Equilibrium Studies of Phytate Ions. 2. Equilibria between Phytate Ions, Sodium Ions and Protons in Sodium Perchlorate Media

Neng Li* and Olof Wahlberg§

Institute of Physical, Inorganic and Structural Chemistry, University of Stockholm, S-106 91 Stockholm, Sweden

Li, N. and Wahlberg, O., 1989. Equilibrium Studies of Phytate Ions. 2. Equilibria between Phytate Ions, Sodium Ions and Protons in Sodium Perchlorate Media. – Acta Chem. Scand. 43: 401–406.

The sodium complexes of phytate have been studied by emf methods at 25 °C in the concentration domain $0.15 \text{ M} \leq [\text{Na}^+] \leq 3 \text{ M}$, $-12 \leq \lg(h/\text{M}) \leq -1.5$ and $C = 5$ mM, $C = 10$ mM. The following species were found: $\text{Na}_7\text{Phyt}^{5-}$, $\text{Na}_7\text{HPhyt}^{4-}$, $\text{Na}_5\text{H}_2\text{Phyt}^{5-}$, $\text{Na}_4\text{H}_3\text{Phyt}^{5-}$, $\text{Na}_3\text{H}_4\text{Phyt}^{5-}$, $\text{Na}_2\text{H}_5\text{Phyt}^{5-}$, $\text{NaH}_6\text{Phyt}^{5-}$, $\text{H}_7\text{Phyt}^{5-}$ and $\text{H}_8\text{Phyt}^{4-}$. An algebraic expression is given for the dependence of the apparent pK_a values on the sodium ion concentration.

In earlier studies reported in this series,[1] we found that the protonation of phytate depends on the counter-ion concentration. This has also been found by other investigators.[2] The aim of the present study was to investigate the role of sodium ions in the acid-base equilibria of phytate. For this purpose the sodium ion concentration has to be varied over a wide range. We have determined the average net charge of the phytate ion to be $z = -5.0 \pm 0.1$ at low sodium ion concentration.[1] At high sodium ion concentrations we can use $\text{Na}_{12}\text{Phyt}(\text{H}_2\text{O})_{38}(s)$ as a model compound, showing that approximately half of the sodium ions are closely bound to the phytate, while the remaining ions are found in the surrounding water shell. The structure of solid sodium phytate has been determined by X-ray diffraction methods.[3] The conformations of phytate ions in solution have been described earlier.[1,4,5]

A major problem in this investigation is posed by the changing activity coefficients. We have considered three ways to handle this problem:

(1) The apparent pK_a values may be determined in a range of media, each of which contains a constant concentration of NaClO_4. Equilibrium constants for the sodium ion complexes are then obtained by comparing the results from the different media. The specific ion interaction theory can be used to estimate the activity coefficients.[6]

(2) The concentration of sodium ions may be varied within each titration and the activity coefficients calculated for each titration point. We have found it difficult to calculate the activity coefficients and vary the sodium ion concentration over a sufficiently large concentration range in this approach.

(3) A third possibility would be to use a medium containing other cations which form weaker complexes with the phytate ions. We have tried Cs^+, but this ion forms insoluble phytate complexes. We have also considered the tetrabutylammonium ion, which was used by Costello, Glonek and Myers.[7] However, one drawback with this approach is that extra acid-base impurities may be introduced together with this ion.

We have used the first method in the present study. However, in a later study we intend to employ the second method to extend the studied sodium range down to lower concentrations. The third method can give valuable information about the sodium complexes at low sodium concentrations.

Symbols

The most common symbols are H, B and C for protons, sodium ions and phytate ions, respectively (cf. Ref. 1). In this article, $\text{H} = \text{H}^+$, $\text{B} = \text{Me}^{z+}$ and $\text{C} = \text{Phyt}^{12-}$. $H =$ the analytical concentration of hydrogen ions in excess of H_2O, Na^+ and C. The total analytical concentrations of B and C are written as B and C. The concentrations of free H, B and C are denoted h, b and c. The symbol for the concentration of the complex $\text{H}_p\text{B}_q\text{C}_r$ is c_{pqr}. The average number of H^+ bound per C is written as Z. V and E are the measured volume and emf, respectively. The symbols are used to denote physical quantities. A superscript $^\circ$ refers to pure water as reference state.
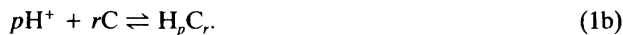
Equilibrium reactions

The equilibria studied in this work can be written as:

$$p\text{H}^+ + q\text{B} + r\text{C} \rightleftharpoons \text{H}_p\text{B}_q\text{C}_r. \tag{1a}$$

---
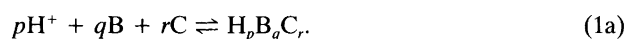
*Permanent address: Department of Chemistry, Peking University, Beijing, China.
§To whom correspondence should be addressed.

The charge of the compex has been omitted in eqn. (1a). The formation constant for a complex $H_pB_qC_r$ is written as β_{pqr}. In this study we determine p, q and r for each species. For a constant sodium ion concentration we can simplify eqn. (1a) to read:

$$pH^+ + rC \rightleftharpoons H_pC_r. \tag{1b}$$

The formation constant for a complex H_pC_r is then denoted β_{pr}. We restrict ourselves to the mononuclear region, thus $r = 1$. In this work we also found it convenient to write the equilibria as stepwise reactions [cf. eqn. (10)].

Experimental

Chemicals and analyses have been described elsewhere.[1]

Apparatus. The emf measurements were performed using an automatic titration system.[8] We measured the emf of the following cell:

$$- \text{RE}|\text{NaClO}_4(\text{aq})|\text{Equilibrium solution}|\text{ME} + \tag{2}$$
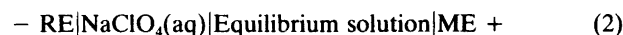
where RE = Ag, AgCl(s) | NaClO$_4$(aq) + 0.01 M NaCl and ME = a hydrogen electrode, freshly prepared for each titration, or an Ingold No. 201 glass electrode. The temperature was kept at (25.00 ± 0.02) °C. We calculated $h = [\text{H}^+]$ from Nernst's equation:

$$E = E_0 + E_Q \lg h + E_j \tag{3}$$

where $E_Q = 59.155$ mV and $E_j = (j_{ac}\, h + j_{alk}\, K_w\, h^{-1})$ mV. For the hydrogen electrode we have to add a small correction term, $E_{H_2} = -1/2\, E_Q \lg\, [p_{H_2}/(100\ \text{kPa})]$. E_0 was determined in separate experiments, one for each medium. The activity coefficients were defined to approach unity when the solution approaches pure medium. They were assumed to be constant within the experimental error limits. For further details on the titration procedure, see Ref. 9. The values for j_{ac}, j_{alk} and K_w were taken from Ref. 10.

The computer programs used in this study are given in Ref. 1.

Equilibrium analysis. From the emf measurements we calculated $h = [\text{H}^+]$, and from the chemical analysis we obtained H, B and C. The equilibrium condition corresponding to reaction (1a) can be written as

$$c_{pqr} = \beta_{pqr} h^p b^q c^r \tag{5}$$

The mass balance and equilibrium equations used in the calculations are:

$$H = h + \Sigma p c_{pqr} \tag{6}$$

$$B = b + \Sigma q c_{pqr} \tag{7}$$

$$C = c + \Sigma r c_{pqr}. \tag{8}$$

The average number of protons bound per phytate was calculated from:

$$Z = (H-h+K_w h^{-1})/C. \tag{9}$$

Treatment of data

We measured 780 experimental points. Some of these are shown in Fig. 1, in which it can be seen that the curves are roughly parallel. In the alkaline region the curves become steeper as [Na$^+$] is lowered. This feature indicates that HC tends to disappear at low values of [Na$^+$], and H$_2$C is favoured instead.

In this study we have used both glass electrodes and hydrogen electrodes to measure $h = [\text{H}^+]$. Values obtained with the two types of electrodes agree very well (cf. Fig. 1).
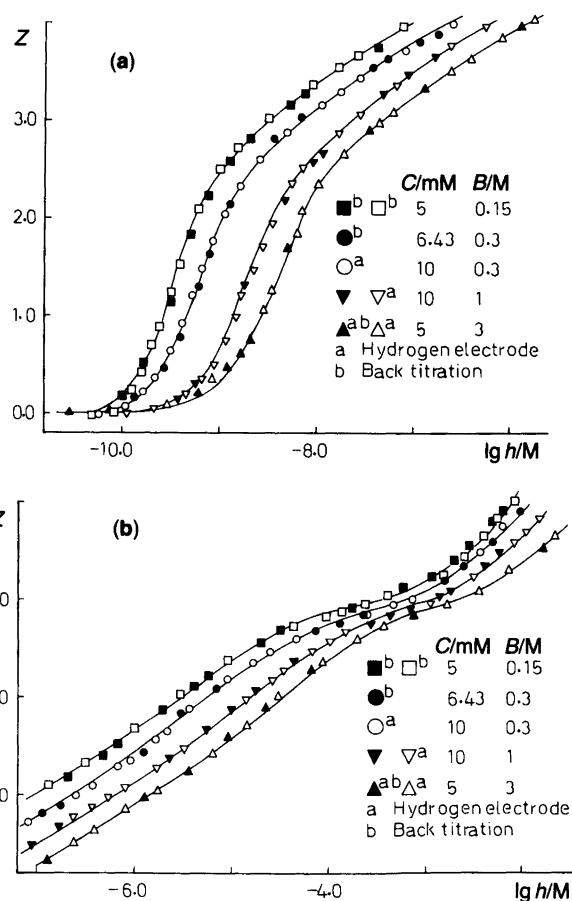

Fig. 1. Z = The average number of H$^+$ bound per C as a function of lg(h/M), at constant B and C. The circles, squares and triangles are experimental points measured using glass or hydrogen electrodes. The solid lines were calculated from the final equilibrium model (cf. Table 3) using the computer program SOLGASWATER.[14]

Table 1. The calculated overall equilibrium constants, given as $\lg(\beta_{p1}+3\sigma)$, for phytate species in different ionic media. σ = the standard deviation. Results from two titrations are reported for each medium except 3 M, for which the final results from Ref. 1b are given.

p	B = 0.15 M	0.15 M	0.3 M	0.3 M	1 M	1 M	3 M
1	8.53±0.26	8.50±0.03	8.45±0.3	8.45±0.3	8.40±0.02	8.40±0.02	8.29±0.03
2	19.00±0.03	18.96±0.03	18.54±0.02	18.46±0.03	17.47±0.01	17.41±0.01	16.94±0.01
3	28.02±0.03	28.05±0.03	27.31±0.04	27.16±0.05	25.90±0.01	25.80±0.01	25.00±0.01
4	35.93±0.03	35.97±0.03	34.81±0.04	34.74±0.08	32.73±0.02	32.69±0.04	31.63±0.03
5	42.20±0.05	42.22±0.05	40.63±0.07	40.71±0.07	38.19±0.03	38.15±0.05	36.69±0.03
6	47.04±0.06	47.10±0.08	45.11±0.04	45.23±0.03	42.23±0.04	42.03±0.04	40.57±0.04
7	49.41±0.05	49.37±0.23	47.04±0.13	47.40±0.06	44.18±0.14	43.97±0.09	42.11±0.04
8	51.35±0.03	51.73±0.10	49.20±0.05	49.17±0.03	46.11±0.03	45.63±0.04	43.7 ±0.2

Forward and back titrations are mutually consistent, thus indicating that the studied reactions are reversible. No free orthophosphate could be detected in the equilibrium solutions. During the LETAGROPVRID[11] calculations, the parameter "dirt acid" was found to correspond to less than 0.1 % of the total *H*. The "best" model for each medium has been determined, and Table 1 contains the results of the least-squares refinement. Table 2 gives the corresponding pK_a values.

The derivation of one model for all data. In order to describe all the data by a single model we have to include both the formation of the sodium complexes and the variation of the activity coefficients. We have postulated unit activity coefficients in each medium, i.e. at each constant sodium level. In order to compare the different media, each with its own reference state, we must define an activity scale common to all data. Therefore, we introduce pure water as the common reference state and denote it by the superscript in $K^{\circ}{}_{Naj}$.

The sodium complexes. We have determined the average charge, z, of the phytate ions in dilute solutions to be $z = -5.0\pm0.1$ for $-10 < \lg(h/M) < -5$, $0.05\,M < [Na^+] < 0.11\,M$ and $C = 10\,mM$ (cf. Ref. 1). Thus, Na_7Phyt^{5-} predominates in alkaline solutions at low sodium ion con-

centrations. When $[Na^+]$ increases, the sodium phytate complexes might bind more Na^+. We have tested this possibility by fitting various models to the data. The successful model was a rather simple one (see below). We can write the studied reactions as stepwise acid-base dissociation equilibria:

$$Na_qH_pPhyt_r^z + s\,Na^+ \rightleftharpoons Na_{q+s}H_{p-1}Phyt_r^{z+s-1} + H^+ \quad (10)$$
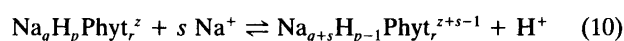
where $p = 1,2,...,8$, $r = 1$ and $z = p+q-12$.

The equilibrium constant for eqn. (10) is written as K_{Naj}, where j is the serial number of H^+ dissociated from the hypothetical acid $H_{12}Phyt$; thus $j = 1,2,...12$. The values of q and s are unknown, except that one species must be Na_7Phyt^{5-}. A constant average net charge would imply that $s = +1$. If this were strictly true, the activity coefficients for the different phytate ions in eqn. (10) should not differ much. The values of q and s are determined below for each species (p,q,r) by comparing the values of the apparent dissociation constants determined for a series of constant sodium levels. The equilibrium constant for reaction (10) can be written:

$$K^{\circ}{}_{Naj} = ([H^+]\,Na_{q+s}H_{p-1}Phyt^{z+s-1})/$$

$$([Na^+]^s\,Na_qH_pPhyt^z)\,K_\gamma \quad (11)$$

ble 2. The experimentally determined pK_{aj} values for phytic acid in $(Na)ClO_4$ medium at 25 °C. Values from two titrations are reported for each ·dium, and also the values calculated from the overall model [eqn. (17)]. The errors are given as 3σ (σ = the standard deviation).

j[a]	B = 0.15 M			0.3 M			1 M			3 M	
	Titr. 1	Titr. 2	Model	Titr. 1	Titr. 2	Model	Titr. 1	Titr. 2	Model	Titr. 1–6	Mc
12	8.53±0.26	8.50±0.03	8.59	8.45±0.3	8.45±0.3	8.53	8.40±0.02	8.40±0.02	8.41	8.29±0.02	8.2
11	10.50±0.03	10.50±0.03	10.53	10.09±0.02	10.01±0.03	10.00	9.07±0.02	9.01±0.02	9.19	8.65±0.03	8.6
10	9.02±0.05	9.09±0.05	9.02	8.77±0.05	8.70±0.06	8.72	8.43±0.02	8.39±0.02	8.29	8.06±0.02	8.0
9	7.91±0.05	7.93±0.05	7.82	7.50±0.06	7.58±0.10	7.51	6.86±0.03	6.89±0.05	7.03	6.63±0.04	6.6
8	6.27±0.07	6.24±0.06	6.13	5.82±0.08	5.97±0.11	5.82	5.46±0.04	5.45±0.07	5.38	5.06±0.05	5.0
7	4.84±0.08	5.1 ±0.10	4.88	4.48±0.08	4.52±0.08	4.57	4.04±0.05	3.88±0.07	4.14	3.88±0.05	3.8
6	2.37±0.08	2.27±0.3	2.49	1.93±0.14	2.17±0.07	2.18	1.95±0.15	1.94±0.10	1.77	1.54±0.07	1.5
5	1.94±0.06	2.36±0.3	1.98	2.16±0.14	1.77±0.07	1.92	1.93±0.15	1.66±0.10	1.80	1.6 ±0.2	1.6

is the number of protons bound to the phytate ion according to eqn. (1), and *j* is the number of protons dissociated from phytic acid, $H_{12}C$.
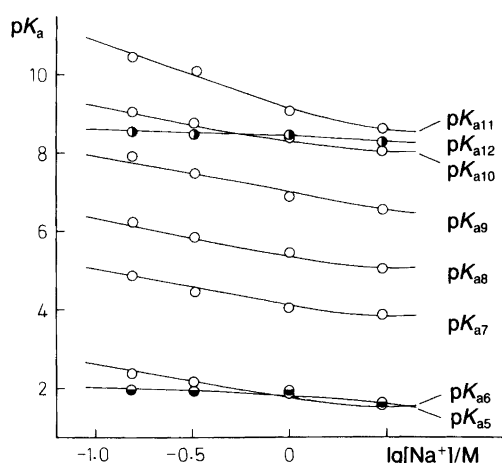
Fig. 2. The apparent pK_{aj} values as functions of $\lg([Na^+]/M)$. The circles correspond to the experimental pK_{aj} values, calculated for each medium (cf. Table 2). The solid lines were calculated from the complete model (eqn. 17).

where K_γ is an activity coefficient quotient. Eqn. (11) can be simplified to read:

$$pK_{aj} = pK^\circ_{Naj} - s\,\lg([Na^+]/M) + \lg K_\gamma \tag{12}$$

where pK_{aj} is the apparent equilibrium constant, which is determined in a series of media, each with a constant sodium ion concentration.

The activity coefficients. The activity coefficient quotient K_γ in eqn. (12) can be expressed by:

$$\lg K_\gamma = \lg \gamma_{H^+} - s\,\lg\gamma_{Na_+} + \lg\gamma_C \tag{13}$$

where γ_C is the ratio of the activity coefficients for the two phytate ions in eqn. (10). We can estimate $\lg\gamma_i$ for each ion "i" in the solution as (cf. Ref. 6):

$$\lg\gamma_i = -z_i^2 D + \Sigma\epsilon_i m_k \tag{14}$$

$$D = 0.5109\ \sqrt{I/m^\ominus}/(1+1.5\ \sqrt{I/m^\ominus}). \tag{15}$$

The ionic strength I was calculated as $I = b + h$ (cf. footnote under Table 4). The summation in eqn. (14) is made over all the counter ions "k" in the solution. The concentration of each counter ion, m_k, is expressed in units of mol per kg H_2O. The first term in eqn. (14) is referred to as the Debye–Hückel term, which accounts for the electrostatic effects. The second term in eqn. (14) accounts for the specific interactions between ions, which are important at high ion concentrations. We will assume that the interactions between ions of the same charge are negligible. Furthermore, we will neglect all interactions with counter ions present in low concentrations, i.e. all except Na^+ and ClO_4^-. The eqns. (13), (14) and (15) can be used to derive the following expression for $\lg K_\gamma$ in eqn. (12):

$$\lg K_\gamma = -K_D D + K_\epsilon m_X. \tag{16}$$

In eqn. (16), $K_D = 1 - s + f(z)$ and $K_\epsilon = \epsilon_{H^+} - s\epsilon_{Na^+} + \Delta\epsilon_C$ where $\Delta\epsilon_C$ is the difference between the interaction coefficients for the two phytate ions in eqn. (10). The function $f(z)$ is dependent on the charges of the phytate ions. We know $\epsilon_{Na^+} = 0.01$ kg mol^{-1} and $\epsilon_{H^+} = 0.14$ kg mol^{-1} (cf. Ref. 6). The concentration of the sodium perchlorate medium is denoted by m_X.

The final model. The equation for the calculation of the apparent pK_{aj} as a function of $[Na^+]$ can now be written as:

$$pK_{aj} = pK^\circ_{Naj} - s\cdot\lg([Na^+]/M) - K_D\cdot D + K_\epsilon\cdot m_X. \tag{17}$$

The constants pK°_{Naj}, s, K_D and K_ϵ were determined by a graphical curve-fitting method (Fig. 2). The theoretical model [eqn. (17)] has been fitted to the observed pK_{aj} values in Table 2. The parameters of the "best" fit are given in Table 3.

Results

We propose the stoichiometric formulae and parameters given in Table 4, which are valid for phytate ions in sodium perchlorate solutions with $0.15\ M \le [Na^+] \le 3\ M$ and $C \le$

Table 3. Parameters describing the sodium complex formation and the variation of the activity coefficients in the sodium-proton phytate system [eqn. (17)].

p	q	r	j	pK°_{Naj}	s	z	f(z)	K_D	$K_\epsilon m^\ominus$	$\Delta\epsilon_C m^{\ominus\,a}$
1	7	1	12	8.85±0.05	0	−4	1	2	0.15	0.01
2	5	1	11	8.65±0.05	2	−5	−1	−2	0.01	−0.11
3	4	1	10	8.20±0.05	1	−5	0	0	0.05	−0.08
4	3	1	9	7.00±0.05	1	−5	0	0	0.11	−0.02
5	2	1	8	5.30±0.05	1	−5	0	0	0.06	−0.07
6	1	1	7	4.05±0.05	1	−5	0	0	0.05	−0.08
7	0	1	6	1.65±0.10	1	−5	0	0	0.03	−0.10
8	0	1	5	2.25±0.2	0	−4	1	2	0.17	0.03

[a] $\Delta\epsilon_C$ is the difference between the interaction coefficients for the two phytate ions in eqn. (10). $m^\ominus = 1$ mol per kg H_2O.
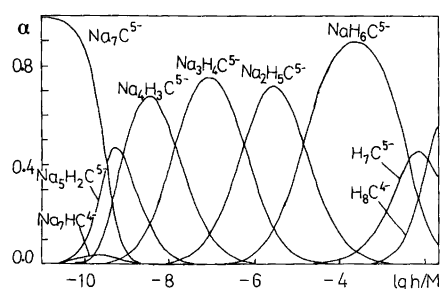
Fig. 3. The distribution of phytate in different species as a function of lg(h/M). The equilibrium constants given in Table 3 were used for the calculations. $C = 5$ mM.

10 m M at 25 °C. All except two of the ions have the net charge $z = -5$. The exceptions are Na_7HPhyt^{4-} and H_8Phyt^{4-}; the former is important only at high sodium concentrations, and the latter occurs at lg(h/M) > -3. Any pK_{aj} for phytic acid at 25 °C can be calculated at various concentrations of sodium ions by eqn. (17) above (cf. Table 4). The distribution of phytate in different species is given in Fig. 3. The values of $pK°_{Naj}$ and the interaction coefficients are given in Table 3.

Discussion

The phytate equilibrium system is a biologically important system in which the role of the sodium ions is crucial (cf. Ref. 12). Since the sodium ion forms complexes with the phytate ion and is also a characteristic component of the ionic atmosphere around the phytate ion, we must include both these aspects in our model. We have therefore derived the necessary theoretical model for our problem (eqn. 17) and fitted model functions to our experimental pK_{aj} values (Fig. 2). The resulting parameters are given in Table 3. The stoichiometries and the stability constants for the sodium complexes have been determined (Table 4). These complexes are stable down to comparatively low sodium ion concentrations, but we had problems with preparing stable

Table 4. Equations for the calculation of pK_{aj} values for phytic acid in the concentration range 0.15 M $\leq$ [Na$^+$] ≤ 3 M.[a]

Species	pK_{aj} value
Na_7HPhyt^{4-}	$pK_{a12} = 8.85 - 2D + 0.15\ m_x/m^{\ominus}$
$Na_5H_2Phyt^{5-}$	$pK_{a11} = 8.65 + 2D + 0.01\ m_x/m^{\ominus} - 2$ lg([Na$^+$]/M)
$Na_4H_3Phyt^{5-}$	$pK_{a10} = 8.20 + 0.05\ m_x/m^{\ominus} -$ lg([Na$^+$]/M)
$Na_3H_4Phyt^{5-}$	$pK_{a9} = 7.00 + 0.11\ m_x/m^{\ominus} -$ lg([Na$^+$]/M)
$Na_2H_5Phyt^{5-}$	$pK_{a8} = 5.30 + 0.06\ m_x/m^{\ominus} -$ lg([Na$^+$]/M)
NaH_6Phyt^{5-}	$pK_{a7} = 4.05 + 0.05\ m_x/m^{\ominus} -$ lg([Na$^+$]/M)
H_7Phyt^{5-}	$pK_{a6} = 1.65 + 0.03\ m_x/m^{\ominus} -$ lg([Na$^+$]/M)
H_8Phyt^{4-}	$pK_{a5} = 2.25 - 2D + 0.17\ m_x/m^{\ominus}$

[a]The values of $m_x/m^{\ominus}$ have been calculated using the empirical relation $m_x/m^{\ominus} = $[Na$^+$]/M/(1 $- 0.0475$[Na$^+$]/M). D is given in eqn. (15). $m^{\ominus} = 1$ mol per kg H_2O.

solutions of phytate below [Na$^+$] $= 10$ mM. However, many biologically important solutions, e.g. human blood, have a relatively high and constant sodium concentration. The nature of the sodium complexes has several interesting implications, and some chemical features are discussed below. The species Na_7HPhyt^{4-} contributes significantly to the model in 3 M $NaClO_4$ medium, where it constitutes about 20 % of the total phytate. It is also important for 1 M $NaClO_4$ medium but of less significance in 0.3 M and 0.15 M $NaClO_4$.

The charge distribution on the phytate ion. The charges of the phytate ions are given in Table 3. According to our results, the charge in eqn. (14) should not be equated with the total charge of the phytate ion. Instead, we have to sum the squared charges of the individual phosphate groups, i.e. phytate ions are too big to be regarded as point charges. From the nmr measurements we could also see that the phosphate groups on the phytate ions behave as individual entities.[1] This is important for the modelling of the long-range electrostatic effects in eqn. (14). We have checked the results by solving the Poisson-Boltzmann equation for a polyelectrolyte.[13] The activity coefficients calculated in these two ways agree to within 3 % for $I < 0.125$ M.

The interaction coefficients for the phytate ions. We did not estimate the individual interaction coefficients for the phytate ions in this study, but rather their differences. It is interesting to note that the interaction coefficients for the phytate ions are correlated with the charges of the ions. From Table 3 we obtain the following values of $\Delta\varepsilon_C$ for single protonation steps [cf. eqn. (10)]:

$\Delta\varepsilon_C = (-0.08\pm0.03)$ kg mol^{-1};

constant charge $z = -5$; the same conformation

$\Delta\varepsilon_C = (0.02\pm0.03)$ kg mol^{-1};

constant charge $z = -5$; changing conformation

$\Delta\varepsilon_C = (-0.11\pm0.03)$ kg mol^{-1};

the charge changes from $z = -5$ to $z = -4$*

$\Delta\varepsilon_C = (-0.02\pm0.03)$ kg mol^{-1};

the charge changes from $z = -4$ to $z = -5$*.

The orders of magnitudes of the pK_{aj} values for phytic acid. An interesting feature of the phytate ion system is the different orders of magnitudes of the apparent pK_{aj} values, depending on the sodium concentration:

---

*Conformation unchanged.

(i) $pK_{a11} > pK_{a10} > pK_{a12}$ for $[Na^+] < 0.6$ M

(ii) $pK_{a11} > pK_{a12} = pK_{a10}$ for $[Na^+] = 0.6$ M

(iii) $pK_{a11} > pK_{a12} > pK_{a10}$ for $[Na^+] > 0.6$ M.

The origin of this phenomenon is the formation of sodium complexes, and especially the stability of $Na_5H_2Phyt^{5-}$, which contains two hydrogen bridges (Fig. 8 and Ref. 1).

References

1. (a) Li, N., Wahlberg, O., Puigdomenech, I. and Öhman, L. O. *Acta Chem. Scand. 43* (1989) 331; (b) Li, N., Puigdomenech, I. and Wahlberg, O. *Chem. Scr. 28* (1989) 111.
2. Hoff-Jørgensen, E. *Kgl. Danske Vidensk. Selskab, Mat.-Fys. Medd. 21* (1944) 27, No. 7.
3. Blank, G. E., Pletcher, J. and Sax, M. *Acta Crystallogr., Sect. B31* (1975) 2584.
4. Isbrandt, L. R. and Oertel, R. P. *J. Am. Chem. Soc. 102* (1980) 3144.
5. (a) Martin, C. J. and Evans, W. J. *Inorg. Biochem. 27* (1986) 17; (b) Martin, C. J. and Evans, W. J. *Inorg. Biochem. 28* (1986) 39.
6. (a) Biedermann, G. In: Goldberg, E. D., Ed., *The Nature of Sea Water*, Report from the Dahlem Konferenzen, Berlin 1975, p. 337; (b) Ciavatta, L. *Ann. Chim. (Rome) 70* (1980) 551.
7. Costello, A. J. R., Glonek, T. and Myers, T. C. *Carbohydr. Res. 46* (1976) 159.
8. Forsberg, O. and Kierkegaard, P. *Chem. Scr. 15* (1980) 110.
9. Forsberg, O., Johansson, K., Ulmgren, P. and Wahlberg, O. *Chem. Scr. 3* (1973) 153.
10. Ulmgren, P. and Wahlberg, O. *To be published.*
11. (a) Arnek, R., Sillén, L. G. and Wahlberg, O. *Arkiv Kemi 31* (1969) 353; (b) Brauner, P., Sillén, L. G. and Whiteker, R. *Arkiv Kemi 31* (1969) 365.
12. May, P. M., Murray, K. and Peaper, D. *2nd International Conference on Bioinorganic Chemistry; Rev. Port. Quim. 27* (1985) 312.
13. Oosawa, F. *Polyelectrolytes*, Marcel Dekker, New York 1971.
14. Eriksson, G. *Anal. Chim. Acta 112* (1979) 375.