. De Stefano, D. Milea, S. Sammartano, Biophys. Chem. 116 (2005) 111.

[114] F. Crea, P. Crea, D. Milea, N. Porcino, S. Sammartano, Ann. Chim. (Rome) 97 (2007) 635.

[115] A. Bencini, A. Bianchi, E. Garcia-Espana, M. Micheloni, J.A. Ramirez, Coord. Chem. Rev. 188 (1999) 97.

[116] A. De Robertis, C. De Stefano, C. Foti, O. Giuffrè, S. Sammartano, Talanta 54 (2001) 1135.

[117] E. Garcia-Espana, P. Diaz, J.M. Llinares, A. Bianchi, Coord. Chem. Rev. 250 (2006) 2952.

[118] C. De Stefano, O. Giuffrè, D. Milea, C. Rigano, S. Sammartano, Chem. Spec. Bioavail. 15 (2002) 29.

[119] B.I. Naik, S.K. Srivastava, Phytochemistry 17 (1978) 1885.

[120] A.E. Martell, R.M. Smith, R.J. Motekaitis, NIST Standard Reference Database 46, version 8, Gaithersburg, 2004.

[121] P.M. May, K. Murray, J. Chem. Eng. Data 46 (2001) 1035.

[122] L. Pettit, K.J. Powell, The IUPAC Stability Constants Database, Academic Software, 2001.