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Sequestering ability of phytate towards protonated BPEI and other polyammonium cations in aqueous solution[☆]

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ABSTRACT

The interaction between protonated branched poly(ethylenimine) [BPEI] and phytate (1,2,3,4,5,6 hexakis (dihydrogen phosphate) *myo*-inositol) [Phy] was studied potentiometrically. The measurements were carried out at $t=25$ °C and at low ionic strength values, without addition of supporting electrolyte, to avoid interferences with other anions and cations. In order to simplify the data treatment, BPEI was considered as a simple tetramine. Different species $Phy(BPEI)H_j$, with $j=6,7,8$, and $Phy(BPEI)_2H_7$ were found, having quite high stability. The ability of phytate to sequester BPEI was quantified by considering the parameter pL_{50}, namely the concentration ($-\log [Phy]_{tot}$) necessary to bind 50% of polyammonium cation (as trace). In our experimental conditions, for the system phytate–BPEI-proton we have $pL_{50}=7.01$, at pH$=7.4$ and $I=0.04$ mol L^{-1}. As for other phytate–polyammonium cation systems, the stability of the phytate–BPEI species is strictly proportional to the charges involved in the formation reactions. Therefore, it was possible to calculate the free energy contribution per bond, $\Delta G_b^U=4.4\pm0.4$ kJ mol^{-1}. The dependence on temperature and ionic strength of the stability of phytate-low/high molecular weight polyammonium cations species, was studied using some semiempirical equations and enthalpy data for the protonation of both components. The dependence on temperature of the stability is quite low and the variation of pL_{50} in the range $15\le t/°C\le37$ is less than 0.5 log units. On the contrary, the effect of ionic strength is highly significant, with a lowering of pL_{50} of ≈2 log units ($I=0$ to 0.15 mol L^{-1}).

1. Introduction

Natural organic matter (constituted to a great extent by macromolecules) is quite rich in functional groups which act as binding sites for inorganic and organic cations or anions over a wide pH range. The study of the physical and chemical properties of natural organic matter is very difficult owing to the fact that in these macromolecules, the protonation and complexation reactions depend on several factors, such as molecular weight, distance between functional groups, distribution of binding sites and structure (in terms of cross linking, etc.). To simplify the thermodynamic study of some natural organic molecules, such as humic and fulvic acids [1], often high molecular weight polyelectrolytes were chosen as model molecules in order to obtain information about the behaviour (acid–base properties and complexing ability) of natural macromolecules. The literature reports several thermodynamic data for the interaction properties of some high molecular weight polyelectrolytes [2–6]; in particular these data regard the acid–base properties of polycarboxylates and their interactions with alkali, alkaline earth metals [2–5] and some low molecular weight polyamines [6]. Natural and biological fluids contain also amino compounds (amines, polyammonium cations, polyaminocarboxylates, etc.) that are among the fundamental molecules of biological interest, widely present in aminoacids, proteins and enzymes, and are responsible for the most of complex functions that make life possible. The biological function of natural polyelectrolytes is often mediated by interactions with other macromolecules such as, for example, proteins and natural surfactants (lipids). A very important example is the interaction of DNA with proteins. Among these macromolecules, polyammoniun cations have a quite relevant role [7]. In the literature the interaction of polyammonium cations with carboxylic anions was widely reported, whilst a lesser number of data is reported for other ligands containing, for example, phosphate groups, despite their widespread involvement in biological systems. Few examples of phosphate complexation by synthetic polyammonium receptors in water were reported by Garcia-España et al. [8]; other papers also report [9–11] the enthalpy and entropy changes, which are important to interpret the main contribution for the stability of anion complexes in water.

The interaction of polyammonium cations with inorganic and organic polyanions [7–13] was mainly studied for low molecular weight molecules. In our previous papers, we already studied the

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[☆] Speciation of phytate ion in aqueous solution. Previous parts of this series [18,28,40–44].

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interaction of different polyphosphates [13–16] and nucleotides [17] with biogenic amines. More recently the interaction of some polyammonium cations [ethylenediamine, 1-4-diaminobutane (putrescine), 1,5-diaminopentane (cadaverine), diethylenetriamine, *N*,*N*'-bis [3-aminopropyl]-1,4-diaminobutane (sperimine), *N*-[3-aminopropyl]-1,4-diaminobutane (spermidine), tetraethylenpentamine and pentaethylenehexamine] with phytate [18], some polymers, such as polyacrylate (MW=2 kDa), polystirenesulphonates (MW=70 and 1000 kDa) and polyvinylsulphonate (MW=9 kDa) [2], and alginic and fulvic acids [19] were studied.

As a further contribution to the study of the acid–base properties and complexing ability of polyelectrolytes already undertaken [2–6], in the present paper we studied the interaction of an high molecular weight ammonium polyelectrolyte (branched polyethylenimine MW=750 kDa) with an high charged polyphosphate, such as phytate. This high molecular weight polyamine has a monomeric unit which contains primary, secondary and tertiary amino groups in the ratio 1:2:1, as shown in Fig. 1. It is a polymer obtained from the aziridine through ring-opening cationic polymerisation.

Poly(ethylenimine) [BPEI], has several applications in the biological, industrial and pharmaceutical fields [20–23]. For example, it is used in bioprocesses to facilitate the transfer of nucleic acids through biological membranes, for purification of DNA binding proteins, to transfer genes and oligonucleotides into living cells, as a flocculating agent and to immobilise enzymes and bacteria, as backbone of artificial enzymes. BPEI binds also heavy metal cations [24–26], and therefore can be used in several environmental applications [27].

Phytate [Phy], is widely present in nature and has an important biological activity; it modifies the bioavailability of several metal ions (such as calcium, iron, zinc, copper: see, e.g., [[28] and references therein]); it is regarded as the primary storage form of both phosphate and inositol in plant seeds and grains, and to be an antioxidant [[28] and references therein]; it shows marked anticarcinogenic/antineoplastic properties; it may reduce and prevent kidney stone formation and it plays key roles in a number of crucial physiological activities, in addition to the treatment of different pathologies. Among its industrial and/or technological applications, very important is the use of phytate in remediation problems, such as the immobilisation and in-situ treatment of soils contaminated by many metals (including heavy metals and radionuclides).

Owing to their high charges, BPEI and phytate can be considered as model molecules for the study of the interactions between natural polycations and polyanions. The problems that can be encountered in such a study are connected to the interpretation/elaboration of the experimental data; different methods were used to explain the acid–base properties of BPEI: by applying the Ising model to short-range interactions [29], by relating the BPEI protonation with ion condensation [30] and by calculating the deprotonation quotient at different pHs [31]. In our previous work [23], we used a new approach to study the acid–base properties of BPEI; using this approach the branched poly (ethylenimine) was treated as a simple aliphatic tetramine. Since this approach is suitable to describe the acid–base properties of BPEI, it was used for studying its complexation with phytate.

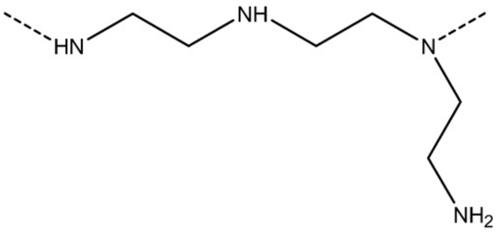

Fig. 1. Representation of BPEI monomeric unit.

Table 1
Protonation constants of BPEI at different ionic strength and temperatures

Species	$\log K_i^{Ha}$						
	$t=25$ °C			$t=15$ °C		$t=37$ °C	
	$I=0$[b]	$I=0.04$[b]	$I=0.15$[b]	$I=0$[c]	$I=0.15$[c]	$I=0$[c]	$I=0.15$[c]
BPEIH	9.36	9.39	9.49	9.65	9.78	9.04	9.17
$BPEIH_2$	7.90	8.09	8.29	8.18	8.57	7.58	7.98
$BPEIH_3$	5.29	5.62	5.85	5.50	6.05	5.06	5.63
$BPEIH_4$	1.80	2.23	2.46	1.88	2.52	1.72	2.39

[a] According to Eq. (4).
[b] Ref. [23].
[c] Calculated by empirical equations (refs. [34–37]).

2. Experimental section

2.1. Chemicals

All the reagents were Fluka analytical grade. A 50% aqueous solution of BPEI was used without further purification; its concentration was determined by potentiometric titrations, assuming the ligand as a monomer. Phytic acid solutions were prepared by weighing pure dipotassium salt $K_2H_{10}Phy$ (Fluka). Hydrochloric acid working solutions were prepared by diluting concentrates, and standardized against sodium carbonate. Tetraethylammonium hydroxide solutions were prepared from concentrated Et_4NOH (Fluka puriss. Electrochemical grade) and standardized against potassium biphthalate. All the solutions were prepared by using analytical grade water ($R \geq 18$ MΩ cm^{-1}) and preserved from atmospheric CO_2 by means of soda lime traps. Grade A glassware was employed.

2.2. Apparatus

The potentiometric measurements were carried out by using a potentiometer (Metrohm mod. E605) coupled with a combinated ISE-H Orion Ross type glass electrode (mod. 8172), and with an automatic titrant dispenser (Metrohm Dosimat mod. 665). The potentiometer and the dispenser were connected to a PC and a suitable home made software allows monitoring of the titrations. The program allows the equilibrium potentials to be read and determination of the titrant volume to be added, based on the actual buffering properties of the solution under titration, so that the differences in successive readings were of ~0.1 as $-\log [H^+]$. The estimated precision of the system was ±0.15 mV in the e.m.f. and ±0.003 ml in the titrant volume. The measurement cell was thermostatted at $t=25 \pm 0.2$ °C, and pure nitrogen presaturated with an ionic media solution at the same ionic strength of the working solution, was bubbled into the solution in order to avoid O_2 and CO_2 interference. The solutions were magnetically stirred.

Table 2
Protonation constants of phytate at different ionic strength and temperatures

Species	$\log K_i^{Ha}$						
	$t=25$ °C			$t=15$ °C		$t=37$ °C	
	$I=0$[b]	$I=0.04$[b]	$I=0.15$[b]	$I=0$[c]	$I=0.15$[c]	$I=0$[c]	$I=0.15$[c]
PhyH	18.17	17.24	9.41	18.25	9.50	18.08	9.31
$PhyH_2$	15.32	14.73	9.67	15.38	9.73	15.25	9.60
$PhyH_3$	12.76	12.41	9.33	12.78	9.35	12.73	9.30
$PhyH_4$	10.05	9.96	7.97	10.06	7.98	10.03	7.95
$PhyH_5$	7.59	7.54	6.35	7.59	6.35	7.58	6.34
$PhyH_6$	6.13	6.12	5.10	6.12	5.10	6.13	5.10
$PhyH_7$	3.52	3.52	2.75	3.49	2.56	3.55	2.62

[a] According to the equilibrium: $H_iPhy^{(12-i)-} + H^+ = PhyH_{i+1}^{(12-i+1)-}$.
[b] Ref. [40].
[c] Calculated by empirical equations (refs. [34–37]).

Table 3
Overall formation constants of phytate–BPEI-proton complexes at t = 25 °C

Species	$\log \beta_{pi}$[a]
$PhyBPEIH_6$	77.16 ± 0.03[b]
$PhyBPEIH_7$	86.20 ± 0.03
$PhyBPEIH_8$	93.75 ± 0.05
$Phy(BPEI)_2H_7$	90.65 ± 0.02

[a] According to Eq. (1).
[b] ±SD.

2.3. Procedure

25.0 ml of solution containing $0.50 \leq C_{BPEI} \leq 1.1$ mmol L^{-1} (calculated as a monomer) and $1 \leq C_{Phy} \leq 2$ mmol L^{-1} (Phy = phytate), were titrated with standard Et_4NOH; titration were carried out in the pH range from ≈7.0 to ≈10.4 and without any addition of supporting electrolyte. The mean ionic strength value of titrated solutions was $I = 0.04 \pm 0.01$ mol L^{-1}. For each titration, a separate calculation of the formal potential (E^0) was carried out by titrating HCl 10 mmol L^{-1} with standard NaOH; this determination was carried out both before and after each experiment. Proton concentration is given as pH = −log [H^+], i.e. the free hydrogen ion concentration scale was used. All the titrations were carried out in duplicate.

2.4. Calculations

The non-linear least squares computer program ESAB2M [32] was used to refine the parameters of the acid–base titrations (species concentrations, formal potential of electrode couple (E^0), water ionic product (K_w)). The formation constant values were refined by the computer program BSTAC [33] which minimize the error squares sum on electromotive force values.

In all the paper, phytate–polyammonium complex formation equilibria refer to the general equations:

$$pA^0 + Phy^{12-} + iH^+ \rightleftarrows A_pPhyH_i^{(12-i)-} \qquad \beta_{pi} \qquad (1)$$

$$pAH_i^{i+} + PhyH_z^{(12-z)-} \rightleftarrows A_pPhyH_j^{(12-j)-} \ (j = pi + z) \qquad K_{pj} \qquad (2)$$

where AH_i^{i+} is a generic polyammonium cation species and $PhyH_z^{(12-z)-}$ a phytate protonated species; A^0 is the neutral polyamine and Phy^{12-} the fully deprotonated form of phytate. Throughout the paper errors are given as ±standard deviation. The dependence of stability constants on temperature can be taken into account by the equation:

$$\log \ K_T = \ \log \ K_\theta + \Delta H_\theta F_1 \qquad (3)$$

where K is a generic formation constant; and

$$F_1 = \left(\frac{1}{\theta} - \frac{1}{T}\right)/R \ \ \ln \ \ 10$$

with $R = 8.309$ J $mol^{-1} \cdot K^{-1}$; θ is the reference temperature in K, and ΔH in J mol^{-1}. ΔH values were obtained from empirical relationships already reported [34,35], whilst the dependence of formation constants on ionic strength was taken into account by an extended Debye–Hückel type equation [36,37].

3. Results

3.1. Protonation constants

For the determination of high molecular weight polyelectrolytes protonation constants [6 and references therein], the dependence of log K^H on the degree of protonation (or on the degree of ionisation) must be taken into account; this implies the use of different calculation methods [38,39]. Nevertheless, in a previous work [23], a simple new approach to calculate the protonation constants, and enthalpy changes, of BPEI was proposed. By this approach BPEI polyelectrolyte can be considered as a simple low molecular weight tetramine, and the protonation steps were studied taking into account the following protonation equilibrium:

$$AH_{i-1}^{(i-1)+} + H^+ \rightleftarrows AH_i^{i+} \, (i = 1 \text{to} \, 4) \ K_i^H. \qquad (4)$$

This approach gives a simple picture of the acid–base proprieties of BPEI. As regards phytate, in some recent papers, we reported its protonation constants [40,41] and enthalpy changes in different ionic media [28,40,42]. In Tables 1 and 2 we report K_i^H values for BPEI and phytate, respectively.

3.2. Phy-BPEI species

The interaction of phytate with some biogenic amines, namely, ethylendiamine (En), 1-4-diaminobutane (putrescine, Ptr), *N*-[3-

Table 4
Thermodynamic parameters for the formation of phytate–BPEI-proton complexes at different temperature and ionic strength

Reaction[a]	ζ[b]	t = 25 °C								
		$\log K_{pj}$	$\log K_{pj}/\zeta$	$\log K_{pj}$	$\log K_{pj}/\zeta$	$\log K_{pj}$	$\log K_{pj}/\zeta$	$-\Delta G$[c]	$T\Delta S$[c]	ΔH[c]
		I = 0[d]		I = 0.04[d]		I = 0.15[d]		I = 0.04[d]		
$PhyH_4^{8-} + BPEIH_2^{2+} = PhyBPEIH_6^{6-}$	16	5.7 ± 0.2	0.36	5.34 ± 0.05	0.33	4.7 ± 0.2	0.30	31	93	62
$PhyH_5^{7-} + BPEIH_2^{2+} = PhyBPEIH_7^{5+}$	14	7.0 ± 0.1	0.50	6.84 ± 0.07	0.49	6.6 ± 0.2	0.47	39	81	42
$PhyH_5^{7-} + BPEIH_3^{3+} = PhyBPEIH_8^{4+}$	21	9.2 ± 0.2	0.44	8.77 ± 0.13	0.41	8.5 ± 0.2	0.40	51	122	71
$PhyH_4^{8-} + BPEIH^+ + BPEIH_2^{2+} = Phy(BPEI)_2H_7^{5+}$	24	10.0 ± 0.2	0.42	9.44 ± 0.10	0.39	8.7 ± 0.1	0.36	54	140	86
Mean value			0.43 ± 0.06		0.41 ± 0.07		0.38 ± 0.07			

	ζ[b]	t = 15 °C				t = 37 °C			
		$\log K_{pj}$	$\log K_{pj}/\zeta$	$\log K_{pj}$	$\log K_{pj}/\zeta$	$\log K_{pj}$	$\log K_{pj}/\zeta$	$\log K_{pj}$	$\log K_{pj}/\zeta$
		I = 0.04[d]		I = 0.15[d]		I = 0.04[d]		I = 0.15[d]	
$PhyH_4^{8-} + BPEIH_2^{2+} = PhyBPEIH_6^{6-}$	16	5.0 ± 0.2	0.31	4.3 ± 0.2	0.27	5.8 ± 0.2	0.36	5.1 ± 0.2	0.32
$PhyH_5^{7-} + BPEIH_2^{2+} = PhyBPEIH_7^{5+}$	14	6.6 ± 0.2	0.47	6.3 ± 0.2	0.45	7.1 ± 0.2	0.51	6.9 ± 0.1	0.49
$PhyH_5^{7-} + BPEIH_3^{3+} = PhyBPEIH_8^{4+}$	21	8.3 ± 0.2	0.39	8.1 ± 0.2	0.39	9.3 ± 0.2	0.44	8.8 ± 0.2	0.42
$PhyH_4^{8-} + BPEIH^+ + BPEIH_2^{2+} = Phy(BPEI)_2H_7^{5+}$	24	8.9 ± 0.2	0.37	8.1 ± 0.2	0.34	10.0 ± 0.2	0.42	9.2 ± 0.2	0.38
Mean value			0.39 ± 0.07		0.36 ± 0.08		0.43 ± 0.06		0.40 ± 0.07

[a] According to Eq. (2).
[b] Product of charges (ζ) involved in the reaction [see Eq. (5)].
[c] kJ mol^{-1}.
[d] I/mol L^{-1}.

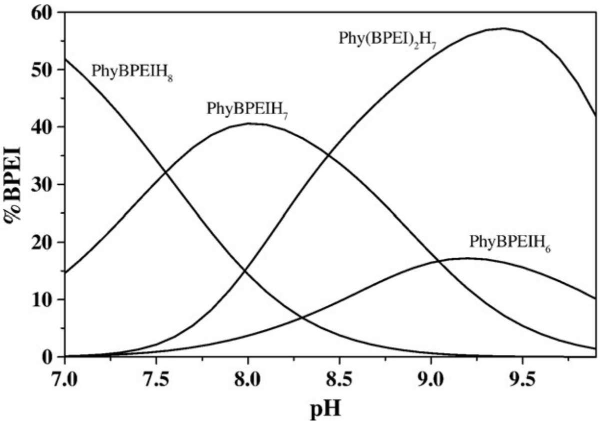

Fig. 2. Speciation diagram for the species of phytate–BPEI system at $I=0$ mol L^{-1} and $t=25$ °C. Experimental condition: $C_{\mathrm{BPEI}}=1$ mmol L^{-1} and $C_{\mathrm{phytate}}=1.5$ mmol L^{-1}.

aminopropyl]-1,4-diaminobutane (spermidine, Spd), and *N,N*'-bis[3-aminopropyl]-1,4-diaminobutane (sperimine, Sper) was already studied [18]. In this study, we observed that phytate forms fairly strong mixed (proton-amine) complexes, in particular when the charges of reactants involved in the formation equilibrium are quite high. In this order, here we investigated the interactions of phytate with an high molecular weight polyammonium cation (MW=750 kDa), such as BPEI; the interactions were studied at low ionic strength values and without addition of supporting electrolyte, owing to the strong interference that phytate anion may undergo with alkali and alkaline earth metal cations [43,44]. Table 3 reports the overall complex formation constants obtained [Eq. (1)], whilst the equilibrium constants, according to the reaction (2), are reported in Table 4. The first three equilibria refer to the generic reaction (2) (1:1 metal–ligand ratio), and the last one refers to the interaction of one mole of phytate with two different protonated species of BPEI, namely mono- and diprotonated species. From the formation constants reported in Tables 3 and 4, it appears that phytate forms with BPEI complexes whose stability is comparable with that of low molecular weight polyammonium cations [18]. It is interesting to observe, as already reported [18], the dependence of log K_{pj} on charges product

$$\zeta = |(\sum Z_{\mathrm{cation}}) \cdot Z_{\mathrm{anion}}| \tag{5}$$

In particular, we can see that the ratio log K_{pj}/ζ is fairly constant for all the species, with a mean value of 0.41±0.07. By using empirical equations [34–37] and enthalpy data for the protonation of BPEI and phytate, we calculated the formation constants of Phy(BPEI)$_p$H$_j$ species at different temperatures (15, 37 °C) and ionic strengths (0, 0.04, 0.15 mol L^{-1}). From the complex formation constants reported in Table 4 and Eq. (5), we calculated the log K_{pj}/ζ values. Therefore it is possible to derive the free energy contribution per bond [34–35] given by

$$\Delta G_{\mathrm{b}}^{0} = \Delta G^{0} / \underline{n}$$

with $\underline{n}=\zeta/2$. At $t=25$ °C and infinite dilution, we have $\Delta G_{\mathrm{b}}^{0}=4.4\pm 0.4$ kJ mol^{-1}. In Fig. 2, we report a speciation diagram for the system studied in this work. Yields of phytate–BPEI-proton species are very high in the whole pH range. In particular, at pH values of seawater (pH≈8.1) or blood (pH≈7.4), the species PhyBPEIH$_7$ and PhyBPEIH$_8$ become the predominant ones. Also the binuclear species, Phy(BPEI)$_2$H$_7$, reaches a formation percentage of about 60% at pH≈9.2, in the reported experimental conditions. In considering these formation constants and formation percentages, one must take into account that they were obtained in the absence of an interacting supporting electrolyte such as NaCl, which is present in all natural and biological fluids. This is particularly important if we take into account that phytate forms stable complexes with alkali metals [28,43], and this interaction may change significantly the speciation of the system. The interaction of a polyammonium cation with phytate is dependent on the concentration of the supporting electrolyte; in fact, in a previous work [18], we calculated the competition between Na$^+$ and two polyammonium cations, in particular ethylenediamine (diamine) and sperimine (tetramine), *vs.* phytate. It was pointed out that in the phytate–ethylenediamine system, phytate there is a strong competition of Na$^+$ with the ammonium cation, at low sodium concentrations ($C_{\mathrm{Na}}\geq 0.05$ mol L^{-1}), whilst in the case of spermine the competition with Na$^+$ can be considered negligible up to $C_{\mathrm{Na}}>0.1$ mol L^{-1}, owing to the high charge (4+) of this amine with respect to ethylenediamine (2+). In the case of the Phy-BPEI system, by taking into account the log K_{pj} and log K_{pj}/ζ values with respect to other phytate–polyammonium cation systems (Table 5), it can be observed that BPEI behaves, in the interaction with phytate, approximately like a diamine or a triamine, rather than a tetramine.

3.3. Sequestering ability of phytate towards BPEI

In a previous paper [44], we proposed a Boltzman type equation able to define the sequestering ability of phytate towards cations, such as Mg^{2+} and Ca^{2+}, by means of a function that takes into account the sum of formation percentages of all cation–ligand complexes, Σ(%), vs. pL, where pL≡−log[L]$_{\mathrm{tot}}$([*L*]$_{\mathrm{tot}}$ is the total ligand concentration). This

Table 5
Literature values of log K_{pj}/ζ ratio for some polyphosphate–polyammonium cation systems at infinite dilution, at $t=25$ °C

Systems	log K_{pj}/ζ	Number of species
BPEI–phytate[a]	0.41±0.07[g]	4
En-phytate[b]	0.40±0.04	9
Ptr-phytate[b]	0.41±0.06	8
Spd-phytate[b]	0.31±0.05	8
Sper-phytate[b]	0.35±0.05	6
En-PO_4^{3-}[c]	0.77±0.03	3
Dien-PO_4^{3-}[c]	0.95±0.20	4
Trien-PO_4^{3-}[c]	1.18±0.43	5
Penten-PO_4^{3-}[c]	0.64±0.11	8
En-$\mathrm{P_2O}_7^{4-}$[c]	0.80±0.15	6
Dien-$\mathrm{P_2O}_7^{4-}$[c]	0.85±0.16	6
Trien-$\mathrm{P_2O}_7^{4-}$[c]	0.86±0.14	8
Tetren-$\mathrm{P_2O}_7^{4-}$[d]	070±0.12	7
Penten-$\mathrm{P_2O}_7^{4-}$[d]	0.82±0.18	9
Dien-$\mathrm{P_3O}_{10}^{5-}$[e]	0.91±0.21	6
Trien-$\mathrm{P_3O}_{10}^{5-}$[e]	0.87±0.13	7
Tetren-$\mathrm{P_3O}_{10}^{5-}$[e]	0.65±0.07	8
Penten-$\mathrm{P_3O}_{10}^{5-}$[e]	0.66±0.10	8
Spd-Tar[f]	0.52±0.10	4
Spd-Cit[f]	0.43±0.03	5
Spd-Glu[f]	0.58±0.08	4
Spd-Tca[f]	0.39±0.04	5
Spd-Btc[f]	0.49±0.05	6
Sper-Tar[f]	0.68±0.15	6
SperCit[f]	0.62±0.13	7
Sper-Tca[f]	0.53±0.08	6
Sper-Btc[f]	0.49±0.06	7
En-Tar[f]	0.48±0.06	3
En-Cit[f]	0.63±0.12	4
En-Tca[f]	0.64±0.11	4
En-Btc[f]	0.56±0.08	5
Ptr-Tar[f]	0.98±0.24	3
Ptr-Cit[f]	0.75±0.14	4
Ptr-Tca[f]	0.64±0.12	4
Ptr-Btc[f]	0.65±0.11	5

[a] This work.
[b] Ref. [18].
[c] Ref. [46].
[d] Ref. [15].
[e] Ref. [16].
[f] Ref. [45].
[g] ±SD.

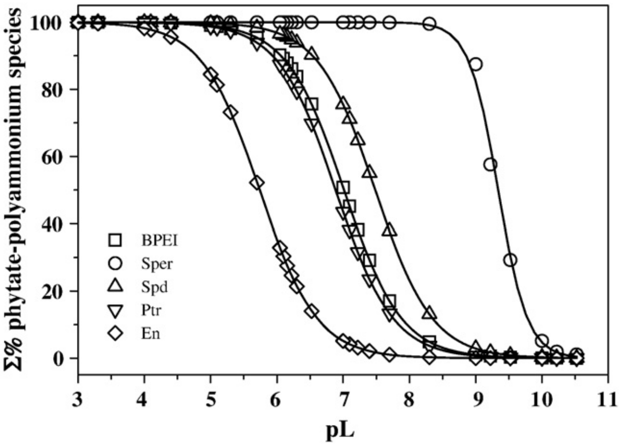

Fig. 3. Sum of percentages of phytate–polyammonium cations species vs. pL at $I=0$ mol L^{-1}, $t=25$ °C and pH=7.4. Concentration: $C_{\text{polyammonium cation}}$=(trace).

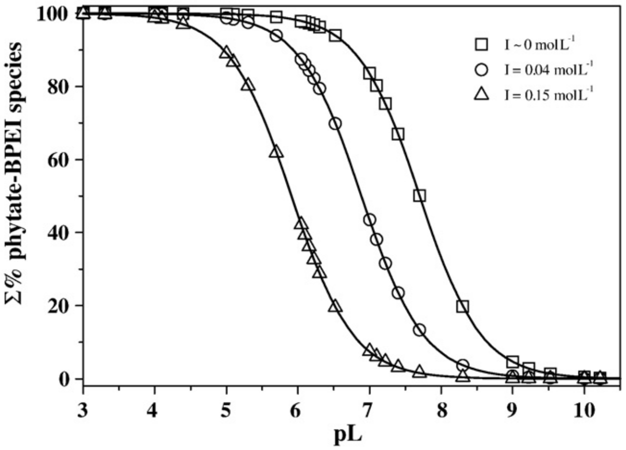

Fig. 4. Sum of percentages of phytate–BPEI species vs. pL at different ionic strengths at pH=7.4 and $t=25$ °C. Concentration: C_{BPEI}=(trace).

function is assimilable to a sigmoid curve (or a dose response curve) with asymptotes of 100 for pL→−∞ and 0 for pL→+∞:

$$\sum(\%) = 100 \times \left[\frac{1}{1+\ exp\ \left[(pL - pL_{50})/S\right]} - 1\right] \tag{6}$$

where pL_{50} and S are empirical parameters. In particular, pL_{50} represents the ligand concentration necessary to sequester 50% of metal ion; therefore this parameter can be used as a measure of the sequestering abilities of different ligands. The parameter S is a measure of the slope in the flex of the function Σ(%) *vs*. pL. The Boltzman type equation was also applied to the phytate–BPEI and other phytate–polyammonium cation systems (namely: En, Ptr, Spd, Sper); the sequestering diagram reported in Fig. 3 was drawn at pH=7.4 and $I\approx 0$ mol L^{-1}, at a very low polyammonium cation concentration (as trace) and phytate concentration from ≈10^{-11} to 10^{-3} mol L^{-1}. From the fit of Eq. (6) we obtained, for example, that the concentration of phytate necessary to sequester 50% of the initial BPEI, in this experimental condition, was $9.77\cdot 10^{-8}$ mol L^{-1} ($pL_{50}=7.01$). The sequestering ability of phytate towards polyammonium cation follows the trend:

Sper >>Spd > BPEI >>Ptr > En

The pL_{50} and S values for each phytate–polyammonium cation system, calculated at pH=7.4 and $I\approx 0$ mol L^{-1}, are reported in Table 6. From Fig. 3 and from the pL_{50} values reported, it can be observed that the sequestering ability of phytate towards BPEI is intermediate between those observed for Spd and Ptr, that are a tri- and a diamine, respectively. This behaviour is due to the partial deprotonation of BPEI at pH>6; in fact two protonation constants of BPEI are in the acidic region (log $K_3^{\mathrm{H}}=5.62$ and log $K_4^{\mathrm{H}}=2.23$, at $I=0.04$ mol L^{-1} and $t=25$ °C). The log K_{pj}/ζ values reported in Table 5, for the phytate–BPEI and phytate–Ptr systems are, within the standard deviation, equal. Since the potentiometric measurements were carried out in the pH range from ≈7.0 to ≈10.4, two aminic groups are deprotonated and BPEI behaves as a discharged cation. In particular we must notice the sequestering behaviour of phytate towards spermine. The stability of the species formed in this systems is evidenced by the high formation constant and pL_{50} values and also by the sharp slope of the curve Σ% *vs*. pL ($S=0.20$ in comparison with $S=0.43$ for the other systems). Two factors contribute to this behaviour: the first one is related to the complete protonation of spermine in a wide pH range, as above discussed, and, very likely to the favourable distance between ammonium groups that render easier the binding to phytate.

Table 6
Empirical parameters of Eq. (6) calculated at pH=7.4 and $I\approx 0$ mol L^{-1} at $t=25$ °C

System	pL_{50}	S
Phy-BPEI	7.01	0.433
Phy-Sper	9.34	0.199
Phy-Spd	7.48	0.432
Phy-Ptr	6.88	0.434
Phy-En	5.74	0.434

Two factors that can influence the sequestering ability of BPEI towards phytate are the variation of ionic strength and temperature; these effects are shown in Figs. 4 and 5, respectively. In Fig. 4 we report a sequestering diagram of phytate–BPEI system at three different ionic strengths and $t=25$ °C; we obtained the following pL_{50} values: $pL_{50}=7.70$ ($I=0$ mol L^{-1}); $pL_{50}=6.89$ ($I=0.04$ mol L^{-1}); $pL_{50}=5.91$ ($I=0.15$ mol L^{-1}) and $S=0.43$. Fig. 5 shows a sequestering diagram at $I=0.15$ mol L^{-1} and $t=15$, 25 and 37 °C, respectively. In this case we obtained: $pL_{50}=5.67$ ($t=15$ °C); $pL_{50}=5.91$ ($t=25$ °C); $pL_{50}=5.99$ ($t=37$ °C) and $S=0.43$. From the pL_{50} values obtained by fitting data of Figs. 4 and 5, it is evident that the sequestering ability of phytate towards BPEI is mostly dependent on the variation of ionic strength rather than the variation of temperature. In fact, from the pL_{50} values above reported it is evident that a variation of temperature from $t=15$ to 37 °C, gives a relatively low variation of 0.5 units in pL_{50}. Instead ionic strength gives a higher contribution in the variation of pL_{50} that

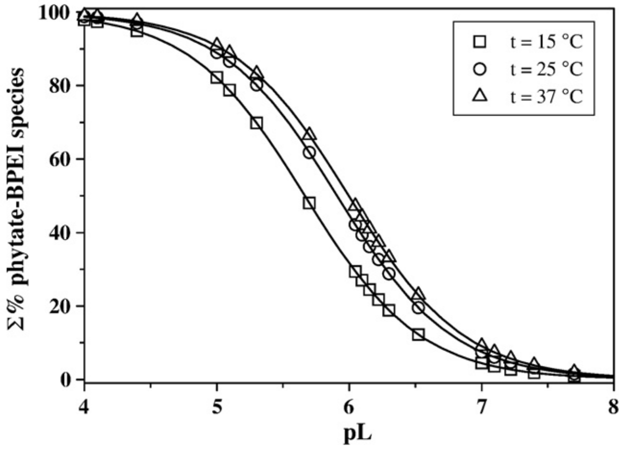

Fig. 5. Sum of percentages of phytate–BPEI species vs. pL at different temperatures at pH=7.4 and $I=0.15$ mol L^{-1}. Concentration: C_{BPEI}=(trace).

differs of ≈2.0 log units in the ionic strength range from $I=0$ to 0.15 mol L^{-1}.

4. Comparison with other similar systems

In the literature several data can be found on the interaction of open chain linear polyammonium cations with several polyanions [13–19]. As reported [2,15,16,18], the stability of these complexes is strictly dependent on the charges involved in the formation reaction, and when the charges are the same, similar values of formation constants were obtained. These behaviours can be observed in the formation constant values and the log K_{pj}/ζ ratio obtained for the interaction of phytate with some biogenic amines (ethylenediamine (En), 1-4-diaminobutane (putrescine, Ptr), 1,5-diaminopentane (cadaverine, 1,5d), diethylenetriamine (Dien), *N*,*N*'-bis[3-aminopropyl]-1,4-diaminobutane (sperimine, Sper), *N*-[3-aminopropyl]-1,4-diaminobutane (spermidine, Spd), tetraethylenpentamine (Tetren) and pentaethylenehexamine (Penten)) [18] and of some polyammonium cations with polycarboxylate (i.e. tartrate (Tar); citrate (Cit); glutamate (Glu); 1,2,3-propanetricarboxylate (Tca) and 1,2,3,4-butanetetracarboxylate (Btc)) [45] (see Table 5). Nevertheless in this last work [45], we also observed that the structure of both polyammonium cations and polyanions plays a less important role. Taking into account the same phytate–polyammonium cation systems, we observe that the log K_{pj}/ζ ratio can be considered constant, with a slight decrease of its value when the number of amino groups in the molecules increases. In the case of phytate–BPEI interaction, we obtained, as above seen, a mean value of log $K_{pj}/\zeta=0.41$, that is comparable with the interaction of phytate with a diamine (such as putrescine). Moreover, comparison can be made with other polyammonium cation–ligand systems reported in Table 5.

5. Free energy contribution per bond

As already seen in the previous sections, there is a relationship between the logarithm of formation constants and the charges involved in the formation reaction; the different log K_{pj}/ζ values observed for the various systems, and the lower log K_{pj}/ζ values of the phytate–BPEI-proton system with respect to other polyammonium cation–polyphosphate systems is imputable to the conformational arrangement of its phosphate groups [28] and to the charge density. For the phytate–BPEI-proton system, we calculated the free energy contribution per bond, that is 4.4 kJ mol^{-1}, whilst for the polyammonium cation–polyphosphate systems reported in Table 5 we calculated a mean value of $\Delta G_b^0=9.4$ kJ mol^{-1}. Similar comparison can be made with the polyammonium cation–polycarboxylate systems; in this case we obtained $\Delta G_b^0=6.7$ kJ mol^{-1}. Greater differences are observed if we take into account high molecular weight molecules; in fact in a previous work [6] we obtained for the interaction of an high molecular weight polycarboxylate (polyacrylate 2 kDa) with polyammonium cations a free energy contribution per bond of $\Delta G_b^0=16.1$ kJ mol^{-1} and for two polysulphonates (9 and 1000 kDa) with polyammonium cations a value of $\Delta G_b^0=13.8$ kJ mol^{-1}. This sharp difference in the stability of low and high molecular weight polyanion and polycation complexes is due to the high charge density of polyelectrolytes with respect to low molecular weight systems. In a recent work some correction for the effective charge (higher than the formal ones) were considered too [19]. This parameter is a simple evidence of the different sequestering ability of several polycations towards polyanions. Similar investigations were carried out by Bazzicalupi et al. [9] on the interaction of phosphate and pyrophosphate with several polyammonium receptors. They observed that although the electrostatic attraction is the driving force in anion interactions with polyammonium receptors, the stability trends of such complexes are not strictly determined by electrostatic contributions, in contrast with the general trend of increasing stability with increasing charge of receptors and anions. In fact considering the stability of the complexes formed by phosphate and pyrophosphate in a given protonation degree, with the same receptors, they did not observe a trend in contrast to electrostatic expectations, and that the less charged anion can form more stable complexes. They concluded that the stability of complexes is strictly dependent on the ability of ligands (phosphate and pyrophosphate) to involve one or more phosphate moieties in the interaction with a single polyammonium cations, and this assumption is in agreement with the results reported in our work, especially for the systems involving phytate.

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