Speciation of Phytate Ion in Aqueous Solution. Protonation Constants in Tetraethylammonium Iodide and Sodium Chloride

Concetta De Stefano, Demetrio Milea, and Silvio Sammartano*

Dipartimento di Chimica Inorganica, Chimica Analitica e Chimica Fisica, Università di Messina,
Salita Sperone, 31, I-98166 Messina (Vill. S. Agata), Italy

Acid−base properties of phytic acid [1,2,3,4,5,6-hexakis(dihydrogen phosphate)-*myo*-inositol] in aqueous solution have been studied, by potentiometric measurements ([H^+]-glass electrode), in tetraethylammonium iodide (Et_4NI) and sodium chloride aqueous media, at different ionic strengths ($0 < I$ mol $L^{-1} \leq 1$ for Et_4NI and $0 < I$ mol $L^{-1} \leq 5$ for NaCl) and at $t = 25$ °C. Protonation of phytate ion proved strongly dependent on both ionic medium and ionic strength. The protonation constants obtained in NaCl are considerably lower than the corresponding ones in Et_4NI (e.g., at $I = 0.5$ mol·L^{-1}, log $K_3^H = 11.7$ and 9.1 in Et_4NI and in NaCl, respectively), owing to the strong interactions occurring between phytate and sodium cations present in the background salt. Some empirical equations are proposed for the dependence of protonation constants on ionic strength for both ionic media. Also, the function log K_i^H versus i (i = protonation step) can be modeled, and this allowed us to guess some protonation constants (in Et_4NI) at very high pH values.

Introduction

1,2,3,4,5,6-Hexakis(dihydrogen phosphate)-*myo*-inositol (IUPAC name), best known as phytic acid, was first identified in 1855. It is widely present in nature as calcium, magnesium, and potassium mixed salts (phytines), and it is the most important member of the wide and complex class of phytates, the major phosphorus-containing constituents of cereal grains. Also, other products have been shown to contain it: potatoes, some fruits, sunflowers, carrots, legumes, and others. Since, in 1903, the existence of this compound in seeds was first announced, it became the subject of investigation for many scientists in different fields. Research is addressed to understand its structure, its functional and nutritional properties, and its chemical behavior. Particularly, it is considered to be the principal source of reactive phosphate groups for living organisms, but it also decreases the bioavailability of dietary metal ions (e.g., calcium, iron, zinc, copper).[1−3] Moreover, owing to its very strong binding to deoxyhemoglobin, it is one of the most effective chemical agents for decreasing the affinity of hemoglobin for oxygen.[4] This behavior is due to the characteristic of phytate to form stable complexes with many metal ions, proteins, and starch. These complexes are often insoluble under physiological conditions or too strong to release nutritive principles, and so these are unavailable for living organisms.[2] Many experimental evidences demonstrate also antioxidant and anticarcinogenic properties of *myo*-inositol exaphosphoric acid. Moreover, it can be used in treating metals to prevent rust, as a replacement for cyanide or ammonium phosphate in etching solutions for offset printing, in toothpastes/mouthwashes, in dentistry (dental cements), and in antifreeze/cooling water or other closed systems for corrosion protection.[1] Owing to its ability to form highly insoluble complexes with many heavy metals and radio nuclides, it is also employed as a sequestering and immobilizing agent toward these dangerous classes of compounds.[5] Despite this, for many years, scientists found it very difficult to understand the real structure of the phytate molecule. There are many reasons for this: (i) commercial phytates often contain lower phosphate derivatives than hexaphosphate, resulting from steps in the biosynthesis or from chemical degradation in the isolation procedure; (ii) among hexaphosphates, nine stereoisomeric inositols are possible; (iii) isolation and purification are very difficult, and there is not a standard procedure; (iv) phytate structure depends strictly on counterion, pH, and system conditions.

However, recent NMR, Raman, and X-ray studies in aqueous solution and in solid state demonstrated that phytate exists in either of two conformations. Actually, is almost universally accepted that, in aqueous solution, the conformation is dependent mainly on pH, ionic medium, and ionic strength. This affects the acid−base properties and therefore all the interactions with the ionic medium.[4]

Many works dealing with the chemical properties of phytic acid are present in the literature.[1−12] Despite this, few papers report quantitative data on the acid−base properties and interactions with alkali metal ions.[3,13−21] To obtain information on the speciation of phytate in aqueous natural media, one must study its protonation in different supporting electrolytes at different ionic strengths and its complexes with major cationic components of natural fluids, that is, alkali and alkaline earth cations in different concentration conditions. On the contrary, most of the studies on the protonation of this polyanion have been performed at one ionic strength in a single ionic medium. Moreover, reported protonation constants from different laboratories are not in agreement.

To start a systematic study of the chemicophysical properties of phytic acid in aqueous solution, first we determined (at $t = 25$ °C by potentiometry, ISE-H^+) its protonation constants in tetraethylammonium iodide (Et_4NI)

---

* To whom correspondence should be addressed. E-mail: sammartano@chem.unime.it. Fax: +39-(0)90-392827.

10.1021/je020124m CCC: $25.00

Table 1. Experimental Values of Phytate Protonation Constants[a] in Et_4NI at Different Ionic Strengths and at $t = 25$ °C

I/mol·L^{-1}	log K_3^H	log K_4^H	log K_5^H	log K_6^H	log K_7^H
0.132	12.08 ± 0.02[b]	9.914 ± 0.009[b]	7.600 ± 0.008[b]	6.14 ± 0.01[b]	3.57 ± 0.01[b]
0.262	11.90 ± 0.01	9.784 ± 0.010	7.423 ± 0.009	6.056 ± 0.008	3.43 ± 0.01
0.478	11.68 ± 0.01	9.82 ± 0.01	7.490 ± 0.009	6.010 ± 0.009	3.57 ± 0.10
0.689	11.30 ± 0.02	9.81 ± 0.02	7.51 ± 0.02	6.17 ± 0.02	3.71 ± 0.03
0.905	11.14 ± 0.02	9.68 ± 0.02	7.50 ± 0.02	6.17 ± 0.02	3.70 ± 0.02

[a] K_i^H refers to the equilibrium $H^+ + H_{i-1}Phy^{(12-i+1)-} = H_iPhy^{(12-i)-}$. [b] ± standard deviation.

and sodium chloride media and at various ionic strengths ($0 < I$/mol·L$^{-1} \leq 1$ for Et_4NI and $0 < I$/mol·L$^{-1} \leq 5$ for NaCl). We chose these salts because the first is considered a noninteracting medium[22] and the second is the most important inorganic salt in natural fluids. Equations for the interpretation of the dependence of protonation constants on ionic strength are proposed too.

Experimental Section

Chemicals. Hydrochloric acid and sodium hydroxide solutions were prepared by diluting concentrated ampules (Fluka); tetraethylammonium hydroxide was prepared from concentrated Fluka solutions. Solutions of HCl and NaOH or Et_4NOH were standardized against sodium carbonate and potassium hydrogen phthalate, respectively. Et_4NI and NaCl were prepared by weighing the pure salts (Fluka) dried in an oven at 110 °C. Et_4NI was previously purified by recrystallization from methanol. Phytic acid solutions were prepared by weighing the dipotassium salt from Aldrich successively passed on a strong exchange cationic resin (Dowex 50W × 8). Concentration was checked potentiometrically, and potassium absence was proved by flame emission spectrometry. All solutions were prepared with analytical grade water ($R = 18$ MΩ·cm^{-1}), using grade A glassware.

Apparatus and Procedure. Potentiometric titrations were carried out (at 25.0 ± 0.1 °C) using an apparatus consisting of a model 713 Metrohm potentiometer, equipped with a combined glass electrode (Ross type 8102, from Orion), and a model 765 Metrohm motorized buret. The estimated accuracy was ±0.2 mV and ±0.003 mL for emf and titrant volume readings, respectively. The apparatus was connected to a PC, and automatic titrations were performed using a suitable computer program to control titrant delivery and data acquisition and to check for emf stability. All titrations were carried out under magnetic stirring and bubbling through the solution purified presaturated N_2, to exclude O_2 and CO_2 inside. Potentiometric measurements were carried out by titrating 25 mL of the solution containing phytic acid (0.8−2 mmol·L^{-1}), an excess of hydrochloric acid (1.5−3 mmol·L^{-1}), and the background salt in order to obtain pre-established ionic strength values (Et_4NI, $0.1 \leq I \leq 1.0$ mol·L^{-1}; NaCl, $0.1 \leq I \leq 5.0$ mol·L^{-1}) with standard Et_4NOH (for measurements in Et_4NI) or NaOH (for measurements in NaCl) solutions up to pH 11.5. For each experiment, independent titrations of strong acid solution with standard base were carried out under the same medium and ionic strength conditions as those of the systems to be investigated, with the aim of determining electrode potential ($E°$) and acidic junction potential ($E_j = j_a[H^+]$). The reliability of the calibration in the alkaline range was checked by calculating pK_w values. Preliminary measures gave evidence for a reduction of electrode performance when an intensive use in phytic acid solutions was made. This aspect is probably due to reversible alterations of the junction membrane of the electrode. We overcame this problem using two different electrodes for two consecutive measures: while one was working, the other one was regenerated with a recommended procedure.

Calculations. The BSTAC[23] and STACO[24] computer programs were used in the refinement of all the parameters of an acid−base titration ($E°$, pK_w, coefficient of junction potential j_a, analytical concentration of reagents) and in the calculation of complex formation constants. The ES4ECI[25] program was used to draw distribution diagrams and to calculate species formation percentages. The LIANA[26] program was used to test the dependence of log K on ionic strength using different equations.

Protonation constants, K_i^H, are given according to the equilibrium

$$H^+ + H_{i-1}Phy^{(12-i+1)-} = H_iPhy^{(12-\mathbf{i})-} \qquad K_i^H$$

and are expressed in the molar (mol·L^{-1}) concentration scale.

Results and Discussion

Protonation Constants in Et_4NI and NaCl. First measurements were carried out using tetraethylammonium iodide as background salt (in the ionic strength range $0.1 \leq I \leq 1.0$ mol·L^{-1}), to obtain protonation constants in a noninteracting medium. Values obtained at various ionic strengths for all constants are reported in Table 1.

In our experimental conditions, we obtained only values of species $H_iPhy^{(12-i)-}$ with $3 \leq i \leq 7$: according to some literature data, in fact, the first two protonation steps are too strong (we just obtained log $K_3^H = 12.08$ at $I = 0.132$ mol·L^{-1}) and the last five steps are very weak (log $K^H \leq 2$).[3,16,18] Protonation constants are a regularly decreasing function of i with Δ log K_i^H = log $K_{(i+1)}^H$ − log $K_i^H \approx 2.2$ at $I = 0.1$ (mol·L^{-1}) and 1.9 at $I = 0.9$ (mol·L^{-1}). A lowering effect of ionic strength on log K_i^H values is also observed which is more effective for protonation steps in the alkaline range.

To evaluate the dependence of phytate protonation on ionic medium, we performed the same measurements using NaCl, the most important inorganic salt in natural fluids (seawater, biological fluids, etc.), as background salt. The wide range of investigated ionic strengths ($0.1 \leq I \leq 5.0$ mol·L^{-1}) allows us to extend all conclusions to other aqueous systems (hypersaline waters, industrial wastes, etc). As expected, and in accordance with literature data, the constants obtained (see Table 2) are considerably lower than the corresponding ones in tetraethylammonium iodide (e.g., at $I = 0.5$ mol·L^{-1}, log $K_3^H = 11.7$ and 9.1 in Et_4NI and in NaCl, respectively), owing to the strong interactions occurring between phytate and sodium cations present in the background salt.[17−19] This aspect made it possible to obtain values of the first two protonation steps that are too strong in Et_4NI. It is also important to put in evidence how in NaCl medium there is an inversion in the strength of the first two protonation constants. This aspect has already been reported in the literature and well explained.[17,27]

Table 2. Experimental Values of Phytate Protonation Constants[a] in NaCl 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
0.186	9.22 ± 0.11[b]	9.89 ± 0.12[b]	9.24 ± 0.05[b]	7.93 ± 0.02[b]	6.28 ± 0.02[b]	5.08 ± 0.03[b]	2.70 ± 0.04[b]
0.268	9.31 ± 0.08	9.40 ± 0.10	9.22 ± 0.06	7.86 ± 0.03	6.23 ± 0.03	5.01 ± 0.03	2.76 ± 0.06
0.501	9.22 ± 0.04	8.86 ± 0.08	9.05 ± 0.06	7.49 ± 0.02	5.91 ± 0.02	4.67 ± 0.02	2.37 ± 0.05
0.503	8.89 ± 0.06	9.24 ± 0.07	8.81 ± 0.04	7.37 ± 0.02	5.81 ± 0.02	4.52 ± 0.02	2.21 ± 0.04
0.711	8.81 ± 0.10	9.04 ± 0.12	8.60 ± 0.07	7.35 ± 0.03	5.74 ± 0.03	4.36 ± 0.04	2.16 ± 0.05
0.929	8.66 ± 0.05	8.95 ± 0.06	8.51 ± 0.03	7.09 ± 0.02	5.57 ± 0.01	4.33 ± 0.02	1.82 ± 0.07
1.378	8.52 ± 0.03	8.87 ± 0.03	8.36 ± 0.02	7.09 ± 0.01	5.57 ± 0.01	4.33 ± 0.01	2.20 ± 0.03
1.825	8.64 ± 0.05	8.69 ± 0.07	8.36 ± 0.04	7.02 ± 0.02	5.51 ± 0.02	4.30 ± 0.02	2.40 ± 0.06
2.746	8.42 ± 0.03	8.73 ± 0.03	8.18 ± 0.02	6.89 ± 0.01	5.41 ± 0.01	4.23 ± 0.02	2.48 ± 0.03
4.559	8.42 ± 0.03	8.79 ± 0.03	8.13 ± 0.02	6.87 ± 0.01	5.46 ± 0.01	4.31 ± 0.01	2.53 ± 0.02

[a] K_i^H refers to the equilibrium $H^+ + H_{i-1}Phy^{(12-i+1)-} = H_iPhy^{(12-i)-}$. [b] ± standard deviation.

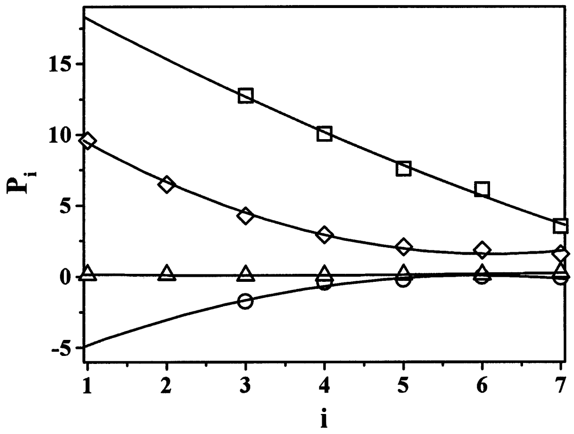

Figure 1. P_i parameters (eq 3) vs phytate protonation step: □, log $^TK_i^H$; ○, B_i; △, C_i; ◇, D_{iNa}.

Table 3. Parameters of the Equation $P_i = P_0 + A_1i + A_2i^2$ (Eq 3)

P_i	P_0	A_1	A_2	σ[b]
log $^TK_i^H$	21.18 ± 0.07[a]	−3.10 ± 0.06[a]	0.086 ± 0.009[a]	0.26
B_i	−6.82 ± 0.06	2.31 ± 0.06	−0.19 ± 0.01	0.16
C_i	0.22 ± 0.01	−0.09 ± 0.01	0.013 ± 0.002	0.02
D_{iNa}	12.78 ± 0.03	−3.67 ± 0.03	0.299 ± 0.008	0.18

[a] ± standard deviation. [b] Standard deviation of the fit.

Table 4. Empirical Parameters of Eqs 1 (log K_i^H = log$^TK_i^H$ + $B_iI^{1/2}$ + C_iI) and 2 (logK_i^H = log$^TK_i^H$ + C_iI − log(1 + $10^{D_{iNa}}I$))

i	log $^TK_i^H$	B_i	C_i	D_{iNa}
1	(18.17)	(−4.70)	0.13 ± 0.02[a]	9.59 ± 0.03[a]
2	(15.32)	(−2.96)	0.12 ± 0.02	6.50 ± 0.04
3	12.76 ± 0.09[a]	−1.76 ± 0.13[a]	0.06 ± 0.01	4.27 ± 0.09
4	10.05 ± 0.08	−0.42 ± 0.12	0.08 ± 0.01	2.91 ± 0.08
5	7.59 ± 0.08	−0.22 ± 0.10	0.13 ± 0.01	2.06 ± 0.08
6	6.13 ± 0.09	−0.10 ± 0.13	0.14 ± 0.02	1.83 ± 0.10
7	3.52 ± 0.10	−0.08 ± 0.12	0.27 ± 0.02	1.56 ± 0.11

[a] ± standard deviation.

Dependence of Protonation Constants on Ionic Medium and on Ionic Strength. By analyzing all the experimental data, the dependence of protonation constants on ionic medium and on ionic strength can be studied. Using the LIANA program, we treated values obtained for single constants in both ionic media (only $H_iPhy^{(12-i)-}$ with $3 \le i \le 7$) altogether and we found (a) that, for Et$_4$NI medium, the dependence can be expressed with the simple relationship

$$\log K_i^H = \log {}^TK_i^H + B_iI^{1/2} + C_iI \qquad (1)$$

and (b) that, for NaCl medium, the dependence is better represented by a similar equation where, in addition, another logarithmic term is to be considered

$$\log K_i^H = \log {}^TK_i^H + C_iI - \log(1 + 10^{D_{iNa}}I) \qquad (2)$$

In these equations K_i^H is the protonation constant, $^TK_i^H$ is the protonation constant at infinite dilution, and B_i, C_i, and D_{iNa} are empirical parameters. The parameter D_{iNa} can be considered as a measure of the interaction between Na$^+$ and $H_iPhy^{(12-i)-}$, by considering the analogy with the equation that describes the lowering effect on the protonation constant for a monoprotic acid, due to the weak association of the cation M$^+$ of the supporting electrolyte[22]

$$\log K_i^H = \log K_i^{H*} - \log(1 + 10^{\log K^M}I)$$

where K^{H*} is the apparent protonation constant and K^M is the formation constant for the species M$^+$−anion of monoprotic acid. For polyprotic acids, slightly more complicated equations must be used,[22] and in particular, when $n > 2$ (n = number of protonable groups), data must be handled with care by considering the different activity coefficients in different ionic media.

The parameters of eqs 1 and 2 are regular functions of the protonation step i, as can be seen in Figure 1 and as expressed by the relationship

$$P_i = P_0 + A_1i + A_2i^2 \qquad (3)$$

with P_i = log $^TK_i^H$, B_i, C_i, and D_{iNa}. The coefficients of eq 3 are reported in Table 3 for all parameters of eqs 1 and 2 with the corresponding standard deviation for each parameter and for the whole fit. By using eq 3, it is also possible to calculate the extrapolated values of log $^TK_i^H$, B_i, and C_i (i = 1, 2) in Et$_4$NI, which are not experimentally accessible. All the empirical parameters for the different protonation steps are reported in Table 4.

Using eqs 1 and 2, it is possible to calculate protonation constants at different ionic strengths. Results are shown in Tables 5 and 6. The good agreement, within experimental errors, between experimental and calculated data is evidenced in Figure 2, where values referred to the third constant in the different media are plotted versus ionic strength. This, in turn, demonstrates the goodness of the proposed model. Differences in the values of protonation constants in the two ionic media confirm also how the nature of the ionic medium affects the speciation of phytate, as can be noted by comparing Figures 3 and 4, where distribution diagrams versus the pH of the protonated phytate species in Et$_4$NI and NaCl, respectively, are shown. As can be seen, speciation diagrams are quite different in the two media: curves for different H_iPhy species in NaCl

Table 5. Calculated Values (Eq 1[a]) of Phytate Protonation Constants[b] in Et_4NI 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	(16.70)	(14.40)	12.21 ± 0.06[c]	9.92 ± 0.04[c]	7.53 ± 0.05[c]	6.11 ± 0.05[c]	3.53 ± 0.06[c]
0.15	(16.37)	(14.19)	12.09 ± 0.05	9.90 ± 0.04	7.52 ± 0.04	6.11 ± 0.05	3.53 ± 0.05
0.25	(15.85)	(13.87)	11.90 ± 0.04	9.86 ± 0.03	7.51 ± 0.03	6.11 ± 0.04	3.55 ± 0.04
0.50	(14.91)	(13.29)	11.55 ± 0.03	9.79 ± 0.03	7.50 ± 0.02	6.12 ± 0.03	3.61 ± 0.03
0.75	(14.20)	(12.85)	11.28 ± 0.03	9.75 ± 0.04	7.50 ± 0.02	6.14 ± 0.04	3.66 ± 0.03
1.00	(13.60)	(12.48)	11.06 ± 0.05	9.71 ± 0.05	7.50 ± 0.03	6.16 ± 0.05	3.72 ± 0.04

[a] $\log K_i^H = \log {}^TK_i^H + B_i I^{1/2} + C_i I$. [b] K_i^H refers to the equilibrium $H^+ + H_{i-1}Phy^{(12-i+1)-} = H_iPhy^{(12-i)-}$. [c] ± standard deviation.

Table 6. Calculated Values (Eq 2[a]) of Phytate Protonation Constants[b] in NaCl 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	9.58 ± 0.03[c]	9.84 ± 0.04[c]	9.50 ± 0.02[c]	8.14 ± 0.02[c]	6.50 ± 0.02[c]	5.25 ± 0.02[c]	2.88 ± 0.03[c]
0.15	9.41 ± 0.03	9.67 ± 0.04	9.33 ± 0.02	7.97 ± 0.02	6.35 ± 0.02	5.10 ± 0.02	2.75 ± 0.03
0.25	9.20 ± 0.03	9.46 ± 0.04	9.11 ± 0.02	7.76 ± 0.02	6.15 ± 0.02	4.90 ± 0.02	2.59 ± 0.03
0.50	8.93 ± 0.02	9.19 ± 0.03	8.83 ± 0.02	7.48 ± 0.02	5.88 ± 0.02	4.65 ± 0.02	2.37 ± 0.03
0.75	8.78 ± 0.02	9.04 ± 0.03	8.67 ± 0.02	7.32 ± 0.02	5.74 ± 0.02	4.51 ± 0.02	2.27 ± 0.03
1.00	8.69 ± 0.02	8.95 ± 0.03	8.56 ± 0.02	7.21 ± 0.01	5.65 ± 0.01	4.42 ± 0.02	2.22 ± 0.03
1.50	8.58 ± 0.02	8.83 ± 0.03	8.41 ± 0.02	7.08 ± 0.01	5.54 ± 0.01	4.32 ± 0.02	2.19 ± 0.03
2.00	8.52 ± 0.03	8.77 ± 0.03	8.32 ± 0.02	6.99 ± 0.01	5.48 ± 0.01	4.26 ± 0.02	2.20 ± 0.03
3.00	8.47 ± 0.04	8.71 ± 0.05	8.21 ± 0.02	6.89 ± 0.02	5.43 ± 0.02	4.22 ± 0.03	2.30 ± 0.04
5.00	8.50 ± 0.08	8.74 ± 0.09	8.12 ± 0.03	6.83 ± 0.04	5.47 ± 0.03	4.27 ± 0.05	2.63 ± 0.07

[a] $\log K_i^H = \log {}^TK_i^H + C_i I - \log(1 + 10^{D_{iNa}} I)$. [b] K_i^H refers to the equilibrium $H^+ + H_{i-1}Phy^{(12-i+1)-} = H_iPhy^{(12-i)-}$. [c] ± standard deviation.

Table 7. Literature Data for Phytate Protonation Constants

ref	technique	ionic medium	I[a]	log K_1	log K_2	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}
13	pot.	Na^+		9.7	9.7	9.7	9.7	6.3	6.3	1.84	1.84	1.84	1.84	1.84	1.84
14[b]	^{31}P NMR	n-Bu_4N^+	0.01	12.0	10.0	10.0	7.60	6.85	5.70	2.1	2.1	1.7	1.5	1.5	1.1
15	pot.	KCl	0.2	9.53	9.53	9.19	7.98	6.25	5.20	3.16	2.38	2.38	1.92	1.92	1.92
16	pot.	Et_4NClO_4	0.1	>12	>12	>12	11.47	8.12	6.51	4.00	2.68	1.80	<1.50	<1.50	<1.50
17	pot.	$NaClO_4$	0.15	8.59	10.53	9.02	7.82	6.13	4.88	2.49	1.98				
17	pot.	$NaClO_4$	0.3	8.53	10.00	8.72	7.51	5.82	4.57	2.18	1.92				
17	pot.	$NaClO_4$	1	8.41	9.19	8.29	7.03	5.38	4.14	1.77	1.80				
17	pot.	$NaClO_4$	3	8.29	8.62	8.01	6.61	5.07	3.86	1.52	1.63				
18[c]	pot.	n-Bu_4NBr	0.1			12.00	11.50	7.97	6.41						
18[c]	pot.	LiCl/n-Bu_4NBr	0.1			9.64	9.02	7.05	5.76						
18[c]	pot.	NaCl/n-Bu_4NBr	0.1			9.64	9.31	7.23	6.01						
18[c]	pot.	KCl/n-Bu_4NBr	0.1			9.81	9.47	7.17	5.78	-					
19	pot.	$NaClO_4$	0.2	9.61	9.41	9.39	8.02	6.23	4.96	2.65					
19	pot.	$NaClO_4$	2	9.24	8.94	8.92	7.57	5.88	4.68	2.71					
20	pot.	$NaClO_4$	0.1	10.60	8.40	8.03	6.03	4.88	4.36	3.89	3.43	2.82	2.48	1.20	
21	pot.	n-Bu_4NBr	0.54	11.76	11.08	10.54	9.08	7.15	5.98	3.63	2.58	1.57	0.82	0.4	−0.14
3[d]	pot.	Et_4NClO_4	0.17	>13	>13	12.3	9.92	7.42	6.13	3.59	2.0	2.4	1.0	<1	<1
tw	pot.	NaCl	1	8.69	8.95	8.56	7.21	5.65	4.42	2.22					
tw	pot.	Et_4NI	0.15	16.37	14.19	12.09	9.90	7.52	6.11	3.53					

[a] mol·L^{-1}. [b] $t = 28$ °C. [c] 0.01 mol·L^{-1} ≤ C_{Li}, C_{Na}, C_K ≤ 0.02 mol·L^{-1}. [d] $t = 20$°; tw = this work.

aqueous solution are systematically shifted to lower values, as a consequence of proton displacement, likely due to Na^+−phytate ion pair formation. Moreover, phytate in Et_4NI is never present in its completely deprotonated (dodecanionic) form, and in NaCl the dodecanionic form exists only at pH > 12. Protonation data reported in this work can be used in speciation studies that refer to both not interacting (Et_4NI) and interacting (NaCl) media. An attempt was made to express the dependence on ionic strength in terms of SIT or Pitzer equations,[28] but owing to the very high charge of phytate, it is necessary to modify these equations. This will be the subject of a further contribution.

Comparisons with Literature. As above-mentioned, literature data concerning the protonation of phytate are often not homogeneous. Differences become clear when comparing the values shown in Table 7, in which the main results of the last 50 years are presented. One of the first ones dates back to 1954.[13] Except for the article of Bieth et al.,[18] all measurements were performed using a single ionic medium. Moreover, only two papers report data at different ionic strengths, but in no one were systematic

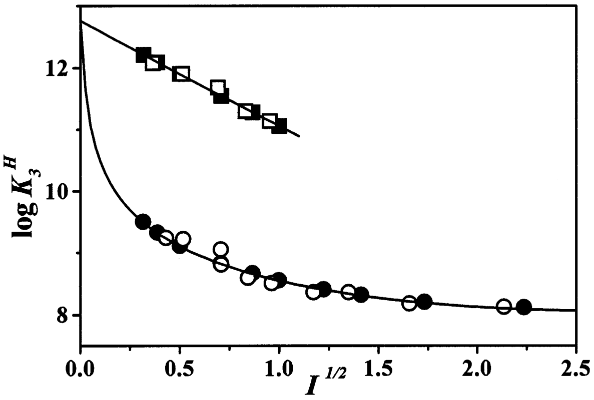

Figure 2. Experimental and calculated data for the third protonation constant vs ionic strength: ■, Et_4NI (calculated); ●, NaCl (calculated); □, Et_4NI (experimental); ○, NaCl (experimental).

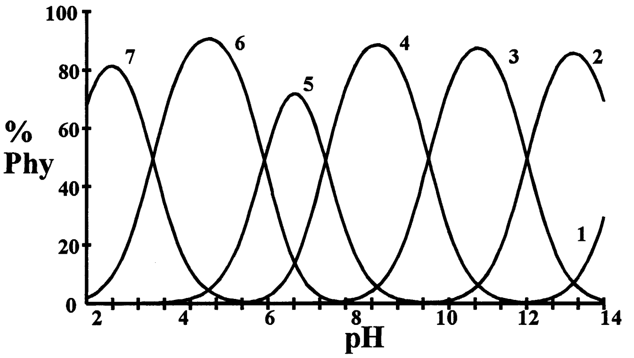

Figure 3. Distribution diagram of the Phy^{12-}−H^{+} species vs pH, in Et$_4$NI at $t = 25$ °C ($C_{\mathrm{Phy}} = 0.01$ mol·L^{-1}; $C_{\mathrm{Et4NI}} = 0.1$ mol·L^{-1}). i refers to the species H$_i$(Phy)$^{(12-i)-}$.

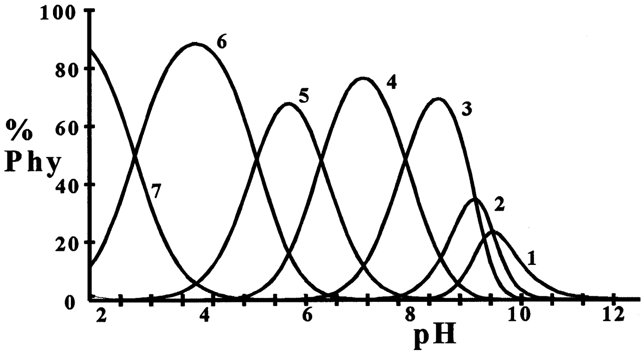

Figure 4. Distribution diagram of the Phy^{12-}−H^{+} species vs pH, in NaCl at $t = 25$ °C ($C_{\mathrm{Phy}} = 0.01$ mol·L^{-1}; $C_{\mathrm{NaCl}} = 0.1$ mol·L^{-1}). i refers to the species H$_i$(Phy)$^{(12-i)-}$.

studies on the phytate dependence on ionic strength made.[17,19] So, this work may be considered as the first scientific contribution in which a systematic approach to the comprehension of the acid−base properties of phytic acid in aqueous solution is proposed.

However, when Table 7 is carefully analyzed, some interesting aspects become clear. For example, the constant values obtained by Li et al.[17] in NaClO$_4$ show the same behavior as those reported by us in NaCl. In both, in fact, an inversion in the strength of first two protonation constants (when Na^{+} is the cation of the background salt) is found. This can be explained in terms of an extra stability of H$_2$Phy^{10-} species with respect to HPhy^{11-}, due to the formation of intramolecular hydrogen bonds in the two-protonated phytate ions, promoted by the conformation that phytate has when sodium ions are present. The two papers are similar with regard to not only the inversion of the strengths of the protonation constants but also the actual values: for example, Li et al. find log $K_4^{\mathrm{H}} = 7.0$ at $I = 1$ mol·L^{-1}, and we find 7.2. Other literature data are fairly consistent with the data included here. Bebot-Brigaud et al.[3] report, at $I = 0.17$ mol·L^{-1} in Et$_4$N^{+} (but at $t = 20$ °C), these values: $>$13 (16.37), $>$13 (14.19), 12.3 (12.09), 9.92 (9.90), 7.42 (7.52), 6.13 (6.11), 3.59 (3.53), and so forth. In parentheses the corresponding values calculated at $I = 0.15$ mol·L^{-1} in this work are reported.

Literature Cited

(1) Oatway, L.; Vasanthan, T.; Helm, J. H. Phytic Acid. *Food Rev. Int.* **2001**, *17*, 419−431 and references therein.

(2) Maga, J. A. Phytate: Its Chemistry, Occurrence, Food Interactions, Nutritional Significance, and Methods of Analysis. *J. Agric. Food Chem.* **1982**, *30*, 1−9 and references therein.

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

(4) Isbrandt, L. R.; Oertel, R. P. Conformational States of *myo*-Inositol Hexakis(phosphate) in Aqueous Solution. A ^{13}C NMR, ^{31}P NMR, and Raman Spectroscopic Investigation. *J. Am. Chem. Soc.* **1980**, *102*, 3144−3148 and references therein.

(5) Jensen, M. P.; Nash, K. L.; Morss, L. R.; Appleman, E. H.; Schmidt, M. A. Immobilization of Actinides in Geomedia by Phosphate Precipitation. In *Humic and Fulvic Acids—Isolation, Structure and Environmental Role*; Gaffney, J. S., Marley, N. A., Clark, S. B., Eds.; ACS Symposium Series 651; American Chemical Society: Washington, DC, 1996; pp 272−285.

(6) Johnson, L. F.; Tate, M. E. Structure of “phytic acids”. *Can. J. Chem.* **1969**, *47*, 63−73.

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

(8) Martin, C. J.; Evans, W. J. Phytic Acid: Divalent Cation Interactions. III. A Calorimetric and Titrimetric Study of the pH Dependence of Copper(II) Binding. *J. Inorg. Biochem.* **1986**, *28*, 39−55.

(9) Paton, G.; Noailly, M.; Mossoyan, J. C. Conformational preferences and intramolecular interactions of *myo*-inositol hexakisphosphoric acid by ^{1}H and ^{31}P NMR studies. *J. Phys. Org. Chem.* **1999**, *12*, 401−407.

(10) Harland, B. F.; Narula, G. Food Phytate and Its Hydrolysis Products. *Nutr. Res.* **1999**, *19*, 947−961.

(11) Bauman, A. T.; Chateauneuf, G. M.; Boyd, B. R.; Brown, R. E.; Murthy, P. P. N. Conformational Inversion Processes in Phytic Acid: NMR Spectroscopic and Molecular Modelling Studies. *Tetrahedron Lett.* **1999**, *40*, 4489−4492.

(12) Blank, G. E.; Pletcher, J.; Sax, M. Hemoglobin Cofactors. I. The Crystal Structure of Myoinositol Hexaphosphate Dodecasodium Salt Octatriacontahydrate. *Acta Crystallogr., Sect. B* **1975**, *31*, 2584−2592.

(13) Barré, R.; Courtois, J. E.; Wormser, G. Etude de la structure de l'acide phytique au moyen de ses courbes de titration et de la conductivité de ses solutions. *Bull. Soc. Chim. Biol.* **1954**, *36*, 455−474.

(14) Costello, A. J. R.; Glonek, T.; Myers, T. C. ^{31}P Nuclear Magnetic Resonance−pH Titrations of *myo*-Inositol Hexaphosphate. *Carbohydr. Res.* **1976**, *46*, 159−171.

(15) Evans, W. J.; McCourtney, E. J.; Shrager, R. I. Titration Studies of Phytic Acid. *J. Am. Oil Chem. Soc.* **1982**, *59*, 189−191.

(16) Bieth, H.; Spiess, B. A Comparative Study of the Protonation of *myo*-Inositol Hexakis(phosphate). *J. Chem. Soc., Faraday Trans.* **1986**, *82*, 1935−1943.

(17) Li, N.; Wahlberg, O. Equilibrium Studies of Phytate Ions. 2. Equilibria Between Phytate Ions, Sodium Ions and Protons in Sodium Perchlorate Media. *Acta Chem. Scand.* **1989**, *43*, 401−406.

(18) Bieth, H.; Jost, P.; Spiess, B.; Wehrer, C. Effect of the Alkalimetal cations on the Protonation Constants of *myo*-Inositol Hexakis(phosphate). *Anal. Lett.* **1989**, *22*, 703−717.

(19) Balogi, Z.; Làsztity, L. S. Potentiometric Evaluation of the Protonation Constants of Phytic Acid. *Acta Chim. Hung.* **1991**, *128*, 393−400.

(20) Siddiqi, K. S.; Shah, S. A.; Aqra, F. M. A. M.; Tabassum, S.; Zaidi, S. A. A.; Benlian, D. Equilibrium studies on interactions of rare earth ions with phytic acid. *Ind. J. Chem.* **1993**, *32A*, 421−423.

(21) Brigando, C.; Mossoyan, J. C.; Favier, F.; Benlian, D. Conformational Preferences and Protonation Sequence of *myo*-Inositol Hexaphosphate in Aqueous Solution; Potentiometric and Multinuclear Magnetic Resonance. *J. Chem. Soc., Dalton Trans.* **1995**, 575−578.

(22) Daniele, P. G.; De Robertis, A.; De Stefano, C.; Sammartano, S.; Rigano, C. On the Possibility of Determining the Thermodynamic Parameters for the Formation of Weak Complexes Using a Simple Model for the Dependence on Ionic Strength of Activity Coefficients. Na^{+}, K^{+} and Ca^{2+} Complexes of Low Molecular Weight Ligands in aqueous Solution. *J. Chem. Soc., Dalton Trans.* **1985**, 2353−2361.

(23) De Stefano, C.; Mineo, P.; Rigano, C.; Sammartano, S. Ionic Strength Dependence of Formation Constants. XVII. The Calculation of Equilibrium Concentrations and Formation Constants. *Ann. Chim. (Rome)* **1993**, *83*, 243−277.

(24) De Stefano, C.; Foti, C.; Giuffrè, O.; Mineo, P.; Rigano, C.; Sammartano, S. Binding of Tripolyphosphate by Aliphatic Amines: Formation, Stability and Calculation Problems. *Ann. Chim. (Rome)* **1996**, *86*, 257−280.

(25) De Robertis, A.; De Stefano, C.; Rigano, C.; Sammartano, S. The Calculation of Equilibrium Concentrations in Large Multimetal/Multiligand Systems. *Anal. Chim. Acta* **1986**, *191*, 385−398.

(26) De Stefano, C.; Sammartano, S.; Mineo, P.; Rigano, C. Computer Tools for the Speciation of Natural Fluids. In *Marine Chemistry—An Environmental Analytical Chemistry Approach*; Gianguzza, A., Pellizzetti, E., Sammartano, S., Eds.; Kluwer Academic Publishers: Amsterdam, 1997; pp 71−83.
(27) Li, N.; Wahlberg, O.; Puigdomenech, I.; Ohman, L. O. Equilibrium Studies of Phytate Ions. 1. Equilibria Between Phytate Ions and Protons in 3M $NaClO_4$ Medium. *Acta Chem. Scand.* **1989**, *43*, 331−339.
(28) Grenthe, I.; Puigdomenech, I. *Modelling in Aquatic Chemistry*; OECD: Paris, 1997; and references therein.

Received for review June 28, 2002. Accepted September 24, 2002. We thank the Italian Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR), for financial support.

JE020124M