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ORIGINAL PAPER

Thermodynamic data for lanthanoid(III) sequestration by phytate at different temperatures

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Abstract The results of an investigation on the interactions between phytate ion (*myo*-inositol hexaphosphate, Phy) and some lanthanoid cations (La^{3+}, Nd^{3+}, Sm^{3+}, Dy^{3+}, and Yb^{3+}) are reported. The stability constants of various LnH_jPhy species (Ln = generic lanthanoid) were determined by potentiometry (ISE-H^+ glass electrode) in $NaCl_{aq}$ at $I = 0.15$ mol dm^{-3} and $t = 25$ °C, and the corresponding formation enthalpies by calorimetric titration. The thermodynamic data obtained were used to provide a speciation scheme for the lanthanoid(III)–phytate systems at different temperatures. The sequestering ability of this ligand toward Ln^{3+} was also evaluated by calculation of pL_{50} values (the total concentration of ligand necessary to bind 50% of a cation present in trace amounts) under different conditions, and equations were formulated to model their dependence on temperature and pH.

Keywords Lanthanoids · Phytic acid · Speciation · Stability constants · Thermodynamics

Introduction

Phytic acid (*myo*-inositol-1,2,3,4,5,6-hexakis(dihydrogenphosphate)) and its salts (the deprotonated form is denoted in this work by "Phy") are widespread biological species of much interest to workers in very different fields, because of the variety of its applications in nutrition, environmental science, medicine, and technology (detailed information may be found, e.g., in Refs. [2–8]). This is also apparent from the large number of scientific articles and reviews dedicated to phytate, published in different journals with a very broad audience. The molecule is also very interesting physicochemically, the quite strong binding ability of phytate toward metal ions being one of its most interesting properties [2]. Because of this last characteristic, for example, phytate may alter the bioavailability of some metals and may interfere with the uptake of some nutrients by organisms. Analogously, because this ligand is a "non-toxic" and "low cost" product, it has also been used for remediation of contaminated sites [9–14]. In this light, some years ago we started investigating the physicochemical behavior of phytate ion in aqueous solution, by determining a series of thermodynamic data (i.e., stability constants, and entropy and enthalpy changes) necessary for definition of phytate speciation and binding ability toward several metal and organometal cations of biological and environmental interest, and toward polyammonium cations (some results are reviewed in Ref. [2]). As an extension of this study, in this paper we report results from an investigation on the interactions between phytate ion and some lanthanoid cations (La^{3+}, Nd^{3+}, Sm^{3+}, Dy^{3+}, and Yb^{3+}; a generic lanthanoid will subsequently be denoted "Ln^{3+}"). The impressive number of fields in which these cations are commonly used, and detailed information about their biological and environmental activity, are described, for example, in Refs. [15–25]. Here it is sufficient to remember that the characteristics that make the lanthanoid(III) cations suitable for application in medicine, industry, and other fields are strictly related to their chemical speciation and their coordination chemistry [16, 19, 25]. Because there is a concrete possibility that both some lanthanoids(III) and

Speciation of phytate ion in aqueous solution. Last contribution to this series: Ref. [1].

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phytate may be simultaneously present in a series of biological and environmental systems (e.g., soils, organisms), a correct scheme for speciation of aqueous systems containing both the lanthanoids(III) and phytate is essential. Moreover, the quite strong binding ability of phytate may be exploited for chelation of these cations, the direct consequences of which may have a large impact in key sectors such as, e.g., medicine (diagnosis and therapy) or environmental science (remediation).

Results and discussion

Before any discussion of the results obtained, an important aspect must be pointed out. In its completely deprotonated form, phytic acid should be regarded as a dodecanion. However, it has been observed [26–28] that this ligand, in the presence of aqueous solutions of alkali metal cations (Na^+ in our case), is never present in this form, and has a variable effective charge ranging from $z = 5-$ to $z = 7-$. For this reason, in this manuscript charges on the various phytate species have been omitted for simplicity.

Formation and stability of LnH_jPhy species

Phytate protonation constants in $NaCl_{aq}$ were taken from a previous paper [29], and the speciation schemes reported by Baes and Mesmer [30] were adopted for Ln^{3+} hydrolysis, considering their recommended hydrolysis constant values with the corresponding data for the dependence on ionic strength. Though these data refer mainly to perchlorate media [30], they were used because preliminary calculations showed that Ln^{3+} hydrolysis is strongly inhibited by phytate complexation, as is also observed for other cations [28, 31–34]. Therefore, hydrolysis constants of low accuracy do not dramatically affect the accuracy of stability constants determined for the LnH_jPhy species. Analysis of potentiometric data gave evidence of the formation of seven mononuclear species for all the lanthanoids investigated, namely: LnPhy, LnHPhy, LnH_2Phy, LnH_3Phy, LnH_4Phy, LnH_5Phy, LnH_6Phy. The corresponding formation constants are reported in Table 1.

Under the experimental conditions adopted (high ligand-to-metal ratios), no polynuclear species were observed. The high stability constants of the LnPhy species reported in Table 1 indicate a need for further checking of these values. In fact, as also reported in the literature [35], the high stability of some metal–ligand complexes may very often lead to unreliable results when the corresponding formation constants are only determined by simple ISE-H^+ potentiometric titrations. This is because of the possible complete displacement of the proton from the ligand when the metal cation is present in the titrand solution. One suitable solution to these undesirable effects is to perform potentiometric titrations in the presence of another ligand whose stability constants with the metal cation under investigation are well known and are comparable with those of the ligand to be studied [35]. In this way, the competition between the two ligands for the metal cation hinders complete proton displacement from the investigated ligand, making its potentiometric titration (ISE-H^+) reliable. For these purposes, in order to be more confident of the formation constants reported in Table 1, some ISE-H^+ potentiometric titrations were also performed for the Phy–La^{3+} system in the presence of ethylenediaminetetraacetate (EDTA). This ligand was chosen because its stability constants with La^{3+}

Table 1 Stability constants of LnH_jPhy species at $t = 25$ °C and $I = 0.15$ mol dm^{-3} in $NaCl_{aq}$

j	La^{3+}	Nd^{3+}	Sm^{3+}	Dy^{3+}	Yb^{3+}	Ln^{3+} [a]
log K^{b}_{1j}						
0	15.42 ± 0.09	15.59 ± 0.16	14.80 ± 0.12	15.63 ± 0.12	15.49 ± 0.24	15.4 ± 0.3
1	14.53 ± 0.11	14.46 ± 0.19	13.76 ± 0.20	14.67 ± 0.16	14.66 ± 0.25	14.6 ± 0.4
2	13.72 ± 0.09	13.87 ± 0.16	13.14 ± 0.15	13.94 ± 0.13	13.85 ± 0.27	13.7 ± 0.3
3	10.60 ± 0.08	10.79 ± 0.15	10.14 ± 0.16	10.87 ± 0.12	10.72 ± 0.24	10.6 ± 0.3
4	7.78 ± 0.06	7.99 ± 0.11	7.41 ± 0.12	8.00 ± 0.09	7.98 ± 0.19	7.8 ± 0.3
5	4.87 ± 0.06	5.13 ± 0.10	4.45 ± 0.11	4.94 ± 0.09	5.34 ± 0.12	4.9 ± 0.3
6	3.32 ± 0.04	3.50 ± 0.08	2.87 ± 0.10	3.41 ± 0.07	3.04 ± 0.10	3.2 ± 0.3
log β^{c}_{1j}						
0	15.42	15.59	14.80	15.63	15.49	15.4
1	23.94	23.87	23.17	24.08	24.07	23.8
2	32.80	32.95	32.22	33.02	32.93	32.8
3	39.01	39.20	38.55	39.28	39.13	39.0
4	44.16	44.37	43.79	44.38	44.36	44.2
5	47.60	47.86	47.18	47.67	48.07	47.7
6	51.15	51.33	50.70	51.24	50.87	51.1

[a] Mean values, for a generic lanthanoid

[b] ±95% confidence interval; log K_{1j} refers to the equilibrium: $La^{3+} + H_jPhy = LaH_jPhy$

[c] log β_{1j} refers to the equilibrium: $La^{3+} + Phy + j\ H^+ = LaH_jPhy$

are well known in the literature, as reported in the most common databases [36–41], and are highly comparable with those of phytate (for the LaEDTA species, log $K = 15.2$ at $t = 25$ °C and $I = 0.15$ mol dm^{-3}). Determination of the stability constants of the LnH$_j$Phy species from these measurements gave, within experimental error, results which were highly comparable with the data reported in Table 1, supporting their reliability.

Formation enthalpy and entropy changes for LaH$_j$Phy species

The experimental conditions for the calorimetric measurements are reported in the Experimental section. The enthalpy changes for La^{3+} hydrolysis were calculated according to Klungness and Byrne [42], and the phytate protonation enthalpies were taken from Ref. [43]. The enthalpy changes calculated by the ES5CMI [44] computer program for the formation of LaH$_j$Phy species, together with the corresponding entropy values, were determined by taking into account the stability constants obtained from the potentiometric measurements and are reported in Table 2.

The thermodynamic data for the formation of the LaPhy species should be regarded as "tentative" only and must be handled with care, owing to the high error associated with the determination of the corresponding enthalpy change. ΔH_{1j} values reported in Table 2 can be also used for calculation of the stability constants of various LaH$_j$Phy species at temperatures other than $t = 25$ °C by applying the Clarke and Glew [45] equation. Assuming that ΔC_p are negligible in a small temperature range, this equation can be written as:

$$\log K_{1jT} = \log K_{1j\theta} + \Delta H_{1j}\,(1/298.15 - 1/T)/2.303R \qquad (1)$$

where $\log K_{1jT}$ is the stability constant at a given temperature (in Kelvin), $\log K_{1j\theta}$ is the corresponding value at $t = 25$ °C and with $R = 8.314472(15)$ J K^{-1} mol^{-1} when ΔH_{1j} is expressed in J mol^{-1}. As an example, the $\log K_{1j}$ values of various LaH$_j$Phy species were calculated at $t = 15$ and 37 °C, and are reported in Table 3.

Distribution and relevance of LnH$_j$Phy species

The stability constants of the LnH$_j$Phy species, reported in Table 1, show that the behavior of various lanthanoids toward phytate ion is quite similar. All these cations form the same species, and their stability is very similar. This suggested the possibility of proposing a common scheme for speciation of a generic lanthanoid in the presence of phytate, where the $\log K$ values of different LnH$_j$Phy species are the "mean" stability constants for the systems investigated in this work, and are reported in the last column of Table 1. The distribution of these species versus pH is shown in Fig. 1, in which the range (at the 95% confidence interval) of the highest formation percentages reached by single species, calculated by taking into account the errors associated to the various stability constants, is reported.

Of course, considering the speciation scheme for the "generic lanthanoid" results in high variability of formation percentages of a single species, but this approximation may be equally useful to derive some important information. First of all, from analysis of Fig. 1 it is clear that LnH$_j$Phy species are formed in appreciable percentages over the whole pH range investigated. Moreover, although the percentage formation of a single species is highly variable (as demonstrated by the wide error bars at the maximum percentages), the amount of a cation complexed by phytate is quite constant at a given pH. Finally, errors associated with the percentage formation of LnH$_2$Phy species are lower than for others. Because this is the main species at pH $\approx$ 7–8, the result is that speciation of the lanthanoid(III)–phytate system is better defined in the typical pH range of several natural waters and biological fluids (e.g., for blood plasma it is pH $\approx$ 7.35, for seawater

Table 2 Thermodynamic data for the formation of LaH$_j$Phy species, at $t = 25$ °C and $I = 0.15$ mol dm^{-3} in NaCl$_{aq}$

j	ΔH_{1j} (kJ mol^{-1})	$T\Delta S_{1j}$ (kJ mol^{-1})	ΔG_{1j} (kJ mol^{-1})
0	−127 ± 3[a] (−127)[b]	−39 ± 3[a] (−39)[b]	−87.96 ± 0.5[a] (−87.96)[b]
1	−103.0 ± 0.7 (−91.2)	33.6 ± 0.3 (−8.3)	−136.57 ± 0.6 (−82.89)
2	−84.8 ± 0.5 (−60.0)	102.3 ± 0.1 (18.3)	−187.11 ± 0.5 (−78.27)
3	−66.4 ± 0.5 (−38.0)	156.1 ± 0.2 (22.5)	−222.53 ± 0.5 (−60.46)
4	−47.4 ± 0.4 (−16.6)	204.5 ± 0.2 (27.8)	−251.91 ± 0.5 (−44.38)
5	−35.1 ± 0.6 (−3.6)	236.4 ± 0.5 (24.2)	−271.53 ± 0.5 (−27.78)
6	−26.0 ± 0.9 (5.5)	265.8 ± 0.9 (24.4)	−291.79 ± 0.2 (−18.94)

[a] Values refer to the equilibrium: $La^{3+} + Phy + j\,H^+ = LaH_jPhy$, ±95% confidence interval

[b] Values in parentheses refer to the equilibrium: $La^{3+} + H_jPhy = LaH_jPhy$

Table 3 Stability constants of LaH_jPhy species in $NaCl_{aq}$ at $I = 0.15$ mol dm^{-3}, at different temperatures, calculated by use of Eq. 1

j	log K^{a}_{1j}		
	$t = 15$ °C	$t = 25$ °C	$t = 37$ °C
0	16.19	15.42	14.56
1	15.08	14.53	13.91
2	14.08	13.72	13.31
3	10.83	10.60	10.34
4	7.88	7.78	7.67
5	4.89	4.87	4.85
6	3.29	3.32	3.36

[a] log K_{1j} refers to the equilibrium: $La^{3+} + H_jPhy = LaH_jPhy$

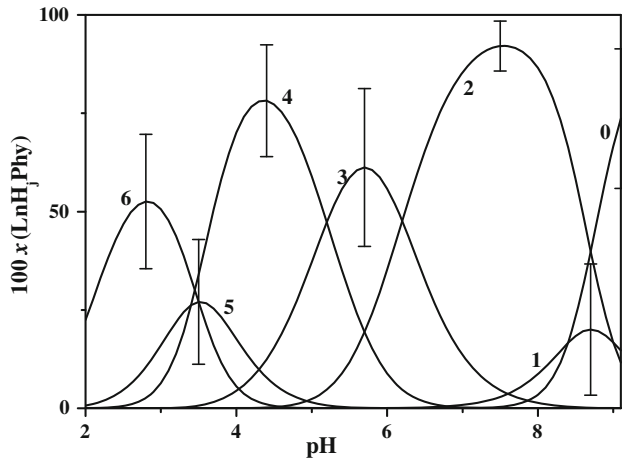

Fig. 1 Distribution diagram of LnH_jPhy species versus pH at $I = 0.15$ mol dm^{-3} in $NaCl_{aq}$ and $t = 25$ °C. Ln^{3+} stands for a generic lanthanoid. Experimental conditions: $c_{Ln} = 5 \times 10^{-4}$ mol dm^{-3}, $c_{Phy} = 2 \times 10^{-3}$ mol dm^{-3}. *j indexes* in the figure refer to LnH_jPhy species, e.g., "0" refers to LnPhy species. *Error bars* for the highest percentage formation of the various species are at the 95% confidence interval

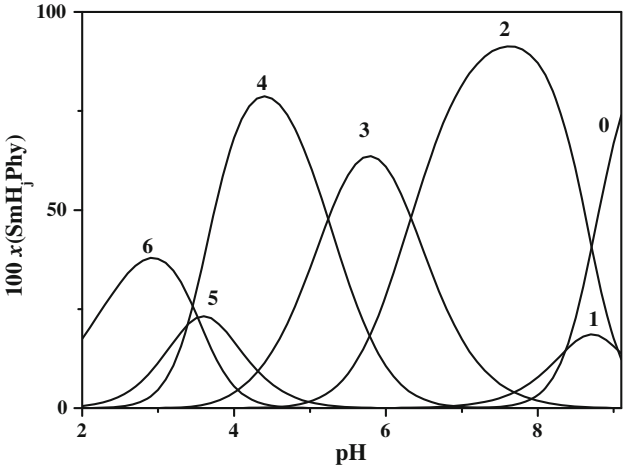

Fig. 2 Distribution diagram of SmH_jPhy species versus pH at $I = 0.15$ mol dm^{-3} in $NaCl_{aq}$ and $t = 25$ °C. Experimental conditions: $c_{Sm} = 5 \times 10^{-4}$ mol dm^{-3}, $c_{Phy} = 2 \times 10^{-3}$ mol dm^{-3}. *j indexes* in the figure refer to SmH_jPhy species, e.g., "0" refers to SmPhy species

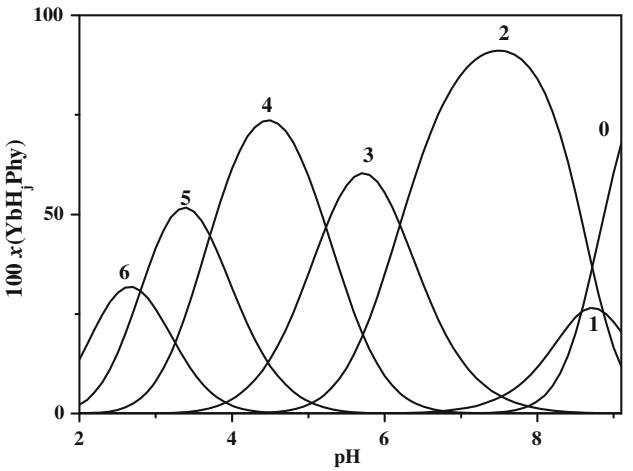

Fig. 3 Distribution diagram of YbH_jPhy species versus pH at $I = 0.15$ mol dm^{-3} in $NaCl_{aq}$ and $t = 25$ °C. Experimental conditions: $c_{Yb} = 5 \times 10^{-4}$ mol dm^{-3}, $c_{Phy} = 2 \times 10^{-3}$ mol dm^{-3}. *j indexes* in the figure refer to YbH_jPhy species, e.g., "0" refers to YbPhy species

it is pH $\approx$ 8.1). However, these considerations on the variability of formation percentage of LnH_jPhy species encouraged us to conduct a more critical analysis of stability constants of single systems, reported in Table 1. This analysis revealed that, although differences in the stability of LnH_jPhy species for the investigated lanthanoids are minor, they follow the trend suggested by the so called "double–double" or "tetrad" effect (originally rationalized by Fidelis and Siekierski [46–48] and by Peppard and coworkers [49, 50]). According to this effect, there is a correlation between the shape of the curves of a generic lanthanoid (or actinide) property (and, therefore, of stability constants also) and atomic number, resulting in division of the lanthanoid series into various subgroups. Looking at the shape of these curves, already reported in the original articles by Fidelis and Siekierski, the lower stability of SmH_jPhy species compared with the other LnH_jPhy species is coherent. The observed minor differences in the stability constants result in slight changes in the speciation diagrams of the Ln^{3+}–Phy systems investigated, shown in Figs. 2 and 3 for Sm^{3+} and Yb^{3+}, respectively.

For example, the high uncertainties of formation percentage that we observed for a generic lanthanoid (Fig. 1) in the acidic pH range are a logical consequence of the fact that the speciation plots of single systems show the highest discrepancies in this range. Formation of LnH_6Phy species

Table 4 Highest formation percentage, with regard to metal cation, of LnH_jPhy species in $NaCl_{aq}$ at $I = 0.15$ mol dm^{-3}, and $t = 25$ °C, and relative pH

j	La^{3+}		Nd^{3+}		Sm^{3+}		Dy^{3+}		Yb^{3+}		Ln^{3+} [a]	
	%	pH	%	pH	%	pH	%	pH	%	pH	%	pH
0	70.7	9.1	77.2	9.1	73.9	9.1	72.5	9.1	67.8	9.1	73.6	9.1
1	25.2	8.7	16.6	8.7	18.6	8.7	22.1	8.7	26.5	8.7	20.1	8.7
2	91.0	7.5	92.2	7.6	91.3	7.6	91.1	7.5	91.1	7.5	92.1	7.5
3	62.8	5.7	63.3	5.7	63.6	5.8	65.5	5.7	60.3	5.7	61.2	5.7
4	76.2	4.4	76.1	4.4	78.7	4.4	78.0	4.3	73.6	4.5	78.2	4.4
5	27.0	3.6	31.2	3.5	23.2	3.6	23.8	3.5	51.7	3.4	27.0	3.5
6	55.4	2.8	62.1	2.7	37.9	2.9	58.4	2.8	31.8	2.7	52.6	2.8

[a] Using stability constants for a generic lanthanoid

is >50% for all the lanthanoids investigated except Sm^{3+} and Yb^{3+}, and is always higher than that of LnH_5Phy for all Ln^{3+} except Yb^{3+}. In contrast, at pH ≈ 7–8, the main species is LnH_2Phy for all the systems investigated, with formation always >90%. The highest formation percentage reached by various Ln_iH_jPhy species in all the investigated systems, and the corresponding pH, are reported in Table 4 to facilitate comparisons among the different systems.

Sequestration of lanthanoids by phytate

Among the large number of direct applications of speciation studies, evaluation of the sequestering ability of a ligand toward a given cation or, better, comparison of the binding ability of two or more ligands is worth mentioning. This evaluation is frequently a challenging task, owing to the difficulties regarding, for example, the different number and/or nature of complexes formed. This last aspect is strictly correlated with the network of interactions occurring in a multicomponent system and, in particular, with the different complexing abilities of different ligand classes in different conditions and with competition of the proton and/or hydroxide ion with metals and ligands involved in the sequestration process. Very often, all these aspects combine to make comparison of sequestering ability very difficult. This problem has been overcome by calculation of pL_{50}, a semi-empirical parameter that, when the conditions (pH, ionic strength, supporting electrolyte, temperature) are fixed, can give an objective representation of this binding ability. This parameter, already tested successfully for other systems [31, 33, 51–53], is obtained by use of the following Boltzmann-type equation:

$$y = \frac{A_1 - A_2}{1 + 10^{(pL - pL_{50})}} + A_2 \quad (2)$$

where y represents the total percentage of not-complexed metal (a Ln^{3+} in our case), $A_1 = 0$, and $A_2 = 100$. It is important to stress again that:

1 this property varies with experimental conditions, but it is independent of the analytical concentration of the metal ion when this is present as a trace amount in the system; and
2 in the calculation of pL_{50}, all the side-interactions occurring in the system (metal hydrolysis, ligand protonation, interactions with other components) are taken into account in the speciation model, but are excluded from estimation of pL_{50} and do not make any contribution.

In this way, the pL_{50} value quantifies the sequestering power of a ligand, "cleaned" from all competitive reactions, simplifying comparisons. The higher the value of pL_{50}, the stronger the binding ability of the ligand. Analogously, for a ligand, the higher the value of pL_{50}, the stronger the binding ability toward a given cation. Moreover, the dependence of pL_{50} on experimental conditions can be modeled by very simple relationships as a function of different variables (pH, temperature, ionic strength).

pL_{50} values calculated for sequestration of the lanthanoids by phytate at different pH are reported in Table 5, pL_{50} values for sequestration of a generic lanthanoid Ln^{3+} are also reported.

Table 5 Calculated pL_{50} for lanthanoid(III) sequestration by phytate at different pH, at $t = 25$ °C and $I = 0.15$ mol dm^{-3} in $NaCl_{aq}$

pH	pL_{50}					
	La^{3+}	Nd^{3+}	Sm^{3+}	Dy^{3+}	Yb^{3+}	Ln^{3+} [a]
4.50	5.35	5.56	4.96	5.57	5.56	5.4
5.50	7.31	7.50	6.86	7.59	7.45	7.3
6.50	9.36	9.52	8.82	9.59	9.48	9.4
7.35	11.02	11.15	10.41	11.22	11.08	11.0
8.10	12.26	12.26	11.51	12.26	11.95	12.1

[a] For a generic lanthanoid

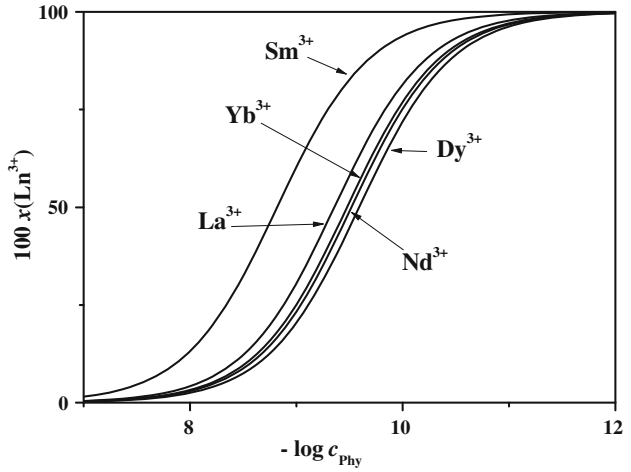

Fig. 4 Lanthanoid(III) sequestration diagrams in presence of phytate. Percentage of La^{3+}, Nd^{3+}, Sm^{3+}, Dy^{3+}, and Yb^{3+} not complexed by phytate as a function of total ligand concentration (as $-\log c_{Phy}$) in corresponding Phy–Ln^{3+} systems at $I = 0.15$ mol dm^{-3} in $NaCl_{aq}$ at $t = 25$ °C, at pH = 6.50. Total concentration of Ln^{3+}, $c_{Ln} = 10^{-15}$ mol dm^{-3}

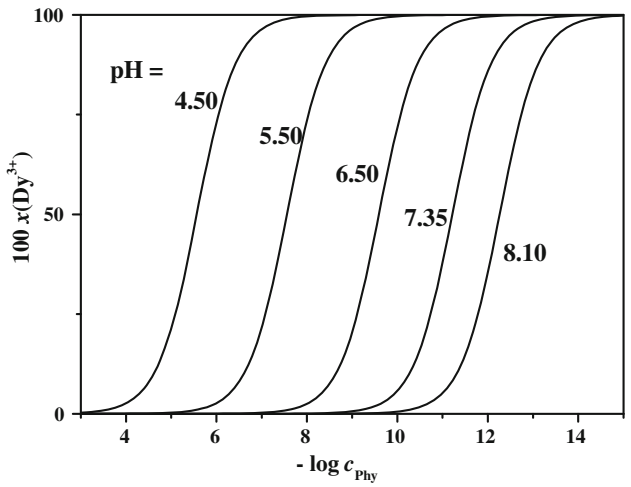

Fig. 5 Dysprosium(III) sequestration diagrams in the presence of phytate. Percentage of Dy^{3+} not complexed by phytate as a function of total ligand concentration (as $-\log c_{Phy}$) in the Phy–Dy^{3+} system at $I = 0.15$ mol dm^{-3} in $NaCl_{aq}$ at $t = 25$ °C, at different pH values. Total concentration of Dy^{3+}, $c_{Dy} = 10^{-15}$ mol dm^{-3}

Figure 4 shows sequestration diagrams for the lanthanoids at pH = 6.50, and Fig. 5 shows diagrams for sequestration of dysprosium(III) by phytate at different pH.

As observed from the analysis both in Table 5 and in these figures, phytate seems to be a good sequestering agent (high pL_{50} values) for all the cations investigated, and its sequestering ability is quite similar for all of them—only toward samarium is the sequestering ability of phytate slightly lower than that toward the other lanthanoids. Nevertheless, by analogy with our procedure for the stability constants of LnH_jPhy species, these similarities enable calculation of pL_{50} values for a generic lanthanoid. With regard to these pL_{50} values (reported in the last column of Table 5), it is important to remark that they may be calculated by two equivalent procedures. In one case, they can be obtained as the arithmetical mean of the pL_{50} values of single investigated lanthanoids; in another case, they can be derived from the sequestration diagrams constructed by use of the stability constants of LnH_jPhy species (reported for a generic lanthanoid in the last column of Table 1).

Concerning the dependence of pL_{50} on pH, it has already been demonstrated for other systems [28, 33, 51] that this can be easily modeled by simple relationships. As shown in Fig. 6 for the investigated lanthanoids also, pL_{50} are a linear function of pH.

The refined intercepts, slopes, and statistical data for the linear fits for the single systems are reported in Table 6, as also are those referred to pL_{50} for a generic lanthanoid.

The data in Table 6 and Fig. 6 clearly indicate that all these datasets can be modeled by a single function and are, within 95% confidence interval, statistically coincident. The coincidence of the linear regressions for the separated and the unique datasets was checked by performing the corresponding "test for the coincidence of regression lines" [54]. The global equation for the dependence of pL_{50} on pH, obtained by fitting together all data in Table 6 (except those for Ln^{3+}), is:

$$pL_{50} = -3.0 + 1.88\,pH \quad (3)$$

with a correlation coefficient of $r = 0.992$ and a standard deviation for the whole fit of $\sigma = 0.31$. As expected, the refined data for the global dataset are statistically coincident also with those obtained for fitting of pL_{50} values of the generic lanthanoid (last row of Table 6).

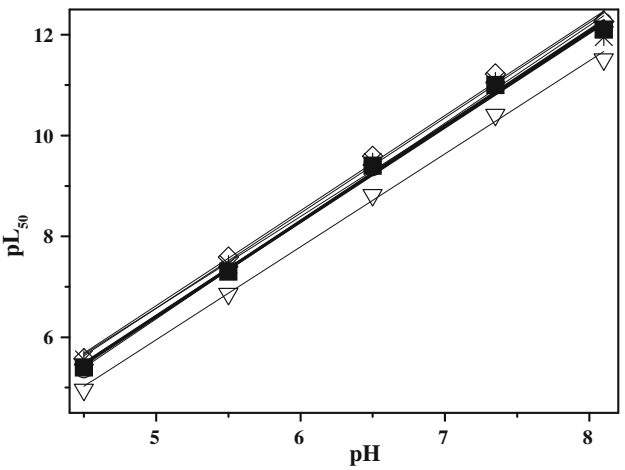

Fig. 6 Dependence on pH of pL_{50} values calculated for lanthanoid(III) sequestration by phytate at $I = 0.15$ mol dm^{-3} in $NaCl_{aq}$ at $t = 25$ °C. The *solid squares* and the *thick line* refer to a generic lanthanoid, Ln^{3+}; other *open symbols* and *thin lines* refer to single investigated lanthanoids, i.e., to La^{3+}, Nd^{3+}, Sm^{3+}, Dy^{3+}, and Yb^{3+}

Table 6 Regression data for the dependence of pL_{50} on pH for lanthanoid(III) sequestration by phytate at $t = 25$ °C and $I = 0.15$ mol dm^{-3} in $NaCl_{aq}$

	a	*b*	r^b	σ^c
La^{3+}	−3.3	1.94	0.999	0.11
Nd^{3+}	−2.9	1.89	0.999	0.15
Sm^{3+}	−3.3	1.84	0.999	0.14
Dy^{3+}	−2.8	1.89	0.998	0.19
Yb^{3+}	−2.5	1.82	0.997	0.24
Ln^{3+} [a]	−3.1	1.90	0.999	0.16

Data refer to the equation: $pL_{50} = a + b$ pH

[a] For a generic lanthanoid

[b] Correlation coefficient

[c] 95% Confidence interval for the whole fit

Effect of temperature on La^{3+} speciation and sequestration

The stability constants of LaH_jPhy species calculated at $t = 15$ and 37 °C by use of Eq. 1 and shown in Table 3 with corresponding values at $t = 25$ °C may be exploited to assess the effect of temperature on the speciation of La^{3+} in the presence of phytate. Moreover, $t = 37$ °C and $I = 0.15$ mol dm^{-3} in $NaCl_{aq}$ really approximate to physiological conditions (for blood plasma $I \approx 0.16$ mol dm^{-3}), and it is well known that both phytate and lanthanum(III) may be present in organisms (phytate occurs mainly in the diet; La(III) and other Ln(III) have several therapeutic applications). Because they can be present simultaneously, knowledge of La^{3+} speciation in the presence of phytate under these conditions may be extremely useful. For example, Ln(III)-based drugs, if consumed orally just before or soon after phytate-rich food (cereals, legumes, etc.), may have their efficacy modified. Figure 7 shows the La^{3+} speciation diagrams in the presence of phytate at $t = 15$, 25, and 37 °C.

As can be noted, the effect of temperature on the speciation of the Phy–La^{3+} system is quite negligible in this small range (i.e., $15 \leq t/$ °C ≤ 37). Only a very small shift ($\sim$0.1–0.2 pH units) toward more basic pH is observed in the formation of the species when the temperature is increased. Similarly, stability constant values at different temperatures may be used to calculate the pL_{50} values for sequestration of La^{3+} by phytate at these temperatures. As an example, Fig. 8 shows the La^{3+} sequestration diagrams at $t = 15$, 25, and 37 °C at pH = 7.35 (blood plasma is pH $\approx$ 7.35).

Corresponding pL_{50} values at this pH are $pL_{50} = 11.39$, 11.02, and 10.70 at $t = 15$, 25, and 37 °C, respectively. This is an indication that the sequestering ability of phytate toward lanthanoids decreases slightly with increasing temperature. The above reported pL_{50} values show that the

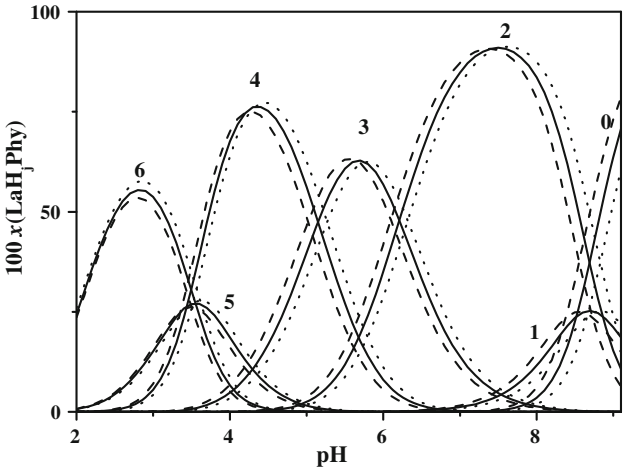

Fig. 7 Distribution diagram of LaH_jPhy species versus pH at $I = 0.15$ mol dm^{-3} in $NaCl_{aq}$ at different temperatures. Experimental conditions: $c_{Ln} = 5 \times 10^{-4}$ mol dm^{-3}, $c_{Phy} = 2 \times 10^{-3}$ mol dm^{-3}. *j indexes* in the figure refer to LaH_iPhy species, e.g., "0" refers to LaPhy species. *Dashed lines* $t = 15$ °C; *continuous lines* $t = 25$ °C; *dotted lines* $t = 37$ °C

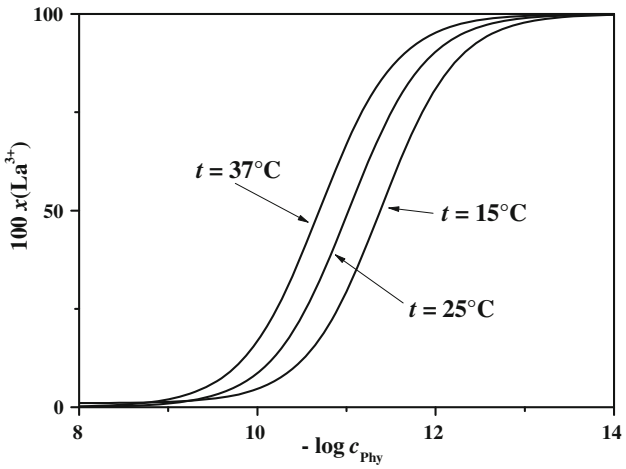

Fig. 8 Lanthanum(III) sequestration diagrams in the presence of phytate. Percentage of La^{3+} not complexed by phytate as a function of total ligand concentration (as $-\log c_{Phy}$) in the Phy–La^{3+} system at $I = 0.15$ mol dm^{-3} in $NaCl_{aq}$ at pH = 7.35, at different temperatures. Total concentration of La^{3+}, $c_{La} = 10^{-15}$ mol dm^{-3}

effect of temperature on phytate sequestering ability is less marked than that expected solely from analysis of the stability constants of LaH_jPhy species at different temperatures (Table 3). This is because, as described in the previous section, a series of phenomena are involved in the sequestration process, so that the binding ability of a ligand toward a given cation cannot be estimated solely by analysis of its stability constants. For example, in the phytate–lanthanoid(III) system, the acid–base properties of both the ligand and the cation are affected by the temperature in such a way that the changes in the stability constants of

metal complexes are mitigated. However, as in other cases [33] the dependence of pL_{50} on temperature may be modeled by a simple linear relationship, for approximate but rapid estimation of phytate sequestering ability at pH = 7.35, in the temperature range $15 \leq t/\,°C \leq 37$:

$$pL_{50} = 11.06 - 0.03\,(t - 25) \quad (4)$$

with a correlation coefficient of $r = 0.996$ and a standard deviation of the fit of $\sigma = 0.05$.

Literature comparisons

In our recent review article on the formation and stability of phytate complexes in solution [2], we critically analyzed the literature published on this topic until early/mid 2007. At that time, few data had been published on phytate interactions with trivalent cations, and only one article was found dealing with phytate–lanthanoid(III) complexes in solution [55]. After some years, to the best of our knowledge no further papers have been published on the speciation of any phytate–lanthanoid(III) system or on the determination of any thermodynamic data related to these systems. Therefore, the data reported in this work may be regarded as original, especially in relation to the fact that Siddiqi et al. [55] investigated the interaction between phytate and Ce^{3+}, Pr^{3+}, Nd^{3+}, Sm^{3+}, Gd^{3+}, Tb^{3+}, Dy^{3+}, and Ho^{3+}, but in their paper reported only one log K value for formation of a generic metal–phytate complex at ten different pH values ($1.5 \leq pH \leq 6.0$), at $t = 25 \pm 1$ °C and $I = 0.1$ mol dm^{-3} in $NaClO_{4aq}$. These authors also found that the stability of this complex decreases with increasing pH, and they justified this result in terms of "interference" of sodium ions. Effectively, previous studies [29, 31, 56, 57] demonstrated that the ionic medium strongly affects the acid–base properties of phytic acid. For example, stable ion pairs formed by phytate with alkali metal cations (e.g. Na^+) have, as a consequence, a substantial effect on the effective stability of other metal complexes. Siddiqi et al. also reported, in their conclusions, an overall order of stability of phytate complexes for the investigated lanthanoids, that is: $Ho^{3+} > Dy^{3+} > Tb^{3+} > Sm^{3+} > Gd^{3+} > Nd^{3+} > Pr^{3+} > Ce^{3+}$. Nevertheless, this order is not that effectively observed by Siddiqi et al. at all the pH values investigated, if one analyzes the log K values they reported in their tables.

Experimental

Chemicals

Phytic acid solutions were prepared by weighing the dipotassium salt ($K_2H_{10}Phy$), and passing it over a strong cationic exchange resin (Dowex 50 W X 8). Concentrations were checked potentiometrically by alkalimetric titrations, and the absence of potassium was established by flame emission spectrometry. Sodium phytate solutions for calorimetric measurements were prepared by weighing the dodecasodium salt ($Na_{12}Phy$), evenly checking the concentration potentiometrically. Lanthanum(III) was used in the form of the nitrate salt, and neodymium(III), samarium(III), dysprosium(III), and ytterbium(III) were used in the form of the chloride salts (hexahydrate), without further purification. Their solutions were standardized against EDTA standard solutions [58] and the purity was always ≥99%. Hydrochloric acid and sodium hydroxide solutions were prepared by diluting concentrated ampoules and were standardized against sodium carbonate and potassium hydrogen phthalate, respectively. NaOH solutions were protected from atmospheric CO_2 by means of soda lime traps. NaCl solutions were prepared by weighing pure salt dried in an oven at 110 °C. All chemicals were of the purest analytical grade, and were purchased from Sigma–Aldrich (and its various brands). All solutions were prepared with analytical grade water ($R = 18$ MΩ cm^{-1}) using grade A glassware.

Apparatus and procedure for potentiometric measurements

Potentiometric measurements were carried out (at $t = 25.0 \pm 0.1$ °C) using an apparatus consisting of a Model 713 Metrohm potentiometer, equipped with a half cell glass electrode (Ross type 8101, from Thermo Orion) and a double-junction reference electrode (type 900200, from Thermo Orion), and a Model 765 Metrohm motorized burette. Estimated precision was ±0.15 mV and ±0.003 cm^3 for e.m.f. 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 e.m.f. stability. Some measurements were also carried out using a Metrohm model 809 Titrando apparatus controlled by Metrohm TiAMO 1.0 software for automatic data acquisition. Potentiometric titrations were carried out in thermostatted cells under magnetic stirring and with purified presaturated N_2 bubbling through the solution to exclude O_2 and CO_2 from inside. The titrand solution consisted of different amounts of metal cation (0.5–1 mmol dm^{-3}), phytate (1–7 mmol dm^{-3}), a slight excess of hydrochloric acid (1–7 mmol dm^{-3}), and NaCl in order to obtain a pre-established ionic strength (0.15 mol dm^{-3}). Most of the measurements were performed considering ligand-to-metal ratios in favor of the former, in order to avoid the formation of scarcely soluble species. Potentiometric measurements

were carried out by titrating 25 cm^3 titrand solution with standard NaOH solutions to pH ≈ 9–9.5. For each experiment, independent titrations of strong acid solution with standard base were carried out under the same medium and ionic strength conditions as the systems to be investigated, with the objective of determining electrode potential (E^0) and the acidic junction potential ($E_j = j_a$ $[H^+]$). In this way, the pH scale used was the total scale, pH ≡ −log $[H^+]$, where $[H^+]$ is the free proton concentration (not activity). The reliability of the calibration in the alkaline range was checked by calculating pK_w values. For each titration, 80–100 data points were collected, and the equilibrium state during titrations was checked by adopting the usual precautions. These included checking the time required to reach equilibrium and performing back titrations.

Apparatus and procedure for calorimetric measurements

Calorimetric measurements were performed at 25.000 ± 0.001 °C by use of a Tronac isoperibolic titration calorimeter model 450 coupled with a Keithley 196 system Dmm digital multimeter. The apparatus was connected to a PC, and automatic titrations were performed by using suitable computer software to control calorimetric data acquisition. The measurements were performed in the pH range 2 ≤ pH ≤ 9 by titrating 50 cm^3 solution containing $La(NO_3)_3$ (0.5–1.0 mmol dm^{-3}), NaCl, and HCl in order to obtain the pre-established ionic strength (I = 0.15 mol dm^{-3}) and initial pH values. The titrant consisted of a solution of dodecasodium phytate (c_{Phy} ≈ 0.2 mol dm^{-3}) and was delivered by a 2.5 cm^3 capacity Hamilton syringe, model 1002TLL. For each experimental condition at least three measurements were performed. The precision of the calorimetric apparatus was Q ± 0.008 J, and was checked by titrating a THAM (tris(hydroxymethyl)amino-methane) buffer with HCl (the heat of protonation was ΔH = −47.60 ± 0.05 kJ mol^{-1}). The precision of titrant volume readings was ±0.001 cm^3. The enthalpy of dilution was measured before each experiment under the same experimental conditions as for the measurements.

Calculations

The non-linear least squares computer software ESAB2M [59] was used for refinement of all the conditions of the acid–base titration (E^0, K_w, liquid junction potential coefficient, j_a, analytical concentration of reagents). BSTAC [60] and STACO [61] computer software were used for calculation of complex formation constants. Both programs can deal with measurements at different ionic strengths. The stability constants determined by these two software products were also checked and confirmed by running the same experimental measurements by use of the Hyperquad 2008 suite [62]. ES4ECI [60] software was used to draw speciation and sequestration diagrams, and to calculate percentage formation of species. LIANA [63] software was used to fit different equations. Calorimetric data were analyzed by use of ES5CMI [44] computer software.

Protonation ($i = 0$) and complex formation ($i = 1$) constants are given according to the equilibrium:

$$iLn^{3+} + jH^+ + Phy = Ln_iH_jPhy\ \beta_{ij} \quad (5)$$

or

$$i\,Ln^{3+} + H_jPhy = Ln_iH_jPhy\ K_{ij} \quad (6)$$

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