Available online at www.sciencedirect.com

SCIENCE DIRECT®

Thermochimica Acta 423 (2004) 63–69

thermochimica acta

www.elsevier.com/locate/tca

# Speciation of phytate ion in aqueous solution Thermodynamic parameters for protonation in NaCl[☆]

Concetta De Stefano, Demetrio Milea, Silvio Sammartano*

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

Received 21 January 2004; received in revised form 19 April 2004; accepted 21 April 2004

## Abstract

Phytate protonation enthalpies and entropies were determined by direct calorimetric titrations, in sodium chloride aqueous media at different ionic strengths ($0.1 \le I/\text{mol L}^{-1} \le 1$) and at $t = 25\,^{\circ}\text{C}$. Only the first seven protonation steps were taken into account, since the remaining five protons of phytic acid could be considered as "strongly acidic" ($\log K_i^H \le 1,\ 8 \le i \le 12$). The analysis of results evidenced slight variations among enthalpy and entropy values, for the same protonation step (i), at different ionic strengths. $T\Delta S_i$ values for each protonation step are significantly higher than the corresponding enthalpy changes, revealing the entropic nature of the bond involved in phytate protonation. The enthalpy changes for phytate protonation generally increase for each protonation step, as typical for phosphate ligands. Dependence on ionic strength for both $\log K_i^H$ and $\Delta H_i$ was taken into account by Specific ion Interaction Theory (SIT). Useful predictive relationships for the dependence of $T\Delta S$ on $\Delta G$ and on protonation step "*i*" were proposed.

*Keywords:* Phytate; Protonation; Dependence on ionic strength; Thermodynamic parameters; Predictive relationships; Calorimetry

## 1. Introduction

Since the announcement, in 1903, of the massive presence, in several seeds, of 1,2,3,4,5,6 hexakis (di-hydrogen phosphate) *myo*-inositol (IUPAC name), best known as phytic acid, many papers and reviews dealing with the chemico-physical properties and the several biological functions have been published [1–3 and references therein]. Despite this, few papers report quantitative results on the acid–base properties or on the interactions with alkali metal ions, and data published by different laboratories are often inconsistent [4–19]. Moreover, most experiments have been performed at a single ionic strength and in a single ionic medium, and, therefore, are of low interest for speciation studies in natural fluids. For this reason, our research group undertook a systematic study on the chemico-physical behavior of phytic acid in aqueous solutions in various ionic media and at different ionic strengths. We determined: (i) the protonation constants [20,21]; (ii) the complex formation constants with alkali metal cations [21]; (iii) the binding ability towards other ligands of biological or environmental interest as, for example, biogenic amines [22]; and (iv) the solubility of some calcium complexes [23]. In these studies, several equations for the dependence of equilibrium constants on ionic medium and/or ionic strength have been proposed, together with many different relationships with predictive purposes. Comparisons among phytic acid and other ligands have also been made.

As a further contribution to phytate speciation in different aqueous media, the results of a calorimetric investigation on phytate protonation in sodium chloride, the most important inorganic salt in natural fluids, are reported in this paper. Phytate protonation enthalpies and entropies were determined by direct calorimetric titrations at different ionic strengths ($0.1 \le I/\text{mol L}^{-1} \le 1$). Some papers dealing with complex formation enthalpies between phytate and metal cations have been published [9–11] and a few data are available on phytate protonation enthalpies and entropies in aqueous solutions [6,9], but, to our knowledge, no experiments have been performed in NaCl media. Here, comparisons

---

[☆] Previous contributions to this series: [20–23].

[*] Corresponding author. Tel.: +39-090-393659; fax: +39-090-392827.
*E-mail address:* sammartano@chem.unime.it (S. Sammartano).

0040-6031/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.tca.2004.04.017

among results obtained for protonation of phytate and for other phosphoric and carboxylic ligands are made, and some useful relationships are proposed.

## 2. Experimental section

### 2.1. Chemicals

Hydrochloric acid and sodium hydroxide solutions were prepared by diluting concentrated ampoules (Riedel de Haen). Solutions of HCl and NaOH were standardized against sodium carbonate and potassium hydrogen phthalate, respectively. NaCl was prepared by weighing pure salt (Fluka) dried in an oven at 110 °C. Phytic acid dodecasodium salt solution (sodium phytate, $Na_{12}Phy$) was prepared by weighing SIGMA product. Concentration was checked by acidimetric titrations. All solutions were prepared with analytical grade water ($R = 18\,M\Omega\,cm^{-1}$) using grade A glassware.

### 2.2. Apparatus and procedure

Calorimetric measurements were carried out at 25.000 ± 0.001 °C using an apparatus consisting of a Model 450 Tronac isoperibol titration calorimeter coupled with a Keithey 196 system digital multimeter. Titrant was delivered by a 2.5 mL capacity Hamilton syringe (model 1002TLL). The apparatus was connected to a PC, and automatic titrations were performed using a suitable computer program to control calorimetric data acquisition. The accuracy of the calorimetric apparatus was checked by titrating Tris [tris-(hydroxymethyl)amino-methane] with HCl, and was estimated to be $Q \pm 0.008$ J and $v \pm 0.001$ mL. Calorimetric measurements were performed by titrating 50 mL of the solution containing $Na_{12}Phy$ (0.8–2 mmol $L^{-1}$) and NaCl (in order to obtain the pre-established ionic strength values, $0.1 = I/\mathrm{mol\,L^{-1}} = 1$) with HCl standard solutions. Before each experiment, the heat of dilution was measured under the same ionic strength condition.

### 2.3. Calculations

Calorimetric titration data were analyzed by the ES5CMI computer program [24]. The LIANA program was used to test the dependence of thermodynamic parameters ($\Delta H$, $T\Delta S$, $\Delta G$) for the protonation of phytate ion on ionic strength and to calculate their values at infinite dilution ($I = 0\,\mathrm{mol\,kg^{-1}}$) [25].

All thermodynamic parameters are referred to the single protonation step.

$$H^+ + H_{i-1}\mathrm{Phy}^{(12-i+1)-} = H_i\mathrm{Phy}^{(12-i)-}$$

or to the overall protonation equilibrium

$$\mathrm{i}H^+ + \mathrm{Phy}^{12-} = H_i\mathrm{Phy}^{(12-i)-}$$

## 3. Results and discussion

### 3.1. Experimental protonation enthalpies

The experimental results of calorimetric measurements are shown in Table 1, where the enthalpies and the entropies for the overall protonation equilibria of phytate ion at different ionic strengths are reported together with the corresponding $\Delta G$ values calculated from the protonation constants in NaCl reported in refs. [20,21] and converted in the molal scale (see Table 2). The same thermodynamic parameters for each stepwise protonation reaction are shown in Table 3. As can be observed from these Tables, only the first seven protonation steps were taken into account, owing to the fact that the remaining five protons of phytic acid could be considered as “strongly acidic” ($\log K_i^H \leq 1$, $8 \leq i \leq 12$). The analysis of Tables 1 and 3 evidences slight variations among enthalpy and entropy values, for the same protonation step (i), at different ionic strengths. This aspect is better clarified in Figs. 1 and 2, where overall $T\Delta S$ and $\Delta H$ for each phytate protonation step are plotted versus the square root of ionic strength. As concerns the stability of the

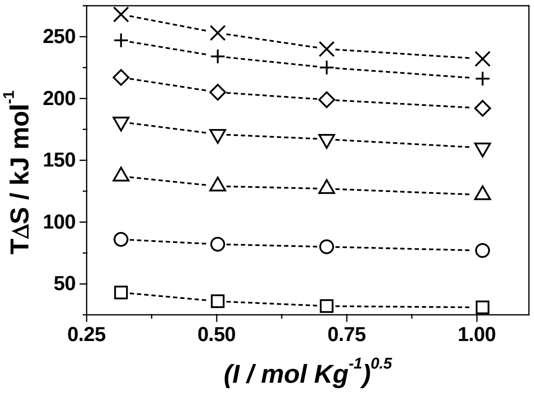

Fig. 1. TΔS values vs. the square root of ionic strength for all protonation steps of phytate ion (overall reaction). □, $i = 1$; ○, $i = 2$; △, $i = 3$; ▽, $i = 4$; ◇, $i = 5$; +, $i = 6$; ×, $i = 7$.

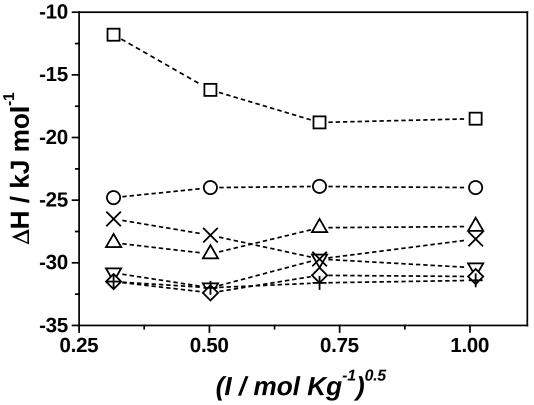

Fig. 2. ΔH values vs. the square root of ionic strength for all protonation steps of phytate ion (overall reaction). □, $i = 1$; ○, $i = 2$; △, $i = 3$; ▽, $i = 4$; ◇, $i = 5$; +, $i = 6$; ×, $i = 7$.

Table 1
Overall thermodynamic parameters[a] for protonation of phytate ion at 25 °C and at different ionic strengths

| $I$ (mol kg$^{-1}$) | Protonation step ($i$) | $\Delta G$ (kJ mol$^{-1}$) | $\Delta H$[b] (kJ mol$^{-1}$) | $T\Delta S$[b] (kJ mol$^{-1}$) |
|---|---|---|---|---|
| 0.100 | 1 | −54.67 | −11.8 ± 2.2 | 43 ± 3 |
| 0.252 | 1 | −52.50 | −16.2 ± 1.6 | 36 ± 2 |
| 0.506 | 1 | −50.94 | −18.8 ± 1.4 | 32 ± 2 |
| 1.022 | 1 | −49.55 | −18.5 ± 2.4 | 31 ± 3 |
| 0.100 | 2 | −110.83 | −24.8 ± 1.3 | 86 ± 2 |
| 0.252 | 2 | −106.48 | −24.0 ± 0.9 | 82 ± 1 |
| 0.506 | 2 | −103.37 | −23.9 ± 0.7 | 80 ± 1 |
| 1.022 | 2 | −100.59 | −24.0 ± 1.1 | 77 ± 2 |
| 0.100 | 3 | −165.04 | −28.4 ± 1.9 | 137 ± 3 |
| 0.252 | 3 | −158.46 | −29.3 ± 1.2 | 129 ± 2 |
| 0.506 | 3 | −153.74 | −27.2 ± 0.8 | 127 ± 1 |
| 1.022 | 3 | −149.40 | −27.1 ± 1.4 | 122 ± 2 |
| 0.100 | 4 | −211.49 | −30.8 ± 2.2 | 181 ± 3 |
| 0.252 | 4 | −202.74 | −32.0 ± 1.5 | 171 ± 3 |
| 0.506 | 4 | −196.41 | −29.7 ± 1.0 | 167 ± 2 |
| 1.022 | 4 | −190.50 | −30.4 ± 1.7 | 160 ± 3 |
| 0.100 | 5 | −248.58 | −31.5 ± 2.5 | 217 ± 4 |
| 0.252 | 5 | −237.82 | −32.4 ± 1.8 | 205 ± 3 |
| 0.506 | 5 | −229.95 | −31.0 ± 1.4 | 199 ± 2 |
| 1.022 | 5 | −222.70 | −31.1 ± 2.3 | 192 ± 3 |
| 0.100 | 6 | −278.54 | −31.5 ± 2.5 | 247 ± 4 |
| 0.252 | 6 | −265.78 | −32.0 ± 1.7 | 234 ± 3 |
| 0.506 | 6 | −256.46 | −31.6 ± 1.5 | 225 ± 3 |
| 1.022 | 6 | −247.88 | −31.4 ± 2.4 | 216 ± 3 |
| 0.100 | 7 | −294.97 | −26.5 ± 2.7 | 268 ± 4 |
| 0.252 | 7 | −280.54 | −27.8 ± 1.9 | 253 ± 3 |
| 0.506 | 7 | −269.96 | −29.7 ± 1.8 | 240 ± 3 |
| 1.022 | 7 | −260.50 | −28.1 ± 3.1 | 232 ± 4 |

[a] Parameters refer to reaction: $iH^+ + \mathrm{Phy}^{12-} = H_i\mathrm{Phy}^{(12-i)-}$.
[b] ± Standard deviation.

Table 2
Overall[a] and stepwise[b] (in parenthesis) protonation constants for phytate ion in NaCl at different ionic strengths and 25 °C

| $I$ (mol kg$^{-1}$) | $\log\beta_1^H$ | $\log\beta_2^H$ | $\log\beta_3^H$ | $\log\beta_4^H$ | $\log\beta_5^H$ | $\log\beta_6^H$ | $\log\beta_7^H$ |
|---|---|---|---|---|---|---|---|
| 0.1 | 9.58 | 19.42 (9.84) | 28.91 (9.49) | 37.05 (8.14) | 43.55 (6.50) | 48.80 (5.25) | 51.68 (2.88) |
| 0.252 | 9.20 | 18.65 (9.45) | 27.76 (9.11) | 35.52 (7.76) | 41.67 (6.15) | 46.56 (4.89) | 49.15 (2.59) |
| 0.506 | 8.93 | 18.11 (9.18) | 26.94 (8.83) | 34.41 (7.47) | 40.29 (5.88) | 44.93 (4.64) | 47.30 (2.37) |
| 1.022 | 8.68 | 17.62 (8.94) | 26.17 (8.55) | 33.37 (7.20) | 39.02 (5.65) | 43.43 (4.41) | 45.64 (2.22) |

[a] $\beta_i^H$, refer to reaction: $iH^+ + \mathrm{Phy}^{12-} = H_i\mathrm{Phy}^{(12-i)-}$.
[b] $K_i^H$, referred to reaction: $H^+ + H_{i-1}\mathrm{Phy}^{(12-i+1)-} = H_i\mathrm{Phy}^{(12-i)-}$.

protonated species of phytate, several discussions have already been made in previous papers [20,21]. However, this contribution better clarifies the nature of this stability; in fact, $T\Delta S$ values for each protonation step are significantly higher than the corresponding enthalpy changes. This indicates the entropic nature of the bond involved in phytate protonation, as usual for electrostatic interactions in aqueous solution. The enthalpy changes generally increase for each protonation step. This behavior is typical for phosphate ligands, as better illustrated in Fig. 3, where $\Delta H$ values (stepwise reaction) for some phosphate ligands are plotted versus the protonation step [26,27]. This trend is opposite to the one shown by some other polyanionic ligands as, for example, some polycarboxylates (malonate, mal; citrate, cit; 1,2,3-propanetricarboxyate or tricarballylate, tca; and 1,2,3,4-butanetetracarboxylate, btc) [28] and is plotted in Fig. 4.

### 3.2. Ionic strength dependence of protonation thermodynamic parameters

The dependence on ionic strength of equilibrium enthalpies is given by:

$$\Delta H = \Delta H^0 + \Sigma_j \nu_j L_j \tag{1}$$

where $\Delta H^0$ is the infinite dilution enthalpy, $L_j$ is the relative partial molar enthalpy for the reactants with stoichiometric coefficients $\nu_j$. The relative partial molar enthalpy is given, in turn, by:

Table 3
Stepwise thermodynamic parameters[a] for protonation of phytate ion at different ionic strengths and 25 °C

| $I$ (mol kg$^{-1}$) | Protonation step ($i$) | $\Delta G$ (kJ mol$^{-1}$) | $\Delta H$ (kJ mol$^{-1}$) | $T\Delta S$ (kJ mol$^{-1}$) |
|---|---|---|---|---|
| 0.100 | 1 | −54.67 | −11.8 | 43 |
| 0.252 | 1 | −52.50 | −16.2 | 36 |
| 0.506 | 1 | −50.94 | −18.8 | 32 |
| 1.022 | 1 | −49.55 | −18.5 | 31 |
| 0.100 | 2 | −56.16 | −13.0 | 43 |
| 0.252 | 2 | −53.98 | −7.8 | 46 |
| 0.506 | 2 | −52.43 | −5.1 | 48 |
| 1.022 | 2 | −51.04 | −5.5 | 46 |
| 0.100 | 3 | −54.21 | −3.6 | 51 |
| 0.252 | 3 | −51.98 | −5.3 | 47 |
| 0.506 | 3 | −50.37 | −3.3 | 47 |
| 1.022 | 3 | −48.81 | −3.1 | 45 |
| 0.100 | 4 | −46.45 | −2.4 | 44 |
| 0.252 | 4 | −44.28 | −2.7 | 42 |
| 0.506 | 4 | −42.67 | −2.5 | 40 |
| 1.022 | 4 | −41.10 | −3.3 | 38 |
| 0.100 | 5 | −37.09 | −0.7 | 36 |
| 0.252 | 5 | −35.08 | −0.4 | 34 |
| 0.506 | 5 | −33.54 | −1.3 | 32 |
| 1.022 | 5 | −32.20 | −0.7 | 32 |
| 0.100 | 6 | −29.96 | 0.0 | 30 |
| 0.252 | 6 | −27.96 | 0.4 | 29 |
| 0.506 | 6 | −26.51 | −0.6 | 26 |
| 1.022 | 6 | −25.18 | −0.3 | 24 |
| 0.100 | 7 | −16.43 | 5.0 | 21 |
| 0.252 | 7 | −14.76 | 4.2 | 19 |
| 0.506 | 7 | −13.50 | 1.9 | 15 |
| 1.022 | 7 | −12.62 | 3.3 | 16 |

[a] Parameters refer to reaction: $H^+ + H_{i-1}\mathrm{Phy}^{(12-i+1)-} = H_i\mathrm{Phy}^{(12-i)-}$.

$$L_j = -RT^2 \left(\partial \ln \frac{\gamma_j}{\partial T}\right) \qquad (2)$$

where $\gamma_j$ is the activity coefficient. According to the Specific ion Interaction Theory (SIT) we have:

$$\log \gamma_j = \frac{-z_j^2 A I^{0.5}}{1 + 1.5 I^{0.5}} + \Sigma_k \varepsilon(j, k) m_k \qquad (3)$$

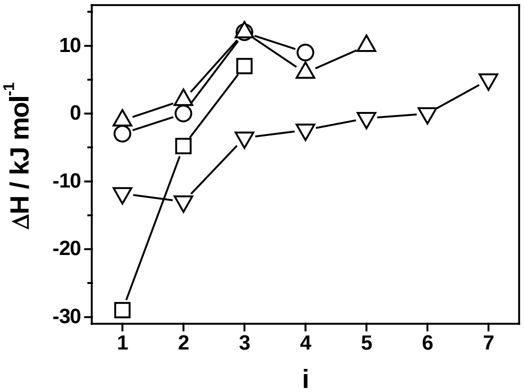

Fig. 3. ΔH values vs. the protonation step (partial reaction) for some phosphate ligands. □, $PO_4^{3-}$ at $I = 0.04\,\mathrm{mol\,L^{-1}}$ in NaCl; ○, $P_2O_7^{4-}$ at $I = 0\,\mathrm{mol\,L^{-1}}$; △, $P_3O_{10}^{5-}$ at $I = 0\,\mathrm{mol\,L^{-1}}$; ▽, $\mathrm{Phy}^{12-}$ at $I = 0.100\,\mathrm{mol\,kg^{-1}}$.

where $\varepsilon(j, k)$ is the interaction coefficient of the $j$-th species with the $k$-th component (of opposite charge) with molality $m_k$ [29]. For a single equilibrium in a solution containing 1:1 salt as supporting electrolyte

$$\Sigma_j \varepsilon(j, k) m_k = \Delta\varepsilon I \qquad (3a)$$

By combining Eqs. (1)–(3a) we have (for phytate protonation):

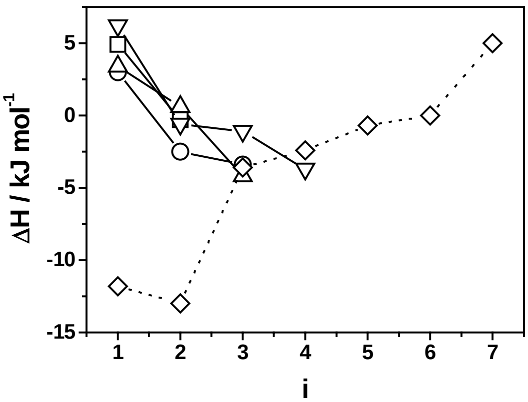

Fig. 4. $\Delta H$ values vs. the protonation step (partial reaction) for phytate ion and some polycarboxylate ligands in NaCl at $I = 0.100\,\mathrm{mol\,kg^{-1}}$. □, $\mathrm{mal}^{2-}$; ○, $\mathrm{cit}^{3-}$; △, $\mathrm{tca}^{3-}$; △, $\mathrm{btc}^{4-}$; ◇, $\mathrm{Phy}^{12-}$.

Table 4
$\Delta\varepsilon_i$ and $\Delta\varepsilon'_i$ values of Eqs. (4) and (5) for each phytate protonation step ($i$)

| *I* | $\Delta\varepsilon_i$ | $\sigma$[a] | $\Delta\varepsilon'_i$ | $\sigma$[a] |
|---|---|---|---|---|
| 1 | 1.3 ± 0.2[b] | 0.15 | 1 ± 2[b] | 1.1 |
| 2 | 1.2 ± 0.2 | 0.12 | 11 ± 3 | 2.9 |
| 3 | 0.9 ± 0.2 | 0.10 | 7 ± 1 | 0.8 |
| 4 | 0.7 ± 0.1 | 0.08 | 4 ± 1 | 0.7 |
| 5 | 0.6 ± 0.1 | 0.07 | 4 ± 1 | 0.5 |
| 6 | 0.5 ± 0.1 | 0.05 | 3 ± 1 | 0.5 |
| 7 | 0.47 ± 0.09 | 0.03 | 2 ± 1 | 0.7 |

[a] Standard deviation of the fit.
[b] ±Standard deviation of refined parameters.

$$\log K_i^H = \log K_i^{\mathrm{H}^0} - 2(12 - i + 1)AI^{0.5}/(1 + 1.5I^{0.5}) + \Delta\varepsilon_i I = \log K_i^{\mathrm{H}^0} - z^* AD.H. + \Delta\varepsilon_i I \tag{4}$$

with

$$\mathrm{A} = -8.945 + \frac{356.0}{T} + 1.4505 \ln T \tag{4a}$$

for $273 \le T\,(\mathrm{K}) \le 333$, and

$$\Delta H_i = \Delta H_i^0 + RT^2 \ln 10 \left[ -z^* \frac{\partial A}{\partial T} \mathrm{D.H.} + \frac{\partial \Delta\varepsilon_i}{\partial T} I \right] = \Delta H_i^0 - z * \mathrm{A}'\mathrm{D.H.} + \Delta\varepsilon'_i I \tag{5}$$

with

$$A' = RT^2 \ln 10 \frac{\partial A}{\partial T} = 1.5 \tag{5a}$$

at $t = 25\,^\circ\mathrm{C}$, and

$$\Delta\varepsilon'_i = RT^2 \ln 10 \frac{\partial \Delta\varepsilon_i}{\partial \mathrm{T}} \tag{5b}$$

$K_i^{H^0}$ and $\Delta H_i^0$ are, respectively, the protonation constant and the protonation enthalpy for the $i$-th protonation step at infinite dilution and at 25 °C.

By fitting data reported in Tables 2 and 3 (for stepwise reactions) to equations 4 and 5 we obtained the difference interaction coefficients $\Delta\varepsilon_i$ and their derivatives $\Delta\varepsilon'_i$, that are reported in Table 4. As expected, $\Delta\varepsilon_i$ values are a decreasing function of the protonation step (this trend is always observed in polyprotic acids) and can be expressed by the linear equation:

$$\Delta\varepsilon_i = 1.4 - 0.15\, i \tag{6}$$

For $\Delta\varepsilon'_i$ values a quite different trend $(i = 1) \ll (i = 2) > (i = 3) >$(other steps), corresponding to that of protonation constants, is observed. This unusual behavior is attributed to a structural change between the first and the second protonated species (or between second and third protonated species, depending on medium and/or ionic strengths, see [13,21] and references therein). The standard deviations on the fit of Eqs. (4) and (5), reported in Table 4, are quite acceptable, for both $\Delta\varepsilon_i$ and $\Delta\varepsilon'_i$, when $4 \le i \le 7$, but increase significantly for the first three protonation steps: this indicates that SIT model is suitable for the protonation of moderately charged polyanions.

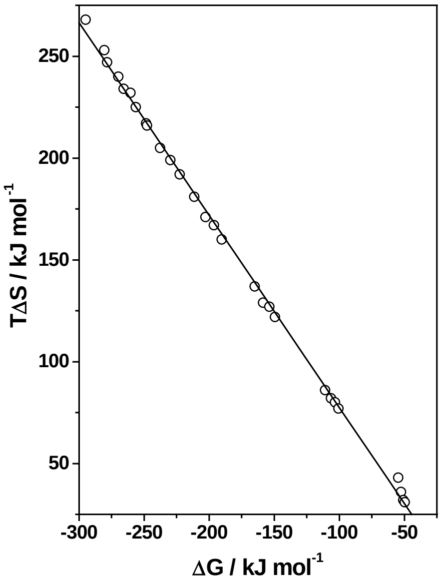

Fig. 5. $\Delta S$ vs. $\Delta G$ for all protonation steps of phytate ion (overall reaction) at all ionic strengths.

### 3.3. Empirical relationships

Thermodynamic parameters for the protonation of some ligands show remarkable regularities. Very interesting is the strict relationship between $T\Delta S$ and $\Delta G$, described by many authors, e.g., for carboxylic ligands [28,30] and references therein. Protonation entropy data collected in this work for phytate ligand allowed us to find similar trends. Fig. 5 shows the existence of a linear relationship between overall protonation $T\Delta S$ and $\Delta G$. These parameters, for all protonation steps and for all the investigated ionic strengths, can be expressed by the empirical equation:

$$T\Delta S = a + b\Delta G \tag{7}$$

with $a = -17 \pm 1$, $b = -0.944 \pm 0.007$ (± standard deviation). The correlation coefficient for the whole fit is $r = -0.999$ and the standard deviation is $\sigma = 3.0$.

Studies already performed in our laboratories, on the protonation (and complex formation) constants of phytic acid in several ionic media, also revealed the existence of very strict and significant links between each protonation step. In Fig. 6, $T\Delta S$ values for phytate protonation (overall reaction) are plotted, for all investigated ionic strengths, versus the protonation step (i). These values, at a given ionic strength, are polynomial function of "$i$", as expressed by the equation:

$$T\Delta S = c + di + ei^2 \tag{8}$$

"$c$", "$d$" and "$e$" are empirical parameters and their values, for each investigated ionic strength, are reported in Table 5

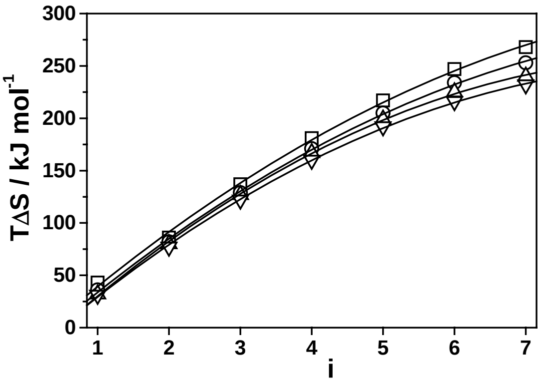

Fig. 6. $T\Delta S$ values vs. the protonation step (i) of phytate ion (overall reaction), for all investigated ionic strengths. □, $I = 0.100\,\mathrm{mol\,kg^{-1}}$; ○, $I = 0.252\,\mathrm{mol\,kg^{-1}}$; △, $I = 0.506\,\mathrm{mol\,kg^{-1}}$; ▽, $I = 1.022\,\mathrm{mol\,kg^{-1}}$.

together with the standard deviations of the fits and their correlation coefficients. By taking into account the same considerations done on experimental errors for previous fit, we can state the goodness of this relationship. As expected, the effect of ionic strength on $T\Delta S$ becomes more significant for higher protonation steps, as evidenced in Fig. 6.

### 3.4. Literature comparisons

As previously mentioned, among several papers published on phytic acid, only a few deals directly with its acid–base properties. Whilst some papers have been published dealing with complex formation enthalpies among phytate and metal cations [9–11], very few data are available on phytate protonation enthalpies and entropies in aqueous solutions [6,9]. Moreover, to our knowledge, no experiments have been previously performed in NaCl media. We found in literature two papers by the same research group dealing with phytate protonation enthalpies determined in KCl at $I = 0.2\,\mathrm{mol\,L^{-1}}$ [6,9]. Despite the different background electrolytes and ionic strengths considered, some comparisons can be made between our data and those reported in these papers. In considering these works, one should take into account that the authors determined ionization enthalpy values for all 12 ionization steps grouping the first five and the last three or four steps into single steps, whilst data related to each single protonation step till seventh are reported in this work. Our considerations are as follows: (i) the stepwise protonation enthalpies follow the same trend for both datasets, i.e. they slightly increase with increasing protonation step; (ii) in both cases $T\Delta S$ is significantly higher than corresponding $\Delta H$, which are 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 of the same order of magnitude. As an example, for the seventh protonation step at $I = 0.252\,\mathrm{mol\,kg^{-1}}$ in NaCl we obtained $\Delta H = 4.2$ and $T\Delta S = 19\,\mathrm{kJ\,mol^{-1}}$, Marini et al. [6] 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.

Table 5
Empirical parameters of equation 8[a] for all investigated ionic strengths

| $I$ (mol kg$^{-1}$) | $c$ | $d$[b] | $e$[b] | σ[c] | $r$[d] |
|---|---|---|---|---|---|
| 0.100 | −18 ± 6 | 60 ± 3 | −2.8 ± 0.4 | 3.82 | 0.998 |
| 0.252 | −23 ± 4 | 60 ± 2 | −2.9 ± 0.3 | 2.42 | 0.999 |
| 0.506 | −29 ± 3 | 62 ± 2 | −3.4 ± 0.2 | 1.99 | 0.999 |
| 1.022 | −27 ± 3 | 59 ± 2 | −3.2 ± 0.2 | 1.74 | 0.999 |

[a] $T\Delta S = c + di + ei^2$; $i$ = phytate protonation step.
[b] ±Standard deviation.
[c] Standard deviation of the whole fit.
[d] Correlation coefficient of the fit.

## Acknowledgements

We thank University of Messina for financial support (PRA).

## References

[1] J.A. Maga, J. Agric. Food Chem. 30 (1982) 1–9.
[2] B.F. Harland, G. Narula, Nutr. Res. 19 (1999) 947–961.
[3] L. Oatway, T. Vasanthan, J.H. Helm, Food Rev. Int. 17 (2001) 419–431.
[4] R. Barré, J.E. Courtois, G. Wormser, Bull. Soc. Chim. Biol. 36 (1954) 455–474.
[5] A.J.R. Costello, T. Glonek, T.C. Myers, Carbohydr. Res. 46 (1976) 159–171.
[6] M.A. Marini, W.J. Evans, C.J. Martin, Anal. Lett. 14 (1981) 707–717.
[7] W.J. Evans, E.J. McCourtney, R.I. Shrager, JAOCS 59 (1982) 189–191.
[8] H. Bieth, B. Spiess, J. Chem. Soc., Faraday Trans. 82 (1986) 1935–1943.
[9] C.J. Martin, W.J. Evans, J. Inorg. Biochem. 26 (1986) 169–183.
[10] C.J. Martin, W.J. Evans, J. Inorg. Biochem. 27 (1986) 17–30.
[11] C.J. Martin, W.J. Evans, J. Inorg. Biochem. 28 (1986) 39–55.
[12] H. Bieth, P. Jost, B. Spiess, C. Wehrer, Anal. Lett. 22 (1989) 703–717.
[13] N. Li, O. Wahlberg, I. Puigdomenech, L.O. Ohman, Acta Chem. Scand. 43 (1989) 331–339.
[14] N. Li, O. Wahlberg, Acta Chem. Scand. 43 (1989) 401–406.
[15] N. Li, O. Wahlberg, I. Puigdomenech, Chem. Scr. 29 (1989) 91–95.
[16] Z. Balogi, L.S. Làsztity, Acta Chim. Hung. 128 (1991) 393–400.
[17] K.S. Siddiqi, S.A. Shah, F.M.A.M. Aqra, S. Tabassum, S.A.A. Zaidi, D. Benlian, Ind. J. Chem. 32A (1993) 421–423.
[18] C. Brigando, J.C. Mossoyan, F. Favier, D. Benlian, J. Chem. Soc., Dalton Trans. (1995) 575–578.
[19] A. Bebot-Brigaud, C. Dange, N. Fauconnier, C. Gérard, J. Inorg. Biochem. 75 (1999) 71–78.
[20] C. De Stefano, D. Milea, S. Sammartano, J. Chem. Eng. Data 48 (2003) 114–119.
[21] C. De Stefano, D. Milea, A. Pettignano, S. Sammartano, Anal. Bioanal. Chem. 376 (2003) 1030–1040.
[22] C. De Stefano, O. Giuffrè, D. Milea, C. Rigano, S. Sammartano, Chem. Speciation Bioavailability 15 (2003) 29–36.
[23] F. Crea, P. Crea, A. De Robertis, S. Sammartano, Chem. Spec. Bioavail. 16 (2004) 53–59.
[24] A. De Robertis, C. De Stefano, C. Rigano, Thermochim. Acta 138 (1986) 141–146.
[25] C. De Stefano, S. Sammartano, P. Mineo, C. Rigano, Computer tools for the speciation of natural fluids, in: A. Gianguzza, E. Pelizzetti,

S. Sammartano (Eds.), Marine Chemistry—An Environmental Analytical Chemistry Approach, Amsterdam, 1997, pp. 71–83.
[26] P.G. Daniele, A. De Robertis, C. De Stefano, A. Gianguzza, S. Sammartano, J. Solut. Chem. 20 (1991) 495–515.
[27] C. De Stefano, C. Foti, A. Gianguzza, J. Chem. Res. (S) 464 (M) (1994) 2639–2661.
[28] C. De Stefano, C. Foti, O. Giuffrè, S. Sammartano, J. Chem. Eng. Data 46 (2001) 1417–1424.
[29] G. Biederman, Ionic Media, in: Presented at Dahlem Workshop, Berlin, 1975, pp. 339–362.
[30] A. De Robertis, C. De Stefano, C. Foti, O. Giuffrè, S. Sammartano, Talanta 54 (2001) 1135–1152.