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Speciation of Phytate Ion in Aqueous Solution.† Sequestering Ability toward Mercury(II) Cation in $NaCl_{aq}$ at Different Ionic Strengths

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As a contribution to understanding the speciation of mercury in the environment and to the study of the sequestering ability of phytate (Phy) toward heavy metal and organometal cations, this paper describes the results of an investigation (at $t = 25$ °C by potentiometry, ISE-H^+ glass electrode) of its interactions with mercury(II) cation in NaCl aqueous solutions at different ionic strengths ($I = 0.15$ and 1.0 mol L^{-1}), in the pH range $2.5 \leq pH \leq 9.5$ and considering metal-to-ligand ratios of $1{:}1 \leq Hg/Phy \leq 4{:}1$. The formation of 11 $Hg_iH_jPhy^{(12-2i-j)-}$ species with $i = 1$ and $0 \leq j \leq 7$ and $i = 2$ and $0 \leq j \leq 2$ was observed. Their complex formation constant values proved to be fairly dependent on ionic strength. The speciation of phytic acid and mercury(II) is also dependent on the metal-to-ligand ratio; the dependence of the stability of phytate−mercury(II) species on the phytate protonation step was modeled, and an empirical predictive relationship was proposed. From the results obtained, phytate has very good sequestering ability toward Hg^{2+}, even in the presence of considerable excesses of chloride ion, that is, another ligand strongly interacting with mercury; this supports future studies both on the use of plants that naturally synthesize it for phytoremediation purposes and on its direct application in remediation techniques.

KEYWORDS: Phytate; mercury; complex formation; effect of ionic strength; phytoremediation; predictive relationships

INTRODUCTION

Since it was first identified in seeds in 1903, scientists have dedicated much effort to the study of phytic acid and its derivatives, due to their important biological activity, their widespread presence in nature, their high number of medical and industrial applications, and, recently, the role they could play in solving remediation problems in environmental chemistry. Huge numbers of papers, reviews, and books have been published [see, for example, references in previous contributions (*1*−*6*) and in reviews (*7*−*10*)], and new findings are continually being reported in the literature (see, e.g., refs *11*−*14*). Of particular interest are, for example, many papers published in recent years on the application of phytates in remediation problems, such as the immobilization and in situ treatment of soils contaminated by many metals (including heavy metals and radionuclides) such as Al, As, Ba, Co, Cr, Cu, Eu, Mn, Ni, Np, Pb, Se, U, and Zn (see, e.g., refs *15*−*17*).

Although most authors provide adequate and exhaustive descriptions of the sequestering ability of phytate and its derivatives toward these metals, few quantitative data are presented on their thermodynamic behavior in aqueous solution. In particular, data regarding the chemical speciation of this ligand in aqueous solution are useful for describing the quantitative and qualitative distribution of phytate in only a given system.

Bearing in mind that natural waters and biological fluids are aqueous solutions, this aspect represents the basis for understanding phytate's peculiar characteristics (nutritional/antinutritional properties, anticarcinogenic activity, sequestering ability, see, e.g., refs *7*−*10*) and for setting up new strategies and methodologies in which this ligand could play an important role such as in remediation techniques (*15*−*17*), as mentioned above. For these reasons, we previously studied the aqueous chemistry of phytic acid in order to understand its chemical speciation in natural waters and biological fluids (*1*−*6*). More recently, we began evaluation of the sequestering ability of this ligand toward heavy metal and organometal cations and described its interactions with dimethyltin(IV) cation (*6*).

In the present paper, we report some results from an investigation (at $t = 25$ °C by potentiometry, ISE-H^+ glass electrode) of its interactions with mercury(II) cation in NaCl

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† For previous contributions to this series, see refs *1*−*6*.

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aqueous solutions at different ionic strengths ($I = 0.15$ and 1.0 mol L^{-1}). Potentiometry was used as the instrumental technique, as recommended by IUPAC for these kinds of speciation studies (*18*). We chose sodium chloride as ionic medium because it is the main inorganic salt dissolved in natural waters and biological fluids. We chose mercury(II) because the present paper aims to contribute to understanding and solving the "mercury problem" by exploring the speciation of mercury in the environment. Worldwide interest in mercury is so high that many national and international institutions are directly involved in research into this element and its compounds and have sponsored many research programs (*19*, *20*). Moreover, despite the most toxic species of mercury being CH_3Hg^+ [where mercury(II) is methylated in waters, sediments, soils, and different organisms mainly by the action of bacteria], mercury(II) is one of the most important inorganic forms in which this element can occur in the environment, and its methylation processes are not so fast as to avoid the presence of appreciable percentages of inorganic Hg(II). Knowledge of its speciation is therefore crucial to understanding its biological activity and chemical−physical behavior (*21*). In fact, speciation plays an important part in the toxicity and exposure of mercury to living organisms and, furthermore, the species influence the physical availability for exposure, the internal transport inside the organism to the tissue on which it has toxic effects, its accumulation, biomodification, and detoxification in the tissues, and its biomagnification on its way up the trophic levels of the food chain. Speciation also influences the transport of mercury within and between environmental compartments, and it is very important for the control of mercury emissions to air (*19*).

Considering the above, our study of the sequestering ability of phytate toward mercury(II) cations (i.e., of their speciation when they occur together) in sodium chloride aqueous media might, from an environmental and biological point of view, prove to be an important tool for determining how this ligand modifies the availability of the pollutant in aqueous systems such as natural waters and biological fluids. In fact, to understand how mercury is sequestered in these complex systems, where other ligands competing with phytate could be simultaneously present, a background knowledge of simpler systems is needed and, therefore, the binding ability of each ligand should be defined independently. The fact that phytate is nontoxic and naturally biosynthesized often in large amounts in many vegetal species (e.g., cereals, legumes, and potatoes) also supports future studies both on the use of plants that naturally synthesize it for phytoremediation purposes and on its direct application in remediation techniques (*15*−*17*).

MATERIALS AND METHODS

Chemicals. Hydrochloric acid and sodium hydroxide solutions were prepared by diluting concentrated ampules (Riedel-deHaën) and were standardized against sodium carbonate and potassium hydrogen phthalate, respectively. NaCl solutions were prepared by weighing pure salts (Fluka) dried in an oven at 110 °C. Phytic acid solutions were prepared by weighing Aldrich dipotassium salt $K_2H_{10}Phy$ and passing it over a strong cationic exchange resin (Dowex 50W X 8 from Fluka). Concentration was checked potentiometrically by alkalimetric titrations, and the absence of potassium was established by flame emission spectrometry. Mercury(II) was used in the form of chloride salt (Fluka); its solutions were standardized against EDTA standard solutions (Fluka), and its purity was always ≥99.5% (*22*). All Fluka, Aldrich, and Riedel-deHaën products were directly purchased by Sigma-Aldrich Italy. All solutions were prepared with analytical grade water ($R = 18$ MΩ cm^{-1}) using grade A glassware.

Apparatus and Procedure. Potentiometric titrations were carried out (at 25.0 ± 0.1 °C) using an apparatus consisting of a model 713 Metrohm potentiometer, equipped with a half-cell glass electrode (Ross type 8101, from Orion), a double-junction reference electrode (type 900200, from Orion), and a model 765 Metrohm motorized burette. Estimated accuracies were ±0.15 mV and ±0.003 mL for emf and titrant volume readings, respectively. The apparatus was connected to a PC, and automatic titrations were performed using a suitable computer program to control titrant delivery and data acquisition and to check for emf stability. All titrations were carried out under magnetic stirring and purified presaturated N_2 bubbled through the solution to exclude O_2 and CO_2 inside.

Table 1. Experimental Conditions for Potentiometric Measurements at $t = 25$ °C[a]

		pH[b]		
I[c]	C_{Phy}[d]	$C_{Hg} = 2$[d]	$C_{Hg} = 3$[d]	$C_{Hg} = 4$[d]
0.15	0.8	9.50	9.20	9.16
0.15	1.0	9.83	9.15	9.14
1.00	0.8	≥10.5	≥10.5	≥10.5
1.00	1.0	≥10.5	≥10.5	≥10.5

[a] HCl (1 mmol L^{-1}) added in each titration; 80–100 experimental points for each titration. [b] pH value at which precipitation starts; no precipitation for $C_{Phy}/C_{Hg} = 1{:}1$. [c] Ionic strength expressed in the molar concentration scale, mol L^{-1}. [d] Total concentrations expressed in the millimolar concentration scale, mmol L^{-1}.

The titrand solution consisted of different amounts of phytic acid (0.8−1 mmol L^{-1}) and mercury(II) dichloride (1−4 mmol L^{-1}), an excess of hydrochloric acid (1 mmol L^{-1}), and the background salt in order to obtain pre-established ionic strength values ($I = 0.15$ and 1.0 mol L^{-1}). Potentiometric measurements were carried out by titrating 25 mL of the titrand solution with standard NaOH solutions up to pH 9.5. Further details of the experimental conditions adopted are given in **Table 1**. In this table, pH values at which the formation of scarcely soluble species occurs are reported, because in some cases it was noted at pH values <9.5. In these conditions, titrations were stopped at that point, whereas, in other experimental conditions, the formation of insoluble species was noted at pH values >9.5, but experimental data were collected up to this pH value.

For each titration, 80−100 points were collected, and the equilibrium state during titrations was checked by adopting standard precautions. These included checking the time necessary to reach equilibrium and performing back-titrations. 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 aim of determining electrode potential ($E°$) and acidic junction potential ($E_j = j_a[H^+]$). In this way, the pH scale used was the total scale, $pH \equiv -\log[H^+]$, where $[H^+]$ is the free proton concentration (not activity). For measurements performed at low ionic strengths, the contribution of the ligand has to be considered: in the most critical conditions (i.e., $I = 0.15$ mol L^{-1}), this contribution to ionic strength is ∼7−8%, which introduces a not dramatic error in calculation. However, this error was taken into account by giving appropriate weights to the results obtained at low ionic strengths in fitting different functions.

Calculations. The nonlinear least-squares computer program ESAB2M (*23*) was used for the refinement of all the parameters of the acid−base titration ($E°$, K_w, liquid junction potential coefficient, j_a, analytical concentration of reagents). The BSTAC (*24*) and STACO (*25*) computer programs were used in the calculation of complex formation constants. Both programs can deal with measurements at different ionic strengths. The ES4ECI (*25*) program was used to draw speciation diagrams and to calculate species formation percentages. The LIANA (*26*) program was used to fit different equations.

Complex formation constants are given according to the equilibrium

$$i\mathrm{Hg}^{2+} + j\mathrm{H}^{+} + \mathrm{Phy}^{12-} = \mathrm{Hg}_i\mathrm{H}_j\mathrm{Phy}^{(12-2i-j)-} \quad \beta_{ij} \qquad (1)$$

or

$$i\mathrm{Hg}^{2+} + \mathrm{H}_j\mathrm{Phy}^{(12-j)-} = \mathrm{Hg}_i\mathrm{H}_j\mathrm{Phy}^{(12-2i-j)-} \quad K_{ij} \qquad (2)$$

Table 2. Phytate Protonation Constants[a] and Mercury(II) Hydrolysis and Complex Formation Constants[b] in NaCl Aqueous Solution at I = 0.15 and 1.0 mol L^{-1} Ionic Strength and at t = 25 °C

	phytate						
I/ mol L^{-1}	log K_1^H	log K_2^H	log K_3^H	log K_4^H	log K_5^H	log K_6^H	log K_7^H
0.15	9.41	9.67	9.33	7.97	6.35	5.10	2.75
1.00	8.69	8.95	8.56	7.21	5.65	4.42	2.22

	mercury(II)								
I/ mol L^{-1}	log β_{110}	log β_{120}	log β_{130}	log β_{210}	log β_{101}	log β_{102}	log β_{103}	log β_{104}	log β_{111}
0.15	–3.61	–6.34	–21.1	–3.62	6.78	13.30	14.38	15.04	3.65
1.00	–3.67	–6.31	–21.1	–3.84	6.70	13.17	14.17	15.10	3.66

[a] K_j^H refers to the equilibrium $H^+ + H_{j-1}Phy^{(12-j+1)-} = H_jPhy^{(12-j)-}$ (*1*, *2*). [b] β_{pqr} refers to the equilibrium $pHg^{2+} + qH_2O + rCl^- = [Hg(OH)_qCl_r]^{(2-q-r)} + qH^+$ (*28*).

Formation constants, concentrations, and ionic strengths are expressed in the molar (mol L^{-1}) concentration scale.

RESULTS AND DISCUSSION

Stability of Proton−Mercury(II)−Phytate Species. To make appropriate calculations from potentiometric data for this system, a preliminary knowledge of the acid−base behavior of both phytate and mercury(II) cation in the same experimental conditions as this system is needed. For phytate, we took previously determined NaCl medium protonation constants (*1*, *2*), whereas for mercury(II) we employed the speciation scheme recommended by Baes and Mesmer for the hydrolysis of mercury(II) in chloride media (*28*), which considers the formation of Hg^{2+}/Cl^- ion pairs. **Table 2** shows the speciation scheme and formation constant values for mercury(II) and phytate protonation in $NaCl_{aq}$ at I = 0.15 and 1.0 mol L^{-1} ionic strengths. The accuracy of the data relative to Hg^{2+} hydrolysis is sufficient for our purposes, because our preliminary calculations showed mercury(II) hydrolysis to be negligible in the presence of phytate (i.e., in our system). There are two main reasons for this: (i) the number chloride species is higher than that of hydrolytic ones [and the chloride−mercury(II) complex formation constants recommended by Baes and Mesmer are consistent with other literature data; see, e.g., refs *29*−*33*]; and (ii) phytate complexation inhibits hydrolysis processes. It is extremely important to take the formation of Hg^{2+}/Cl^- ion pairs into account during calculations, not only because, as we have already pointed out, the chloride ion strongly interacts with mercury(II) to form several complex species but because in our experimental conditions the chloride concentration shifts between ∼190 and ∼1250 times the concentration of Hg^{2+} itself.

Analysis of experimental data in the pH range 2.5 ≤ pH ≤ 9.5 using both STACO and BSTAC computer programs evidenced the formation of 11 phytate−proton−mercury(II) species: $HgPhy^{10-}$, $HgHPhy^{9-}$, HgH_2Phy^{8-}, HgH_3Phy^{7-}, HgH_4Phy^{6-}, HgH_5Phy^{5-}, HgH_6Phy^{4-}, HgH_7Phy^{3-}, Hg_2Phy^{8-}, Hg_2HPhy^{7-}, and $Hg_2H_2Phy^{6-}$. In particular, there are 8 mononuclear (with $0 \le j \le 7$) and three dinuclear (with $0 \le j \le 2$) species. Although titrations of solutions prepared with higher metal-to-ligand ratios were performed [up to Hg(II)/Phy = 4:1], the formation of trinuclear and/or tetranuclear species was not observed.

Complex formation constant values are reported in **Table 3**. Their importance can be evaluated by looking at formation percentages for phytate species versus pH in the phytate−mercury(II) system; these are shown in the speciation diagrams in **Figures 1** and **2**, calculated in NaCl medium at I = 0.15 (**Figure 1**) and I = 1 mol L^{-1} (**Figure 2**) and considering different Hg(II)/Phy ratios [Hg(II)/Phy = 1:1 for **Figures 1a** and **2a**; Hg(II)/Phy = 4:1 for **Figures 1b** and **2b**]. Analysis of these figures reveals that different experimental conditions (i.e., ionic strength and metal-to-ligand ratios) mainly affect phytate speciation in the alkaline pH range, at which a wide number of phytate species are present simultaneously with formation percentages ranging between a few percentage pointsunits and ∼40%.

A more detailed picture of phytate speciation in these conditions is given in **Figures 3** and **4**, where the pH region 7.5 ≤ pH ≤ 9.5 of the diagrams shown in previous figures is enlarged. In this pH range, for example, dinuclear species (as expected) reach higher formation percentages in **Figures 3b** and **4b**, where metal-to-ligand ratios are higher than in the corresponding **Figures 3a** and **4a**, where the metal-to-ligand ratio is Hg/Phy = 1:1. In the same pH range, the effect of ionic strength is also evident, as can be observed by comparing the diagrams in **Figures 3** and **4** obtained under the same metal-to-ligand ratio (i.e., comparing panels **a** and **b** of **Figure 3** with panels **a** and **b** of **Figure 4**, respectively). In fact, lowering the ionic strength (i.e., of sodium and chloride ion concentration) favors

Table 3. Complex Formation Constants for Phytate–Mercury(II) Species in $NaCl_{aq}$ at I = 0.15 and 1.0 mol L^{-1} Ionic Strength and t = 25 °C

	log β_{ij}[a] (log K_{ij})[b]							
I/mol L^{-1})	$HgPhy^{10-}$	$HgHPhy^{9-}$	HgH_2Phy^{8-}	HgH_3Phy^{7-}	HgH_4Phy^{6-}	HgH_5Phy^{5-}	HgH_6Phy^{4-}	HgH_7Phy^{3-}
0.15	16.35 ± 0.09[c]	26.04 ± 0.03[c] (16.63)	35.24 ± 0.03[c] (16.16)	44.05 ± 0.06[c] (15.64)	52.32 ± 0.06[c] (15.94)	58.99 ± 0.09[c] (16.26)	64.28 ± 0.03[c] (16.45)	67.36 ± 0.09[c] (16.78)
1.0	15.47 ± 0.12	24.30 ± 0.09 (15.61)	32.86 ± 0.09 (15.22)	40.94 ± 0.12 (14.74)	48.58 ± 0.09 (15.17)	54.60 ± 0.09 (15.54)	59.18 ± 0.03 (15.70)	61.82 ± 0.09 (16.12)

	log β_{ij}[a] (log K_{ij})[b]		
I/mol L^{-1})	Hg_2Phy^{8-}	Hg_2HPhy^{7-}	$Hg_2H_2Phy^{6-}$
0.15	32.25 ± 0.06[c]	40.68 ± 0.03[c] (31.27)	49.51 ± 0.06[c] (30.43)
1.0	30.23 ± 0.15	38.10 ± 0.09 (29.41)	46.30 ± 0.12 (28.66)

[a] log β_{ij} refers to the reaction $iHg^{2+} + jH^+ + Phy^{12-} = Hg_iH_jPhy^{(12-2i-j)-}$. [b] Values in parentheses; log K_{ij} refers to the reaction $iHg^{2+} + H_jPhy^{(12-j)-} = Hg_iH_jPhy^{(12-2i-j)-}$. [c] ±3 standard deviations.

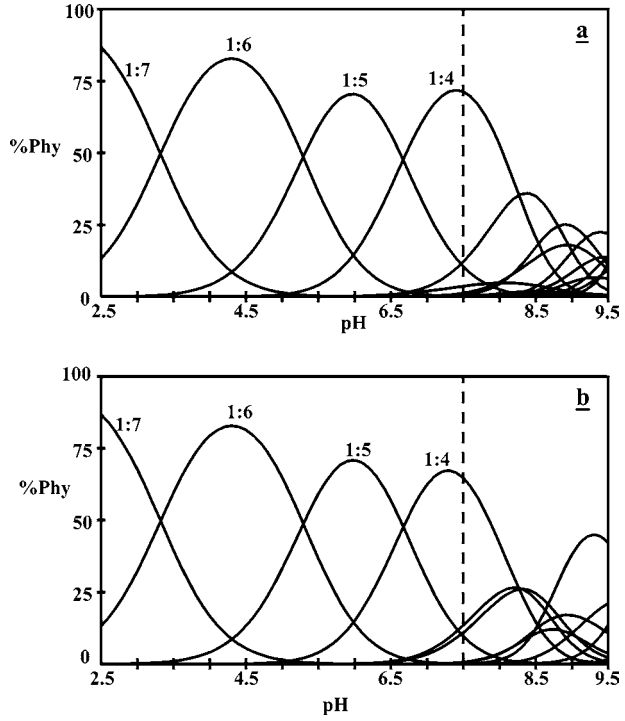

Figure 1. Distribution diagram of formation percentage of phytate species versus pH in the Phy/Hg(II) system at $I = 0.15$ mol L^{-1} ionic strength in $NaCl_{aq}$ and at $t = 25$ °C. *ij* indices in the figures refer to $Hg_iH_jPhy^{(12-2i-j)-}$ species; for example, 1:6 refers to the HgH_6Phy^{4-} species. Experimental conditions: total concentration of phytate, $C_{Phy} = 0.001$ mol L^{-1}; total concentration of Hg(II), $C_{Hg} = 0.001$ (**a**) or 0.004 mol L^{-1} (**b**).

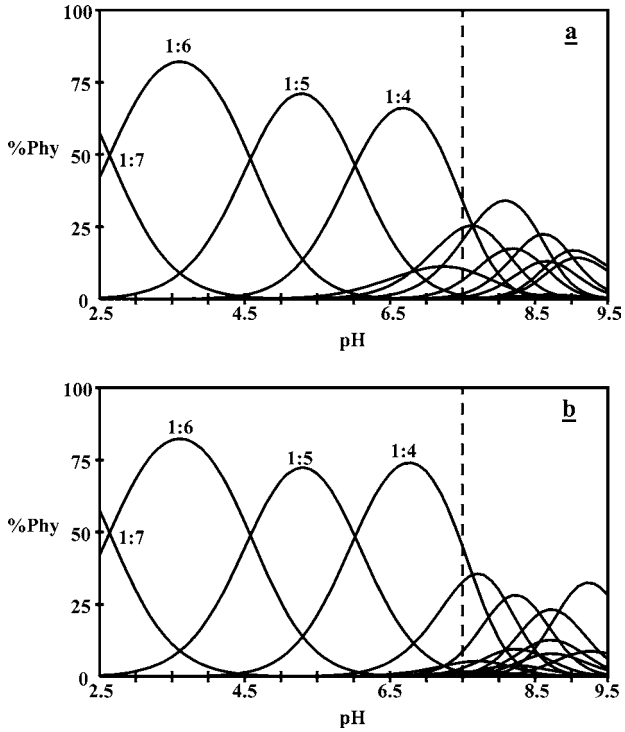

Figure 2. Distribution diagram of formation percentage of phytate species versus pH in the Phy/Hg(II) system at $I = 1.0$ mol L^{-1} ionic strength in $NaCl_{aq}$ and at $t = 25$ °C. *ij* indices in the figures refer to $Hg_iH_jPhy^{(12-2i-j)-}$ species; for example, 1:6 refers to the HgH_6Phy^{4-} species. Experimental conditions: total concentration of phytate, $C_{Phy} = 0.001$ mol L^{-1}; total concentration of Hg(II), $C_{Hg} = 0.001$ (**a**) or 0.004 mol L^{-1} (**b**).

the formation of species with higher "nuclearity" [i.e., the number of mercury(II) cations bound to phytate]. For example,

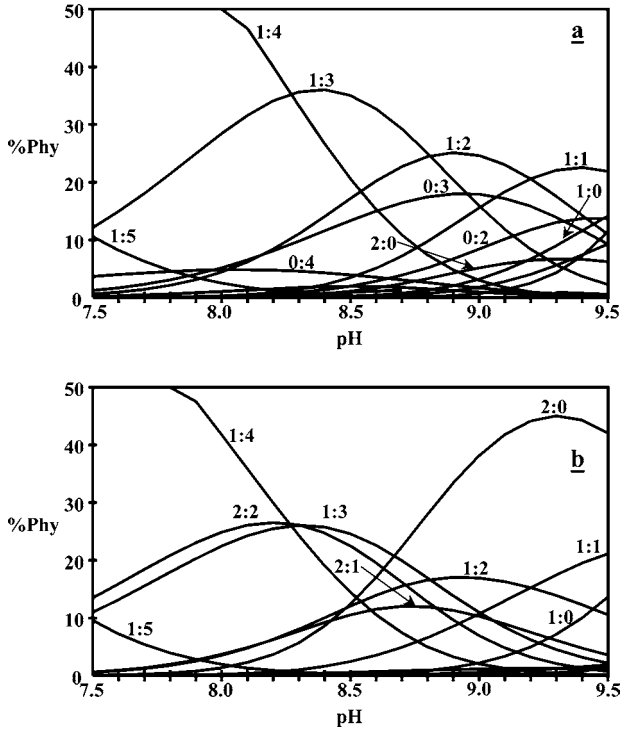

Figure 3. Distribution diagram of formation percentage of phytate species versus pH in the Phy/Hg(II) system at $I = 0.15$ mol L^{-1} ionic strength in $NaCl_{aq}$ and at $t = 25$ °C. *ij* indices in the figures refer to $Hg_iH_jPhy^{(12-2i-j)-}$ species; for example, 1:4 refers to the HgH_4Phy^{6-} species. Experimental conditions: total concentration of phytate, $C_{Phy} = 0.001$ mol L^{-1}; total concentration of Hg(II), $C_{Hg} = 0.001$ (**a**) or 0.004 mol L^{-1} (**b**).

at pH >9 in the **b** diagrams Hg_2Phy^{8-} is the main species at $I = 0.15$ mol L^{-1}, whereas formation of the mononuclear $HgPhy^{10-}$ is favored at $I = 1$ mol L^{-1} even if the metal-to-ligand ratio is the same (Hg/Phy = 4:1 in both diagrams). In the **a** diagrams, too, where Hg/Phy = 1:1, in the pH range $8.0 \leq pH \leq 8.5$, HgH_3Phy^{7-} is the main species at $I = 0.15$ mol L^{-1}, whereas most phytate is present as H_3Phy^{9-} at $I = 1$ mol L^{-1}.

Ionic strength also affects the species distribution curves in the whole investigated pH range (see **Figures 1** and **2**). In fact, at lower ionic strength values, the distribution curves are shifted by ~0.4 unit toward alkaline pH regions. This means that a given species is formed in more acidic pH regions at higher ionic strength values, and, as a consequence, the maximum formation percentage of this species is achieved at lower pH values.

However, from an environmental and biological point of view, two main observations emerge from the analysis of all the diagrams shown in **Figures 1**−**4**: the first is that in all of the pH ranges investigated, phytate is almost always present as mercury ion pairs, with formation percentages of some species exceeding 80% (e.g., HgH_6Phy^{4-}) of total phytate; the second is that the speciation of phytate and mercury is extremely dependent on experimental conditions. As stressed several times in this paper, to better understand the mechanisms underlying the sequestering ability of the former toward the latter, it is of fundamental importance to know in which form they are present in aqueous solution (e.g., natural or waste waters and/or biological fluids); that is, it is necessary to know their speciation in those particular conditions. In this context, it is also important to remember that, as concerns **Figures 1a** and **2a**, when the metal-to-ligand ratio is 1:1, the formation percentage of phytate−mercury complexes is the same with respect to both

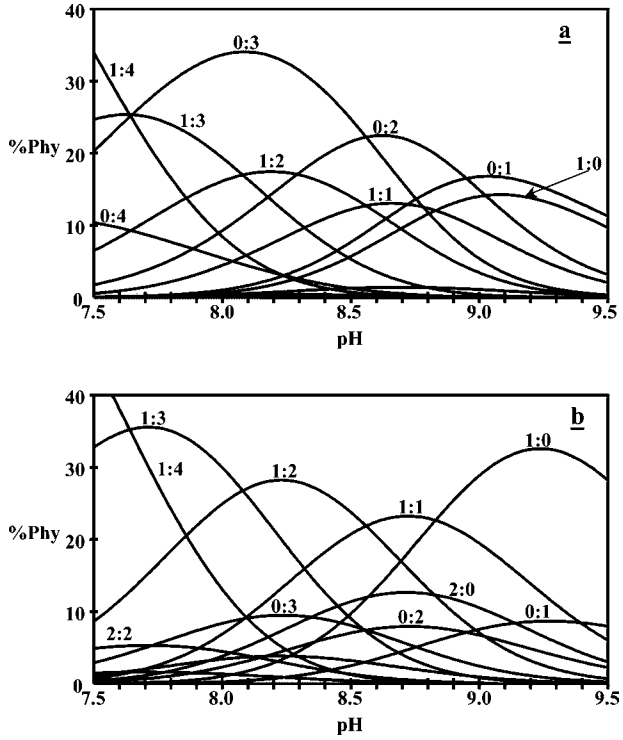

Figure 4. Distribution diagram of formation percentage of phytate species versus pH in the Phy/Hg(II) system at $I = 1.0$ mol L^{-1} ionic strength in $NaCl_{aq}$ and at $t = 25$ °C. *ij* indices in the figures refer to $Hg_iH_jPhy^{(12-2i-j)-}$ species; for example, 1:4 refers to the HgH_4Phy^{6-} species. Experimental conditions: total concentration of phytate, $C_{Phy} = 0.001$ mol L^{-1}; total concentration of Hg(II), $C_{Hg} = 0.001$ (**a**) or 0.004 mol L^{-1} (**b**).

the total ligand and the metal. This clearly means, for example, that the HgH_4Phy^{6-} species represents ~75% of total mercury(II) cation in the experimental conditions illustrated in **Figures 1a** and **2a** (i.e., $C_{Phy} = C_{Hg} = 0.001$ mol L^{-1}) and at pH ~7 (as in some natural waters). This result is extremely important if one considers, for example, that in **Figure 2a** mercury(II) is present in this form despite the presence of another strong complexing ligand (i.e., chloride ion; the diagram is drawn at $I = 1$ mol L^{-1} in $NaCl_{aq}$) in far higher concentrations than phytate (~1000 times higher).

Phytate Concentration Limits for Mercury(II) Sequestration. To evaluate the suitability of phytate as a sequestering agent toward mercury(II) in real aqueous systems, such as biological fluids and natural waters, it is useful to determine the lowest phytate concentration limits at which it can express a "significant" binding ability toward mercuric cation. This is very important for a rough determination of the total amount of ligand likely to be necessary for the removal of a given cation [mercury(II) in this case] from polluted aqueous matrices. Although the estimation of this amount is not absolutely accurate, because it is performed in "model systems", it is very helpful when working in real systems. In fact, this estimation is very difficult because it needs the exact knowledge of the sequestering ability of all other ligands that may be present in these systems (at even higher concentrations than phytate), and this involves very long and difficult studies. An example of competition among phytate and other ligands might be that reported in **Figure 5**, where the percentage of mercury(II) species is plotted versus total phytate concentration (in mol L^{-1}, as $-\log C_{Phy}$, C_{Phy} = analytical concentration of phytate) at $I = 0.15$ mol L^{-1} in NaCl, at pH 7.35 and at a total mercury(II) concentration of $C_{Hg} = 1$ mmol L^{-1}. In these ionic strength and pH conditions (which are similar, for example, to those of

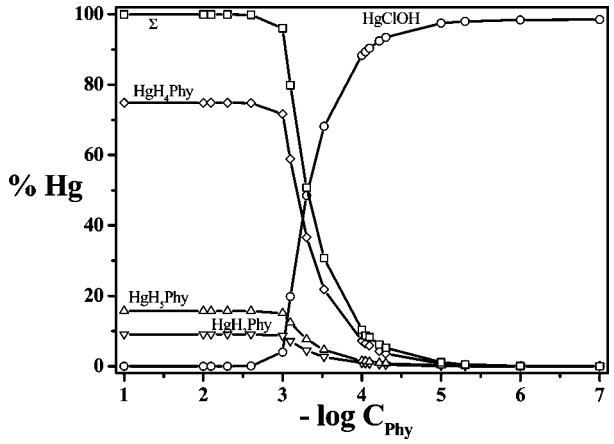

Figure 5. Distribution diagram of formation percentage of mercury(II) species versus $-\log C_{Phy}$ (C_{Phy} = total phytate concentration) in the Phy/Hg(II) system at $I = 0.15$ mol L^{-1} ionic strength in $NaCl_{aq}$ and at $t = 25$ °C. Σ in the figure refers to total percentage of mercury bound to phytate. Experimental conditions: total mercury concentration, $C_{Hg} = 0.001$ mol L^{-1}, pH 7.35.

human blood, where $I \sim 0.16$ mol L^{-1}, the main dissolved salt is NaCl, and pH ~7.35), when $-\log C_{Phy} \sim 3.5$, >50% of mercury is still complexed by phytate. This example is a useful further reminder that, even if chloride anion, which strongly interacts with mercury(II), is present with a total concentration ~500 times higher than that of phytic acid, this ligand is still able to significantly bind >50% of the total mercury(II) present.

Predictive Relationships. Scientists involved in thermodynamic studies of aqueous solutions know that the determination of thermodynamic parameters (such as activity coefficients, interaction constants, enthalpies, entropies) is strongly dependent on the experimental conditions adopted (e.g., temperature, ionic strength, ionic medium). This is particularly important in speciation studies of natural waters and biological fluids, owing to the extreme variability of both the composition and conditions of these aqueous solutions. In these systems, a wide number of interactions may occur, and the thermodynamic behavior of an element or compound might vary considerably from one system to another. The above observations show that the determination of all the thermodynamic parameters in all the different experimental conditions of these systems is, in practice, impossible. For these reasons, many models have been proposed for the quantitative description of the dependence of these parameters on the many chemicophysical properties of aqueous solutions such as the above-mentioned temperature, ionic strength, and ionic medium (*34*). One of the most important characteristics of these models is that they can very often be used predictively to estimate thermodynamic parameters that are not readily available, as happens, for example, when direct experimental analysis is not possible. Unfortunately, sometimes these models alone are not sufficient, and identification of new relationships, for example, between two or more parameters, is therefore necessary. In this light, our systematic study of the thermodynamic behavior of phytic acid in aqueous solutions enabled us to identify many useful empirical relationships for the calculation of some thermodynamic parameters as a function of a wide number of variables (*1*−*6*). Regularities found in the stability of ion pairs formed by phytate and dimethyltin(IV) cation with an increased ligand protonation step (*j*) suggested that complex formation constant values for $Hg_iH_jPhy^{(12-2i-j)-}$ species might follow the same trend (*6*). By plotting log β_{1j} (**Table 3**) at different ionic strengths versus the phytate protonation step (*j*) (see **Figure 6**) we can observe that, at

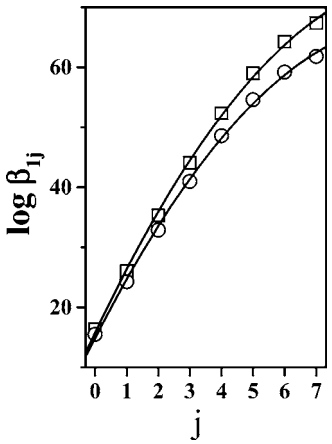

Figure 6. Complex formation constants (eq 3) in $NaCl_{aq}$ at $t = 25$ °C and different ionic strengths (I, in mol L^{-1}) for mononuclear $HgH_jPhy^{(12-2-j)-}$ species versus phytate protonation step (j): □, $I = 0.15$ mol L^{-1}; ○, $I = 1.00$ mol L^{-1}.

different ionic strengths, these constants are a regular function of "j" and can be expressed by the same relationship found for $DMT_iH_jPhy^{(12-2i-j)-}$ species

$$\log \beta_{1j} = a + bj + cj^2 \qquad (3)$$

where a, b, and c are empirical parameters. In particular, after refinements we obtained a reasonably good fit for our data (standard deviation, σ, = 0.27) by constraining the c parameter to the same value for each set of constants at different ionic strengths, that is (±standard deviation), $c = -0.52 \pm 0.02$. Other refined parameters of eq 3 are $a = 15.72$ and 14.69 and $b = 11.11$ and 10.45, at $I = 0.15$ and 1 mol L^{-1}, respectively. Due to the small number of species with $i > 1$, we could not fit the complex formation constants of species with more than one mercury(II) cation in a separate dataset. However, regularities shown by phytate−mercury(II) mononuclear species, together with those shown by the analogue dimethyltin(IV) species, suggest that all of the complex formation constant values of mono- and dinuclear $Hg_iH_jPhy^{(12-2i-j)-}$ species could be represented by a single equation that simultaneously takes into account dependence on the (i) ionic strength, (ii) phytate protonation step, and (iii) nuclearity of the species. This would be particularly important, as previously stated, in speciation studies of natural waters and biological fluids where an approximate (but immediate) knowledge of phytate speciation would be necessary to rapidly determine its sequestering ability toward Hg(II) in experimental conditions other than those adopted in the present paper before more detailed studies are performed.

Literature Comparisons. Despite the high number of papers dealing with the bioavailability of mercury and other metal cations in the presence of phytate (see, e.g., refs cited in refs *7*−*10*, *15*−*17*, and *35*−*43*), few quantitative data are reported on the thermodynamics of phytate−metal cation interactions in aqueous solution (see, e.g., refs *44*−*47*) and, to our knowledge at the present time, none exist for phytate−mercury(II) complexes. These data, on the other hand, are of fundamental importance in understanding the processes involved in metal cation sequestration by this and other ligands used in remediation. Therefore, although the phytate−mercury(II) system has been previously investigated from different points of view, the results reported in this work must be considered as original.

As for the phytate−dimethyltin(IV) system (*6*), it might be useful to make comparisons between phytic acid and other ligands more frequently used as classical sequestering agents for the removal of heavy metal and organometal cations from aqueous matrices, such as ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid (EDTA). Many data can be found in some thermodynamic constant databases (*29*−*33*) for EDTA−Hg(II) complexes, although most of them refer mainly to the formation of HgL species (L = EDTA). For example, the critically selected stability constants in the NIST Standard Reference Database 46 (vers. 8.0) (*33*) for EDTA complexes with mercury(II) cover the formation of just ML, MHL, and MH_2L species [M = Hg(II)] and indicate, for example, a complex formation constant of $\log K = 21.5$ at $t = 25$ °C and $I = 0.1$ mol L^{-1} (ionic medium is not specified, but most of the measurements cited from the bibliography were performed in nitrate media), for the equilibrium

$$Hg^{2+} + L^{4-} = HgL^{2-}$$

against a value of ∼16.4 for the formation of the analogue $HgPhy^{10-}$ at $I = 0.15$ mol L^{-1}.

A quick comparison of the complex formation constants of HgL species for the two ligands reveals that the stability of the $Hg(EDTA)^{2-}$ ion pair is ∼5 units (in the logarithmic scale) greater than that of $HgPhy^{10-}$, suggesting that EDTA has a stronger sequestering ability than phytate. However, as stated in our previous paper on phytate−dimethyltin(IV) interactions (*6*), at least three further factors should be considered in favor of phytate for the removal of heavy metal cations from aqueous matrices: (i) phytic acid is commonly present in the environment and naturally synthesized in significant amounts by several vegetal species (*7*−*10*); the environmental impact of its use is therefore lower than that of EDTA; (ii) commercial phytates, extracted by these plants, are cheaper than EDTA and other sequestering agents and easily available in large quantities; and (iii) at higher pH values, phytate forms with Hg(II) scarcely soluble species that can be more easily removed from polluted matrices with high pH values.

Unfortunately, it has not been possible to further compare the binding ability of phytate toward mercury(II) cation with that of other phosphoric ligands such as phosphate. This is due to the formation of scarcely soluble phosphate−mercury(II) species, as reported, for example, by Högfeldt in his compilation (*31*), where he gives a value of $\log K_s = -6$ at $t = 20$ °C [taken by Gyunner and Orlova (*48*)] for the equilibrium

$$Hg_3(PO_4)_2\,(s) + 4H^+ = 3Hg^{2+} + 2H_2PO_4^-$$

Final Remarks. Our main conclusions on phytate/mercury(II) interactions in $NaCl_{aq}$ at different ionic strengths can be summarized as follows:

(a) Results for the speciation of a phytate/mercury(II) system in $NaCl_{aq}$ are reported here for the first time.

(b) In the pH range $2.5 \le pH \le 9.5$ the formation of 11 phytate−proton−mercury(II) species has been hypothesized; in particular, there are 8 mononuclear (with $0 \le j \le 7$) and three dinuclear (with $0 \le j \le 2$) species.

(c) Complex formation constants for phytate/mercury(II) species [and, therefore, phytic acid and mercury(II) speciation] are fairly dependent on ionic strength and the phytate protonation step; this last dependence has been modeled.

(d) The phytate ligand has been shown to be a very good sequestering agent toward Hg^{2+}, indicating its potential for use in the remediation of sites polluted by this cation; distribution diagrams for mercury species in the presence of phytate have

also been reported as a function of both pH and the analytical concentration of the ligand.

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