1028 J. Chem. Eng. Data **2007,** *52,* 1028−1036

Modeling the Dependence on Medium and Ionic Strength of Glutathione Acid−Base Behavior in $LiCl_{aq}$, $NaCl_{aq}$, KCl_{aq}, $RbCl_{aq}$, $CsCl_{aq}$, $(CH_3)_4NCl_{aq}$, and $(C_2H_5)_4NI_{aq}$

Pasquale Crea, Alessandro De Robertis, Concetta De Stefano,* Demetrio Milea,† and Silvio Sammartano

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

Acid−base properties of γ-L-glutamyl-L-cysteinyl-glycine (glutathione, GSH) in $LiCl_{aq}$, $NaCl_{aq}$, KCl_{aq}, $RbCl_{aq}$, $CsCl_{aq}$, $(CH_3)_4NCl_{aq}$, and $(C_2H_5)_4NI_{aq}$ were investigated at $T = 298$ K, by potentiometry (ISE-H^+, glass electrode), and at different ionic strengths [$I_c \leq 5.0$ mol·L^{-1} for $LiCl_{aq}$ and $NaCl_{aq}$, $I_c \leq 3.0$ mol·L^{-1} for KCl_{aq}, $RbCl_{aq}$, and $(CH_3)_4NCl_{aq}$, $I_c \leq 4.0$ mol·L^{-1} for $CsCl_{aq}$, and $I_c \leq 1.0$ mol·L^{-1} for $(C_2H_5)_4NI_{aq}$]. The dependence on medium and ionic strength of protonation constants was modeled by an extended Debye−Hückel (EDH) type equation and by specific ion interaction theory (SIT) and Pitzer models. All three approaches give comparable and satisfactory results. Protonation constants in various ionic media at different ionic strengths, EDH parameters, SIT interaction coefficients, and Pitzer parameters were calculated, together with protonation constants at infinite dilution: log ${}^{T}K_1^{H} = p^{T}K_{a4} = 10.135 \pm 0.004$, log ${}^{T}K_2^{H} = p^{T}K_{a3} = 9.077 \pm 0.004$, log ${}^{T}K_3^{H} = p^{T}K_{a2} = 3.713 \pm 0.004$, and log ${}^{T}K_4^{H} = p^{T}K_{a1} = 2.124 \pm 0.004$ (in the molar concentration scale, ± 3 standard deviation).

Introduction

Glutathione (γ-L-glutamyl-L-cysteinyl-glycine, GSH) is one of the most important and ubiquitous small biomolecules present in cells of all organisms at millimolar concentrations. It possesses a variety of physiological functions and plays a key role in several biochemical processes in the human body and in plants. New functions and properties of glutathione are being continuously discovered and give a good explanation for the huge number of studies on this ligand. Here we can just remark that GSH represents, with its oxidized form GSSG, the most important buffer system for intracellular redox reactions: it acts as a detoxifying agent toward xenobiotics; it protects organisms from carcinogenic, radical agents of oxidative stress and lipid peroxidation; and, thanks to its high binding ability, it participates in transport, mobilization, and/or elimination of many metal cations, with important effects in toxicology and homeostasis. Some further information about its properties can be found, e.g., in refs 1−5 and in refs reported therein. Despite the several papers published on glutathione, only few deal directly with the modeling of its thermodynamic behavior in aqueous solution or with its chemical speciation, even if it is well-known that these aspects are essential for a thorough understanding of its reactions in natural waters and biological fluids. In fact, the presence in the literature of thermodynamic studies on glutathione (including those on its acid−base properties and on its binding ability toward many metal and organometal cations published over many years (see, e.g., refs 1 and 3−17)) is still not sufficient to give a complete picture of the network of interactions of this ligand. This happens also because the experimental determination of its chemical speciation in all natural and biological systems where it plays a role is, in practice, impossible. At the same time, most of the literature protonation and complexation data were obtained without taking into account their dependence on parameters such as medium, ionic strength, and/or temperature, which highly differentiate all natural systems.

The present work is a first step in this direction, by modeling the dependence on medium and ionic strength of glutathione acid−base behavior in aqueous solutions. GSH protonation constants were determined in alkali metal chlorides and in tetraalkylammonium halides ionic media, from low ($I_c \leq 0.1$ mol·L^{-1}) to high ionic strengths ($I_c \leq 5.0$ mol·L^{-1}). The dependence on medium and ionic strength was modeled by an extended Debye−Hückel (EDH) type equation and by simplified specific ion interaction theory (SIT)[18−20] and Pitzer[21−24] models. Protonation constants at infinite dilution were also determined.

Although most of the natural waters and biological fluids show ionic strength values below $I_c = 1$ mol·L^{-1} (e.g., $I \sim 0.16$ mol·L^{-1} for blood and $I \sim 0.7$ mol·L^{-1} for seawater at $S = 35$), protonation constants at $I_c \gg 1$ mol·L^{-1} were also determined. These values are necessary to obtain more accurate and reliable parameters when modeling the dependence of log K on ionic strength by different models. On the other hand, protonation constants at infinite dilution represent common reference data when making comparisons among various ligands and/or the stability of various complex species.[20,25,26]

Experimental Section

Chemicals. Glutathione (Fluka) was used without further purification, and its purity, checked alkalimetrically, was found to be >99 %. Alkali metal chlorides, tetramethylammonium chloride [$(CH_3)_4NCl$], and tetraethylammonium iodide [$(C_2H_5)_4NI$] aqueous solutions were prepared by weighing pure salts (Fluka) dried in an oven at $T = 383$ K. $(CH_3)_4NCl$ and $(C_2H_5)_4NI$ were previously purified by recrystallization from methanol.

---

* Corresponding author. E-mail: cdestefano@unime.it. Tel: +39-090-6765749. Fax: +39-090-392827.

† Current address: Dipartimento di Biotecnologie per il Monitoraggio Agroalimentare ed Ambientale (BIO.M.A.A.), Università "Mediterranea" di Reggio Calabria, Polo Universitario di Feo di Vito, I-89060 Reggio Calabria, Italy.

10.1021/je6005899 CCC: \$37.00

Table 1. Experimental Ionic Strength Ranges Used in Potentiometric Measurements Carried out for the Determination of Glutathione Protonation Constants in Different Ionic Media at T = 298 K

ionic medium	ionic strength range ($mol \cdot L^{-1}$)
$LiCl_{aq}$	$0.49 \leq I_c \leq 4.81$
$NaCl_{aq}$	$0.10 \leq I_c \leq 4.49$
KCl_{aq}	$0.15 \leq I_c \leq 2.81$
$RbCl_{aq}$	$0.49 \leq I_c \leq 2.91$
$CsCl_{aq}$	$0.49 \leq I_c \leq 3.89$
$(CH_3)_4NCl_{aq}$	$0.12 \leq I_c \leq 2.83$
$(C_2H_5)_4NI_{aq}$	$0.08 \leq I_c \leq 0.85$

Hydrochloric acid, sodium, potassium, and tetraethylammonium hydroxide (Et_4NOH) solutions were prepared by diluting concentrated ampoules (Riedel−deHaën). Acid and hydroxide solutions were standardized against sodium carbonate and potassium hydrogen phthalate, respectively. Hydroxide solutions were preserved from atmospheric CO_2 by means of soda lime traps. All solutions were prepared with analytical grade water ($R = 18\ M\Omega \cdot cm^{-1}$) using grade A glassware.

Apparatus and Procedure. To minimize systematic errors, potentiometric titrations were carried out (at $T = 298 \pm 0.1$ K) using two different apparatuses: the first one consisted of a model 713 Metrohm potentiometer, equipped with a combination glass electrode (Ross type 8102, from Orion), or a half cell glass electrode (Ross type 8101, from Orion) and a double junction reference electrode (type 900200, from Orion), and a model 765 Metrohm motorized burette; the second one was a Crison micro-pH 2002 potentiometer, with the same kind of equipment as the first apparatus. The estimated precision was ± 0.15 mV and ± 0.003 mL for electromotive force (emf) and titrant volume readings, respectively, and was the same for both systems. They were 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. Some measurements were also carried out using a Metrohm model 809 Titrando apparatus controlled by Metrohm TiAMO 1.0 software for the automatic data acquisition.

All potentiometric titrations were carried out under magnetic stirring and by bubbling purified presaturated N_2 through the solution, to exclude O_2 and CO_2 inside. Titrand solutions were prepared by adding different amounts of glutathione (2−5 $mmol \cdot L^{-1}$), hydrochloric acid (3−7.5 $mmol \cdot L^{-1}$), and ionic medium to obtain pre-established ionic strength values, as reported in Table 1. Potentiometric measurements were carried out by titrating 25 or 50 mL of the titrand solutions with standard basic solutions (NaOH for measurements in $LiCl_{aq}$ and $NaCl_{aq}$; KOH in KCl_{aq}, $RbCl_{aq}$, and $CsCl_{aq}$; Et_4NOH in Me_4NCl_{aq} and Et_4NI_{aq}) up to pH ∼ 10.5. The reason we used NaOH instead of LiOH in $LiCl_{aq}$ measurements is that commercial LiOH is not as pure as NaOH (∼98 % of LiOH vs >99.9 % of NaOH) and so it does not serve our purposes; moreover, this choice does not affect the reliability of data obtained in $LiCl_{aq}$ for two reasons: (i) the strength of GSH interactions with lithium and sodium is similar, and (ii) the percentage of Na^+ added is far lower than the corresponding percentage of Li^+ already present in solution. Similar considerations can also be made for the use of KOH in $RbCl_{aq}$ and $CsCl_{aq}$ measurements and of Et_4NOH in Me_4NCl_{aq} measurements. For each titration, 80−100 points were collected, and the equilibrium during titrations was checked using common precautions. These include monitoring the time necessary to reach equilibrium and performing back-titrations. For each experiment, independent titrations of a strong acidic solution with a standard base were carried out under the same medium and ionic strength conditions as those in the systems to be investigated, with the aim of determining electrode potential (E^0) and 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.

Calculations. The nonlinear least-squares computer program ESAB2M[27] was used for the refinement of all the parameters of the acid−base titration (E^0, K_w, liquid junction potential coefficient, j_a, and analytical concentration of reagents). The BSTAC[28] and STACO[29] computer programs were used for the calculation of protonation constants. The ES4ECI[29] program was used to draw speciation diagrams and to calculate species formation percentages. The LIANA[30] program was used to fit different functions.

Details on models used for the dependence on ionic strength are given in the following sections. Formation constants, concentrations, and ionic strengths are expressed in the molar (c, $mol \cdot L^{-1}$) or molal (m, $mol \cdot kg^{-1}$ $[H_2O]$) concentration scales. "c" or "m" subscripts in various symbols refer to molar or molal scales, respectively. Molar to molal conversions were made using appropriate density values.

Protonation equilibria are expressed as

$$H_{i-1}(GSH)^{(i-4)} + H^+ = H_i(GSH)^{(i-3)} \tag{1}$$

with the protonation constant

$$K_i^{H} = \frac{[H_i(GSH)^{(i-3)}]}{[H_{i-1}(GSH)^{(i-4)}][H^+]} \tag{2}$$

$$\log K_i^{H} = pK_{a(5-i)}, \text{ with } 1 \leq i \leq 4$$

Theoretical Approach to the Analysis of Protonation Data

Extended Debye−Hückel (EDH) and Specific Ion Interaction Theory (SIT) Models for Glutathione Protonation Constants. Dependence on ionic strength of glutathione protonation constants was taken into account by an extended Debye−Hückel (EDH) type equation

$$\log K_i^{H} = \log {}^{T}K_i^{H} - z^{*}DH + C_i I \tag{3}$$

with

$$z^{*} = \Sigma(\text{charges})^2_{\text{reactants}} - \Sigma(\text{charges})^2_{\text{products}}$$

and where DH is the Debye−Hückel term

$$DH = AI^{1/2}(1 + 1.5I^{\,1/2})^{-1} \tag{4}$$

with $A = 0.510$ at $T = 298$ K in water. C may be a true constant, or the expression

$$C_i = c_{\infty i} + (c_{0i} - c_{\infty i})F(I) \tag{5}$$

can be used, with

$$F(I) = (I + 1)^{-1} \tag{6}$$

where c_{∞} is the value of C for $I \rightarrow \infty$ and c_0 is the value of C for $I \rightarrow 0$. Moreover, in some cases, a further term for eq 5 is necessary,[31] i.e.

$$c_{\infty i} = c_{\infty i}^{(0)} + c_{\infty i}^{(1)}I \tag{7}$$

In the present work, we adopted this last solution for the first protonation constants in tetraalkylammonium halides.

When protonation constants and ionic strengths are expressed in the molal concentration scale, eq 3 corresponds to the classical and widely used specific ion interaction theory (SIT) equation,[18−20] with $C_i = \Delta\epsilon_i$:

$$\Delta\epsilon_i = \Sigma_j \epsilon(j,k) \tag{8}$$

where $\epsilon(j,k)$ is the SIT interaction coefficient of the *j*-th species (involved in the equilibrium represented by the formation constant *K*) with the *k*-th component (of opposite charge). The specific interaction coefficients $\epsilon(j,k)$ are, in the original model, true constants, but this approximation is valid only for some electrolytes and for some ionic strength ranges. In the modified version of the SIT approach,[31−33] the specific coefficients are expressed, in analogy with eqs 5 and 6 of the EDH-type equation previously shown, as a function of *I*

$$\Delta\epsilon_i = \Delta\epsilon_{\infty i} + (\Delta\epsilon_{0i} - \Delta\epsilon_{\infty i})(I + 1)^{-1} \tag{9}$$

For the first protonation constants in tetraalkylammonium halides, we have

$$\Delta\epsilon_{\infty i} = \Delta\epsilon_{\infty i}{}^{(0)} + \Delta\epsilon_{\infty i}{}^{(1)} I \tag{10}$$

In particular, for the dependence on ionic strength of glutathione protonation constants by the SIT model, the referred to equilibrium shown in eq 2, we have

$$\Delta\epsilon_1 = \epsilon((\mathrm{GSH})^{3-}, \mathrm{M}^+) + \epsilon(\mathrm{X}^-, \mathrm{H}^+) - \epsilon(\mathrm{H(GSH)}^{2-}, \mathrm{M}^+) \tag{11}$$

$$\Delta\epsilon_2 = \epsilon(\mathrm{H(GSH)}^{2-}, \mathrm{M}^+) + \epsilon(\mathrm{X}^-, \mathrm{H}^+) - \epsilon(\mathrm{H_2(GSH)}^{-}, \mathrm{M}^+) \tag{12}$$

$$\Delta\epsilon_3 = \epsilon(\mathrm{H_2(GSH)}^{-}, \mathrm{M}^+) + \epsilon(\mathrm{X}^-, \mathrm{H}^+) - k_\mathrm{m} \tag{13}$$

$$\Delta\epsilon_4 = k_\mathrm{m} + \epsilon(\mathrm{X}^-, \mathrm{H}^+) - \epsilon(\mathrm{H_4(GSH)}^{+}, \mathrm{X}^-) \tag{14}$$

with $\mathrm{M}^+ = \mathrm{Li}^+$, Na^+, K^+, Rb^+, Cs^+, $(\mathrm{CH_3})_4\mathrm{N}^+$, or $(\mathrm{C_2H_5})_4\mathrm{N}^+$ and $\mathrm{X}^- = \mathrm{Cl}^-$ or I^-. The parameter k_m is a coefficient that takes into account the dependence on ionic strength of neutral $\mathrm{H_3(GSH)}^0$ species (e.g., the Setschenow coefficient in solubility measurements; see refs 33−35 for more details). Unfortunately, because the number of equations that could be written is smaller than the number of parameters (i.e., SIT interaction coefficients and/or activity coefficients of species) to be calculated and owing to the lack of many of these values in the literature, we could just refine $\Delta\epsilon$ for each species: further studies are in progress to determine some of these parameters (e.g., k_m values by distribution coefficient measurements; see refs 34−36 for details). However, an extensive discussion on these topics can be found in Bretti et al.[33] and in De Stefano et al.[31] and refs reported therein.

Pitzer Model for Glutathione Protonation Constants. The dependence on ionic strength of glutathione protonation constants was also taken into account by the Pitzer model (for a description of historical aspects and the theoretical basis, one can refer, e.g., to Pitzer, 1973 and 1991,[21−22] and Millero, 1982 and 2001,[23−24] whereas for its direct application to protonation data, one can also refer, e.g., to refs 31 and 33). In their simplified form, Pitzer equations for glutathione protonation constants in an MX ionic medium, expressed as in eq 2, can be written as follows:

$$\log K^\mathrm{H}_{\mathrm{m}i} = \log {}^\mathrm{T}K^\mathrm{H}_{\mathrm{m}i} + [z^* f^\gamma + 2p_{1i} I_\mathrm{m} + p_{2i} I_\mathrm{m}{}^2 + p_{3i}(2I_\mathrm{m} f(2I_\mathrm{m}{}^{1/2})) + 1/2 z^*(2I_\mathrm{m} f'(2I_\mathrm{m}{}^{1/2}))\beta^{(1)}{}_\mathrm{MX}]/\ln 10 \tag{15}$$

with

$$f^\gamma = -0.3915[I_\mathrm{m}{}^{1/2}(1 + 1.2I_\mathrm{m}{}^{1/2})^{-1} + (2/1.2)\ln(1 + 1.2I_\mathrm{m}{}^{1/2})] \tag{16}$$

$$f(x) = 2[1 - (1 + x)\exp(-x)]/x^2 \tag{17}$$

$$f'(x) = -2[1 - (1 + x + x^2/2)\exp(-x)]/x^2 \tag{18}$$

and where, for $\log K^\mathrm{H}_{\mathrm{m}1}$

$$p_{11} = \beta^{(0)}{}_\mathrm{HX} + \beta^{(0)}{}_\mathrm{M(GSH)} - \beta^{(0)}{}_\mathrm{MH(GSH)} + \Theta_\mathrm{HM} \tag{19a}$$

$$p_{21} = C^\Phi{}_\mathrm{HX} + C^\Phi{}_\mathrm{M(GSH)}/\sqrt{3} - C^\Phi{}_\mathrm{MH(GSH)}/\sqrt{2} + C^\Phi{}_\mathrm{MX} + \Psi_\mathrm{HMX} \tag{19b}$$

$$p_{31} = \beta^{(1)}{}_\mathrm{HX} + \beta^{(1)}{}_\mathrm{M(GSH)} - \beta^{(1)}{}_\mathrm{MH(GSH)} \tag{19c}$$

Analogously, for $\log K^\mathrm{H}_{\mathrm{m}2}$

$$p_{12} = \beta^{(0)}{}_\mathrm{HX} + \beta^{(0)}{}_\mathrm{MH(GSH)} - \beta^{(0)}{}_\mathrm{MH_2(GSH)} + \Theta_\mathrm{HM} \tag{20a}$$

$$p_{22} = C^\Phi{}_\mathrm{HX} + C^\Phi{}_\mathrm{MH(GSH)}/\sqrt{2} - C^\Phi{}_\mathrm{MH_2(GSH)} + C^\Phi{}_\mathrm{MX} + \Psi_\mathrm{HMX} \tag{20b}$$

$$p_{32} = \beta^{(1)}{}_\mathrm{HX} + \beta^{(1)}{}_\mathrm{MH(GSH)} - \beta^{(1)}{}_\mathrm{MH_2(GSH)} \tag{20c}$$

and, for $\log K^\mathrm{H}_{\mathrm{m}3}$

$$p_{13} = \beta^{(0)}{}_\mathrm{HX} + \beta^{(0)}{}_\mathrm{MH_2(GSH)} - \lambda + \Theta_\mathrm{HM} \tag{21a}$$

$$p_{23} = C^\Phi{}_\mathrm{HX} + C^\Phi{}_\mathrm{MH_2(GSH)} + C^\Phi{}_\mathrm{MX} + \Psi_\mathrm{HMX} \tag{21b}$$

$$p_{33} = \beta^{(1)}{}_\mathrm{HX} + \beta^{(1)}{}_\mathrm{MH_2(GSH)} \tag{21c}$$

Finally, for $\log K^\mathrm{H}_{\mathrm{m}4}$

$$p_{14} = \beta^{(0)}{}_\mathrm{HX} + \lambda - \beta^{(0)}{}_\mathrm{MH_4(GSH)} + \Theta_\mathrm{HM} \tag{22a}$$

$$p_{24} = C^\Phi{}_\mathrm{HX} - C^\Phi{}_\mathrm{H_4(GSH)X} + \Psi_\mathrm{HMX} \tag{22b}$$

$$p_{34} = \beta^{(1)}{}_\mathrm{HX} - \beta^{(1)}{}_\mathrm{H_4(GSH)X} \tag{22c}$$

For the same reasons mentioned in the last part of the previous section, "classical" Pitzer interaction parameters were not determined.

Results and Discussion

Glutathione Protonation Constants in Various Ionic Media at Different Ionic Strengths. Analysis of the experimental data by both the STACO and BSTAC programs allowed us to determine glutathione apparent protonation constants in different media and ionic strengths, as reported in Tables 2−8. Glutathione acid−base behavior is strongly influenced by medium and ionic strength, as better evidenced in Figures 1−4. In these Figures, protonation constant values are plotted for all four steps in all investigated ionic media versus the square root of ionic strength (in the molar scale, curves in Figures represent dependence on ionic strength by the EDH model; see next

Table 2. Protonation Constants[a] of Glutathione in $\mathrm{LiCl_{aq}}$ at Different Ionic Strengths, at T = 298 K

I_c	I_m	$\log K^{\mathrm{H}}_{c1}$	$\log K^{\mathrm{H}}_{m1}$	$\log K^{\mathrm{H}}_{c2}$	$\log K^{\mathrm{H}}_{m2}$	$\log K^{\mathrm{H}}_{c3}$	$\log K^{\mathrm{H}}_{m3}$	$\log K^{\mathrm{H}}_{c4}$	$\log K^{\mathrm{H}}_{m4}$
0.487	0.493	9.271 ± 0.012[b]	9.266	8.535 ± 0.017[b]	8.530	3.412 ± 0.014[b]	3.407	2.139 ± 0.016[b]	2.134
1.878	1.957	9.292 ± 0.009	9.274	8.760 ± 0.022	8.742	3.518 ± 0.015	3.500	2.285 ± 0.037	2.267
2.861	3.043	9.449 ± 0.005	9.422	8.984 ± 0.017	8.957	3.636 ± 0.011	3.609	2.416 ± 0.056	2.389
4.805	5.337	9.806 ± 0.018	9.760	9.516 ± 0.013	9.470	3.922 ± 0.012	3.876	2.686 ± 0.094	2.640

[a] K^{H}_i refers to equilibrium: $\mathrm{H}_{i-1}(\mathrm{GSH})^{(i-4)} + \mathrm{H}^+ = \mathrm{H}_i(\mathrm{GSH})^{(i-3)}$. [b]± 3 standard deviation.

Table 3. Protonation Constants[a] of Glutathione in $\mathrm{NaCl_{aq}}$ at Different Ionic Strengths, at T = 298 K

I_c	I_m	$\log K^{\mathrm{H}}_{c1}$	$\log K^{\mathrm{H}}_{m1}$	$\log K^{\mathrm{H}}_{c2}$	$\log K^{\mathrm{H}}_{m2}$	$\log K^{\mathrm{H}}_{c3}$	$\log K^{\mathrm{H}}_{m3}$	$\log K^{\mathrm{H}}_{c4}$	$\log K^{\mathrm{H}}_{m4}$
0.104	0.105	9.498 ± 0.012[b]	9.496	8.701 ± 0.012[b]	8.699	3.557 ± 0.015[b]	3.555	2.188 ± 0.015[b]	2.186
0.151	0.152	9.447 ± 0.010	9.445	8.633 ± 0.011	8.631	3.487 ± 0.014	3.485	2.119 ± 0.015	2.117
0.244	0.246	9.368 ± 0.008	9.365	8.606 ± 0.010	8.603	3.493 ± 0.012	3.490	2.162 ± 0.014	2.159
0.468	0.473	9.298 ± 0.006	9.293	8.553 ± 0.009	8.548	3.461 ± 0.009	3.456	2.173 ± 0.014	2.168
0.721	0.733	9.284 ± 0.007	9.277	8.564 ± 0.010	8.557	3.462 ± 0.008	3.455	2.190 ± 0.013	2.183
0.926	0.945	9.302 ± 0.008	9.293	8.582 ± 0.011	8.573	3.491 ± 0.007	3.482	2.241 ± 0.013	2.232
1.412	1.455	9.374 ± 0.009	9.361	8.634 ± 0.012	8.621	3.524 ± 0.007	3.511	2.281 ± 0.013	2.268
1.836	1.908	9.430 ± 0.009	9.413	8.705 ± 0.011	8.688	3.580 ± 0.006	3.563	2.333 ± 0.014	2.316
2.589	2.735	9.594 ± 0.007	9.570	8.826 ± 0.010	8.802	3.679 ± 0.006	3.655	2.420 ± 0.018	2.396
2.811	2.984	9.646 ± 0.006	9.620	8.896 ± 0.009	8.870	3.698 ± 0.006	3.672	2.433 ± 0.019	2.407
3.050	3.256	9.694 ± 0.005	9.666	8.930 ± 0.009	8.902	3.751 ± 0.007	3.723	2.489 ± 0.021	2.461
3.596	3.889	9.827 ± 0.003	9.793	9.058 ± 0.009	9.024	3.807 ± 0.009	3.773	2.466 ± 0.024	2.432
4.040	4.417	9.955 ± 0.002	9.916	9.126 ± 0.011	9.087	3.849 ± 0.011	3.810	2.477 ± 0.027	2.438
4.310	4.745	10.034 ± 0.003	9.992	9.192 ± 0.012	9.150	3.898 ± 0.012	3.856	2.528 ± 0.029	2.486
4.490	4.966	10.081 ± 0.004	10.037	9.230 ± 0.013	9.186	3.930 ± 0.013	3.886	2.574 ± 0.030	2.530

[a] K^{H}_i refers to equilibrium: $\mathrm{H}_{i-1}(\mathrm{GSH})^{(i-4)} + \mathrm{H}^+ = \mathrm{H}_i(\mathrm{GSH})^{(i-3)}$. [b]± 3 standard deviation.

Table 4. Protonation Constants[a] of Glutathione in $\mathrm{KCl_{aq}}$ at Different Ionic Strengths, at T = 298 K

I_c	I_m	$\log K^{\mathrm{H}}_{c1}$	$\log K^{\mathrm{H}}_{m1}$	$\log K^{\mathrm{H}}_{c2}$	$\log K^{\mathrm{H}}_{m2}$	$\log K^{\mathrm{H}}_{c3}$	$\log K^{\mathrm{H}}_{m3}$	$\log K^{\mathrm{H}}_{c4}$	$\log K^{\mathrm{H}}_{m4}$
0.149	0.150	9.477 ± 0.011[b]	9.474	8.635 ± 0.015[b]	8.632	3.483 ± 0.013[b]	3.480	2.144 ± 0.015[b]	2.141
0.482	0.490	9.350 ± 0.010	9.343	8.558 ± 0.026	8.551	3.434 ± 0.014	3.427	2.180 ± 0.013	2.173
0.976	1.006	9.375 ± 0.012	9.362	8.580 ± 0.031	8.567	3.457 ± 0.016	3.444	2.256 ± 0.012	2.243
1.876	1.989	9.532 ± 0.009	9.507	8.711 ± 0.022	8.686	3.564 ± 0.012	3.539	2.381 ± 0.015	2.356
2.810	3.076	9.734 ± 0.010	9.695	8.905 ± 0.024	8.866	3.702 ± 0.013	3.663	2.503 ± 0.021	2.464

[a] K^{H}_i refers to equilibrium: $\mathrm{H}_{i-1}(\mathrm{GSH})^{(i-4)} + \mathrm{H}^+ = \mathrm{H}_i(\mathrm{GSH})^{(i-3)}$. [b]± 3 standard deviation.

Table 5. Protonation Constants[a] of Glutathione in $\mathrm{RbCl_{aq}}$ at Different Ionic Strengths, at T = 298 K

I_c	I_m	$\log K^{\mathrm{H}}_{c1}$	$\log K^{\mathrm{H}}_{m1}$	$\log K^{\mathrm{H}}_{c2}$	$\log K^{\mathrm{H}}_{m2}$	$\log K^{\mathrm{H}}_{c3}$	$\log K^{\mathrm{H}}_{m3}$	$\log K^{\mathrm{H}}_{c4}$	$\log K^{\mathrm{H}}_{m4}$
0.492	0.499	9.344 ± 0.008[b]	9.338	8.526 ± 0.012[b]	8.520	3.515 ± 0.022[b]	3.508	2.300 ± 0.015[b]	2.294
1.460	1.524	9.395 ± 0.005	9.376	8.538 ± 0.013	8.519	3.619 ± 0.023	3.600	2.622 ± 0.025	2.603
2.428	2.610	9.530 ± 0.012	9.499	8.627 ± 0.018	8.596	3.784 ± 0.014	3.753	2.895 ± 0.040	2.864
2.913	3.179	9.616 ± 0.018	9.578	8.658 ± 0.025	8.620	3.853 ± 0.017	3.815	3.085 ± 0.048	3.047

[a] K^{H}_i refers to equilibrium: $\mathrm{H}_{i-1}(\mathrm{GSH})^{(i-4)} + \mathrm{H}^+ = \mathrm{H}_i(\mathrm{GSH})^{(i-3)}$. [b]± 3 standard deviation.

Table 6. Protonation Constants[a] of Glutathione in $\mathrm{CsCl_{aq}}$ at Different Ionic Strengths, at T = 298 K

I_c	I_m	$\log K^{\mathrm{H}}_{c1}$	$\log K^{\mathrm{H}}_{m1}$	$\log K^{\mathrm{H}}_{c2}$	$\log K^{\mathrm{H}}_{m2}$	$\log K^{\mathrm{H}}_{c3}$	$\log K^{\mathrm{H}}_{m3}$	$\log K^{\mathrm{H}}_{c4}$	$\log K^{\mathrm{H}}_{m4}$
0.492	0.503	9.361 ± 0.035[b]	9.351	8.502 ± 0.013[b]	8.492	3.472 ± 0.012[b]	3.462	2.163 ± 0.017[b]	2.153
0.972	1.016	9.368 ± 0.044	9.349	8.505 ± 0.016	8.486	3.521 ± 0.012	3.502	2.303 ± 0.018	2.284
2.929	3.370	9.666 ± 0.029	9.605	8.800 ± 0.011	8.739	3.870 ± 0.010	3.809	2.638 ± 0.062	2.577
3.892	4.712	9.883 ± 0.036	9.800	8.984 ± 0.013	8.901	4.063 ± 0.012	3.980	2.864 ± 0.082	2.781

[a] K^{H}_i refers to equilibrium: $\mathrm{H}_{i-1}(\mathrm{GSH})^{(i-4)} + \mathrm{H}^+ = \mathrm{H}_i(\mathrm{GSH})^{(i-3)}$. [b]± 3 standard deviation.

sections). This different behavior is a clear indication that interactions between GSH species and components of the background salt vary considerably by changing the ionic medium. In general, at a given ionic strength, the lower the apparent protonation constant value, the higher the strength of interactions with other components in solution is. Unfortunately, although for those molecules having only one functional group (e.g., carboxylic acids or amines) it is easy to choose a "noninteracting" ionic medium and to build a sort of "scale of strength" between different ionic media (see, e.g., refs 37 and 38), for "polyfunctional" ligands these operations are quite complicated. Moreover, in the specific case of glutathione we have three binding groups: two carboxylic, one thiolic, and one amino group. Therefore, a deeper evaluation of these effects on GSH acid−base behavior is necessary, and the analysis of each single protonation step is desirable. For these reasons, the protonation/deprotonation sequence must be known as accurately as possible, to assign each protonation constant to a specific functional group. In this circumstance, the literature is very helpful because many papers have been published on this topic:[3−5,10,14,39] now, it is almost universally accepted that, starting from completely deprotonated glutathione, the first proton is bound by the amino group, the second by the thiolic group, and the last two by carboxylic groups (in particular, the third by the "glycinic" and the fourth by the "glutamic" carboxylate). This order is consistent with that shown in Figures 1−4. In fact,

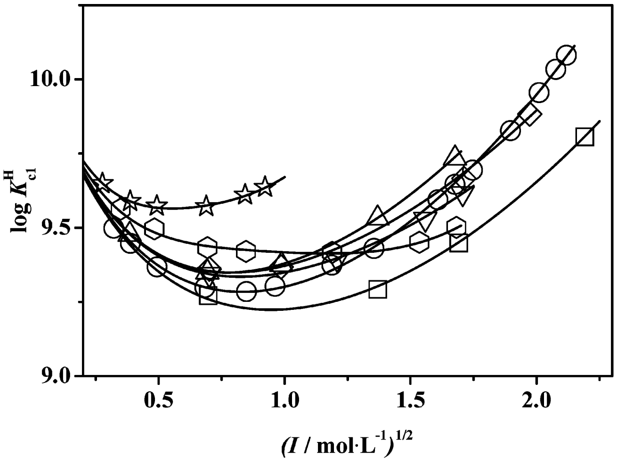

Figure 1. Dependence on medium and ionic strength of the first protonation constant of GSH vs the square root of ionic strength (in mol•L^{-1}), at T = 298 K. Symbol, ionic medium: □, $LiCl_{aq}$; ○, $NaCl_{aq}$; △, KCl_{aq}; ▽, $RbCl_{aq}$; ◇, $CsCl_{aq}$; ⬡, $(CH_3)_4NCl_{aq}$; ☆, $(C_2H_5)_4NI_{aq}$.

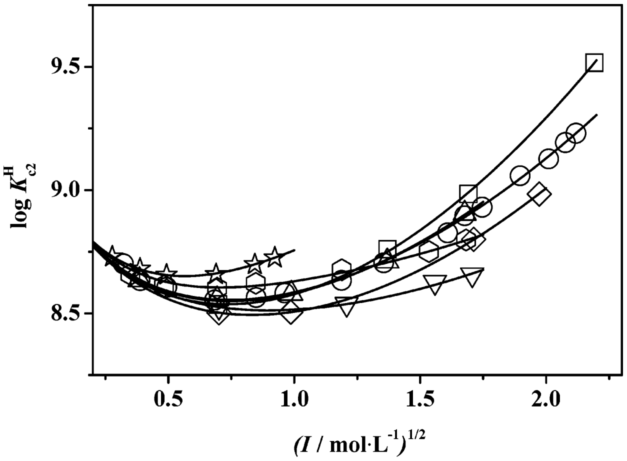

Figure 2. Dependence on medium and ionic strength of the second protonation constant of GSH vs the square root of ionic strength (in mol•L^{-1}), at T = 298 K. Symbol, ionic medium: □, $LiCl_{aq}$; ○, $NaCl_{aq}$; △, KCl_{aq}; ▽, $RbCl_{aq}$; ◇, $CsCl_{aq}$; ⬡, $(CH_3)_4NCl_{aq}$; ☆, $(C_2H_5)_4NI_{aq}$.

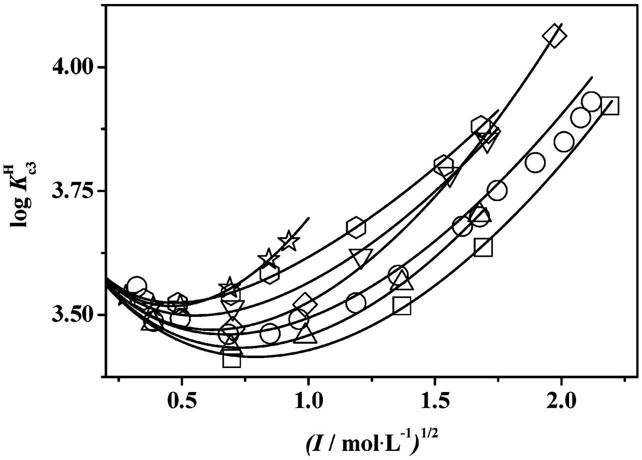

Figure 3. Dependence on medium and ionic strength of the third protonation constant of GSH vs the square root of ionic strength (in mol•L^{-1}), at T = 298 K. Symbol, ionic medium: □, $LiCl_{aq}$; ○, $NaCl_{aq}$; △, KCl_{aq}; ▽, $RbCl_{aq}$; ◇, $CsCl_{aq}$; ⬡, $(CH_3)_4NCl_{aq}$; ☆, $(C_2H_5)_4NI_{aq}$.

with regard to log K^{H}_{c1} (Figure 1), this equilibrium involves a trivalent anion with simultaneous characteristics of O-donor (noninteracting with tetraalkylammonium cations[37]) and N-donor ligands (noninteracting with Na^+ and K^+ salts[38]). The sum of

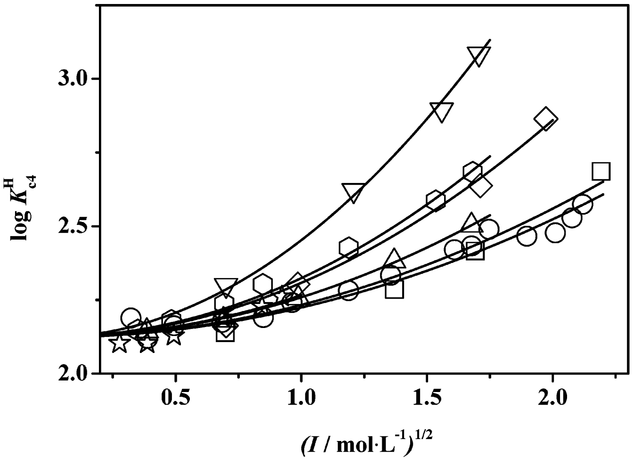

Figure 4. Dependence on medium and ionic strength of the fourth protonation constant of GSH vs the square root of ionic strength (in mol•L^{-1}), at T = 298 K. Symbol, ionic medium: □, $LiCl_{aq}$; ○, $NaCl_{aq}$; △, KCl_{aq}; ▽, $RbCl_{aq}$; ◇, $CsCl_{aq}$; ⬡, $(CH_3)_4NCl_{aq}$; ☆, $(C_2H_5)_4NI_{aq}$.

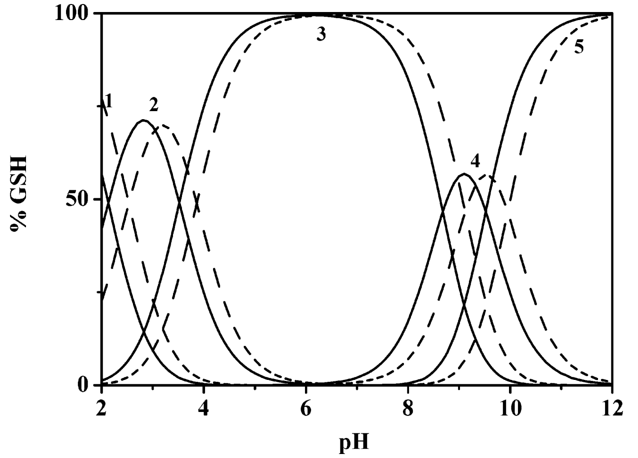

Figure 5. Speciation diagrams of glutathione vs pH in $NaCl_{aq}$ at T = 298 K and I = 0.1 mol•L^{-1} (solid line) or I = 4.0 mol•L^{-1} (dashed line). C_{GSH} = 0.001 mol•L^{-1}. Species: 1, $H_4(GSH)^+$; 2, $H_3(GSH)^0$; 3, $H_2(GSH)^-$; 4, $H(GSH)^{2-}$; 5, $(GSH)^{3-}$.

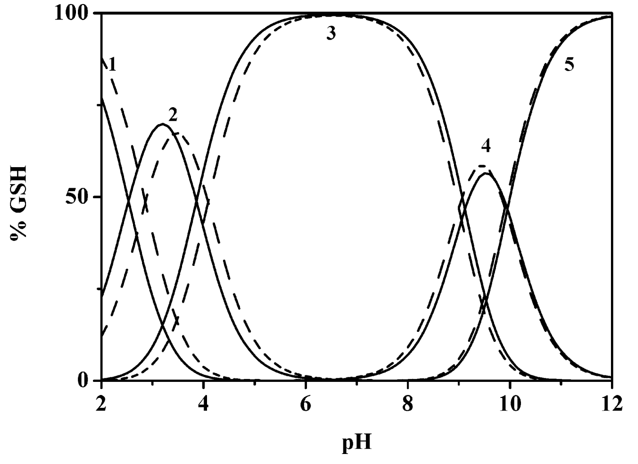

Figure 6. Speciation diagrams of glutathione vs pH in $NaCl_{aq}$ (solid line) and $CsCl_{aq}$ (dashed line) at T = 298 K and I = 4.0 mol•L^{-1}. C_{GSH} = 0.001 mol•L^{-1}. Species: 1, $H_4(GSH)^+$; 2, $H_3(GSH)^0$; 3, $H_2(GSH)^-$; 4, $H(GSH)^{2-}$; 5, $(GSH)^{3-}$.

these effects on the first protonation step yields the following considerations: (i) very weak interactions occur between GSH and tetraalkylammonium salts (especially with $(C_2H_5)_4NI_{aq}$); (ii) interactions with Li^+ are stronger than other alkali metal cations that show similar behavior toward GSH. Similar considerations

Table 7. Protonation Constants[a] of Glutathione in $(CH_3)_4NCl_{aq}$ at Different Ionic Strengths, at $T = 298$ K

I_c	I_m	$\log K^{H}_{c1}$	$\log K^{H}_{m1}$	$\log K^{H}_{c2}$	$\log K^{H}_{m2}$	$\log K^{H}_{c3}$	$\log K^{H}_{m3}$	$\log K^{H}_{c4}$	$\log K^{H}_{m4}$
0.122	0.124	9.563 ± 0.013[b]	9.556	8.658 ± 0.012[b]	8.651	3.531 ± 0.013[b]	3.524	2.148 ± 0.015[b]	2.141
0.234	0.241	9.496 ± 0.015	9.484	8.611 ± 0.012	8.599	3.523 ± 0.012	3.511	2.181 ± 0.014	2.169
0.481	0.509	9.433 ± 0.019	9.409	8.602 ± 0.016	8.578	3.541 ± 0.013	3.517	2.237 ± 0.014	2.213
0.717	0.779	9.420 ± 0.018	9.384	8.622 ± 0.018	8.586	3.584 ± 0.015	3.548	2.302 ± 0.016	2.266
1.413	1.671	9.417 ± 0.014	9.344	8.677 ± 0.017	8.604	3.676 ± 0.014	3.603	2.427 ± 0.026	2.354
2.356	3.161	9.451 ± 0.010	9.323	8.751 ± 0.013	8.623	3.801 ± 0.015	3.673	2.586 ± 0.043	2.458
2.828	4.065	9.501 ± 0.012	9.343	8.797 ± 0.017	8.639	3.880 ± 0.021	3.722	2.683 ± 0.053	2.525

[a] K_i^{H} refers to equilibrium: $H_{i-1}(GSH)^{(i-4)} + H^+ = H_i(GSH)^{(i-3)}$. [b] ± 3 standard deviation.

Table 8. Protonation Constants[a] of Glutathione in $(C_2H_5)_4NI_{aq}$ at Different Ionic Strengths, at $T = 298$ K

I_c	I_m	$\log K^{H}_{c1}$	$\log K^{H}_{m1}$	$\log K^{H}_{c2}$	$\log K^{H}_{m2}$	$\log K^{H}_{c3}$	$\log K^{H}_{m3}$	$\log K^{H}_{c4}$	$\log K^{H}_{m4}$
0.077	0.078	9.649 ± 0.009[b]	9.642	8.730 ± 0.009[b]	8.723	3.538 ± 0.012[b]	3.531	2.103 ± 0.014[b]	2.095
0.150	0.155	9.589 ± 0.011	9.576	8.680 ± 0.008	8.667	3.517 ± 0.012	3.504	2.103 ± 0.012	2.090
0.243	0.255	9.573 ± 0.012	9.552	8.656 ± 0.009	8.635	3.520 ± 0.014	3.499	2.128 ± 0.012	2.107
0.475	0.522	9.572 ± 0.017	9.531	8.658 ± 0.010	8.617	3.554 ± 0.014	3.513	2.189 ± 0.016	2.148
0.711	0.821	9.611 ± 0.014	9.549	8.698 ± 0.006	8.636	3.612 ± 0.013	3.550	2.227 ± 0.024	2.165
0.852	1.014	9.636 ± 0.012	9.561	8.725 ± 0.006	8.650	3.648 ± 0.018	3.573	2.261 ± 0.029	2.186

[a] K_i^{H} refers to equilibrium: $H_{i-1}(GSH)^{(i-4)} + H^+ = H_i(GSH)^{(i-3)}$. [b] ± 3 standard deviation.

Table 9. Protonation Constants[a] of Glutathione at Infinite Dilution and $T = 298$ K

i	$\log {}^{T}K^{H}_{ci}$	$\log {}^{T}K^{H}_{mi}$
1	10.135 ± 0.004[b]	10.134
2	9.077 ± 0.004	9.075
3	3.713 ± 0.004	3.712
4	2.124 ± 0.004	2.123

[a] K_i^{H} refers to equilibrium: $H_{i-1}(GSH)^{(i-4)} + H^+ = H_i(GSH)^{(i-3)}$. [b] ± 3 standard deviation.

can also be made for $\log K^{H}_{c2}$ (Figure 2), but in this case, the strongest interactions can be observed with Rb^+ (and Cs^+, to a lower extent) instead of with Li^+. When considering constants referred to in the protonation of the first carboxylic group, $\log K^{H}_{c3}$ (Figure 3), $(C_2H_5)_4NI_{aq}$ yields the weakest interactions, whereas among the alkali metal cations, the weakest and strongest interactions are those of Cs^+ and Li^+, respectively. However, all these differences are more evident at high ionic strength values and are not so significant at $I < 0.3-0.4$ mol·L^{-1}. Finally, for $\log K^{H}_{c4}$ (Figure 4), protonation constant values always increase with ionic strength for all media, depending on the charges involved in the formation reaction. However, one of the most interesting aspects is that the weakest interactions are observed, in this case, not for tetraalkylammonium salts but for Rb^+, whereas Li^+ and Na^+ yield the strongest interactions.

Influence of Ionic Strength and Ionic Medium on Glutathione Speciation. All differences, evidenced in the previous section, among protonation constant values yield to a different acid−base behavior of glutathione and, therefore, have a direct effect on its chemical speciation in various ionic media and at different ionic strengths. As an example, in Figures 5 and 6, speciation diagrams of glutathione are reported versus pH: the first shows two diagrams obtained in the same ionic medium ($NaCl_{aq}$) but at different ionic strengths ($I = 0.1$ and $I = 4.0$ mol·L^{-1}); the last shows two diagrams at the same ionic strength ($I = 4.0$ mol·L^{-1}) but in different ionic media ($NaCl_{aq}$ and $CsCl_{aq}$). At pH values typical of natural waters and biological fluids (pH ∼ 6−8), glutathione is predominantly present as $H_2(GSH)^-$. However, Figure 5 shows that an increase in ionic strength causes a shift of curves representing formation percentages of various GSH species toward higher pH values (∼0.6−

Table 10. Empirical Parameters of Equation 5 for the Dependence of Glutathione Protonation Constants on Ionic Strength (in the Molar Scale) by Equation 3 (EDH Model), in Different Ionic Media and at $T = 298$ K

medium	$c_{\infty1}$	c_{01}	$c_{\infty2}$	c_{02}	$c_{\infty3}$	c_{03}	C_4
$LiCl_{aq}$	0.228 ± 0.003[a]	0.397 ± 0.006[a]	0.304 ± 0.003[a]	0.331 ± 0.006[a]	0.168 ± 0.003[a]	0.075 ± 0.009[a]	0.109 ± 0.015[a]
$NaCl_{aq}$	0.300 ± 0.003	0.476 ± 0.006	0.230 ± 0.003	0.416 ± 0.006	0.148 ± 0.003	0.223 ± 0.006	0.100 ± 0.006
KCl_{aq}	0.278 ± 0.003	0.656 ± 0.009	0.242 ±0.009	0.402 ± 0.018	0.187 ± 0.003	0.121 ± 0.006	0.135 ± 0.006
$RbCl_{aq}$	0.207 ± 0.003	0.666 ± 0.003	0.129 ± 0.006	0.382 ± 0.012	0.170 ± 0.003	0.350 ± 0.006	0.329 ± 0.012
$CsCl_{aq}$	0.231 ± 0.006	0.687 ± 0.024	0.232 ± 0.003	0.260 ± 0.009	0.221 ± 0.003	0.218 ± 0.003	0.184 ± 0.015
$(CH_3)_4NCl_{aq}$	f_1[b]	1.132 ± 0.003	0.109 ± 0.006	0.631 ± 0.009	0.143 ± 0.006	0.478 ± 0.009	0.200 ± 0.012
$(C_2H_5)_4NI_{aq}$	f_2[c]	1.633 ± 0.012	0.252 ± 0.012	0.737 ± 0.009	0.387 ± 0.021	0.392 ± 0.015	0.142 ± 0.027

[a] ± 3 standard deviation. [b] $f_1 = (-0.181 \pm 0.015) + (0.062 \pm 0.006)I_c$. [c] $f_2 = (-0.498 \pm 0.050) + (0.390 \pm 0.061)I_c$.

Table 11. Empirical Parameters of Equation 9 for the Dependence of Glutathione Protonation Constants on Ionic Strength (in the Molal Scale) by Equation 3 (SIT Model), in Different Ionic Media and at $T = 298$ K

medium	$\Delta\epsilon_{\infty1}$	$\Delta\epsilon_{01}$	$\Delta\epsilon_{\infty2}$	$\Delta\epsilon_{02}$	$\Delta\epsilon_{\infty3}$	$\Delta\epsilon_{03}$	$\Delta\epsilon_{4}$
$LiCl_{aq}$	0.187 ± 0.003[a]	0.438 ± 0.006[a]	0.250 ± 0.006[a]	0.390 ± 0.006[a]	0.134 ± 0.003[a]	0.108 ± 0.003[a]	0.092 ± 0.012[a]
$NaCl_{aq}$	0.253 ± 0.003	0.515 ± 0.003	0.191 ± 0.003	0.446 ± 0.006	0.120 ± 0.003	0.244 ± 0.003	0.083 ± 0.006
KCl_{aq}	0.230 ± 0.003	0.673 ± 0.006	0.199 ±0.003	0.316 ± 0.009	0.150 ± 0.006	0.129 ± 0.003	0.113 ± 0.012
$RbCl_{aq}$	0.171 ± 0.003	0.676 ± 0.006	0.102 ± 0.006	0.387 ± 0.012	0.136 ± 0.003	0.355 ± 0.012	0.291 ± 0.009
$CsCl_{aq}$	0.164 ± 0.003	0.712 ± 0.009	0.163 ± 0.003	0.290 ± 0.015	0.153 ± 0.003	0.240 ± 0.015	0.138 ± 0.009
$(CH_3)_4NCl_{aq}$	f_1[b]	1.042 ± 0.009	0.029 ± 0.006	0.615 ± 0.006	0.047 ± 0.003	0.458 ± 0.009	0.106 ± 0.009
$(C_2H_5)_4NI_{aq}$	f_2[c]	1.572 ± 0.009	0.090 ± 0.012	0.690 ± 0.009	0.195 ± 0.021	0.343 ± 0.015	0.049 ± 0.012

[a] ± 3 standard deviation. [b] $f_1 = (-0.135 \pm 0.015) + (0.026 \pm 0.003)I_m$. [c] $f_2 = (-0.591 \pm 0.034) + (0.319 \pm 0.036)I_m$.

Table 12. Simplified Pitzer Interaction Parameters, Determined for Glutathione Protonation in Different Ionic Media, at $T = 298$ K

medium	p_{1i}	p_{2i}	p_{3i}
		$i = 1$	
$LiCl_{aq}$	0.492 ± 0.003[a]	−0.032 ± 0.003[a]	2.348 ± 0.015[a]
$NaCl_{aq}$	0.583 ± 0.003	−0.039 ± 0.003	2.254 ± 0.009
KCl_{aq}	0.587 ± 0.003	−0.059 ± 0.003	2.464 ± 0.003
$RbCl_{aq}$	0.441 ± 0.003	−0.031 ± 0.003	2.708 ± 0.018
$CsCl_{aq}$	0.348 ± 0.003	−0.008 ± 0.003	2.852 ± 0.009
$(CH_3)_4NCl_{aq}$	0.018 ± 0.003	0.038 ± 0.003	3.875 ± 0.009
$(C_2H_5)_4NI_{aq}$	−0.527 ± 0.003	0.358 ± 0.003	5.009 ± 0.009
		$i = 2$	
$LiCl_{aq}$	0.514 ± 0.003	−0.032 ± 0.003	1.341 ± 0.015
$NaCl_{aq}$	0.406 ± 0.003	−0.023 ± 0.003	1.687 ± 0.009
KCl_{aq}	0.367 ± 0.003	−0.008 ± 0.003	1.697 ± 0.003
$RbCl_{aq}$	0.321 ± 0.003	−0.039 ± 0.003	1.602 ± 0.024
$CsCl_{aq}$	0.355 ± 0.003	−0.026 ± 0.003	1.270 ± 0.003
$(CH_3)_4NCl_{aq}$	0.222 ± 0.003	−0.035 ± 0.003	2.021 ± 0.024
$(C_2H_5)_4NI_{aq}$	−0.105 ± 0.003	0.157 ± 0.009	2.480 ± 0.009
		$i = 3$	
$LiCl_{aq}$	0.276 ± 0.003	−0.018 ± 0.003	0.444 ± 0.015
$NaCl_{aq}$	0.250 ± 0.003	−0.017 ± 0.003	0.775 ± 0.006
KCl_{aq}	0.283 ± 0.003	−0.023± 0.003	0.494 ± 0.003
$RbCl_{aq}$	0.213 ± 0.003	0.001 ± 0.003	1.065 ± 0.018
$CsCl_{aq}$	0.281 ± 0.003	−0.019 ± 0.003	0.620 ± 0.006
$(CH_3)_4NCl_{aq}$	0.144 ± 0.003	−0.015 ± 0.003	1.244 ± 0.024
$(C_2H_5)_4NI_{aq}$	0.328 ± 0.003	−0.137 ± 0.003	0.600 ± 0.006
		$i = 4$	
$LiCl_{aq}$	0.148 ± 0.003	−0.004 ± 0.003	−0.295 ± 0.009
$CNaCl_{aq}$	0.164 ± 0.003	−0.028 ± 0.003	−0.097 ± 0.018
KCl_{aq}	0.180 ± 0.003	−0.024 ± 0.003	−0.110 ± 0.003
$RbCl_{aq}$	0.268 ± 0.006	0.013 ± 0.003	0.320 ± 0.027
$CsCl_{aq}$	0.150 ± 0.003	0.005 ± 0.003	−0.020 ± 0.015
$(CH_3)_4NCl_{aq}$	0.122 ± 0.003	−0.017 ± 0.003	0.226 ± 0.018
$(C_2H_5)_4NI_{aq}$	1.130 ± 0.012	−0.864 ± 0.024	−2.119 ± 0.003

[a] ± 3 standard deviation.

0.7 pH units). With regard to Figure 6, in this specific case, a different ionic medium yields to a variation of pH range where the $H_2(GSH)^-$ species is formed (in $CsCl_{aq}$, the formation of this species occurs in a narrower pH range than in $NaCl_{aq}$), with small differences also in the formation percentages of $H(GSH)^{2-}$ and $H_3(GSH)^0$ species (e.g., $H(GSH)^{2-}$ reaches a maximum of 56.4 % of total GSH at pH = 9.5 in $NaCl_{aq}$, and 58.3 % is reached in $CsCl_{aq}$ at the same pH value; $H_3(GSH)^0$ reaches 69.8 % in $NaCl_{aq}$ at pH = 3.2 and 67.3 % in $CsCl_{aq}$ at pH = 3.5).

Dependence on Medium and Ionic Strength by EDH, SIT, and Pitzer Approaches. The dependence of glutathione protonation constants on medium and ionic strength was modeled by EDH and SIT approaches, as described above. Values of log K^{H}_{ci} reported in Tables 2−8 were fitted to eq 3 to determine protonation constant values at infinite dilution, log ${}^{T}K^{H}_{ci}$, and empirical parameters of eq 5. The use of the LIANA program allowed us to treat each set of constants independently for each step but considering simultaneously all ionic media: this procedure increases the accuracy of calculated protonation constants at $I = 0$ mol·L^{-1}. These values are shown in Table 9 with corresponding constants in the molal scale, and refined $c_{\infty i}$ and c_{0i} parameters are reported in Table 10 for all investigated ionic media. As can be noted from this table, refined parameters for the fourth protonation constant values are just C_i values: the use of eq 5 for this set of values did not significantly improve the goodness of total fit. On the contrary, as already stated, the application of eq 7 to log K^{H}_{c1} values in tetraalkylammonium salts was necessary. However, the quality of calculations performed on all four sets of protonation constants can be better appreciated by looking at the standard deviations of total fits: σ_{fit} (log K^{H}_{c1}) = 0.010, σ_{fit} (log K^{H}_{c2}) = 0.011, σ_{fit} (log K^{H}_{c3}) = 0.011, σ_{fit} (log K^{H}_{c4}) = 0.030.

Analogously, values in the molal scale reported in Tables 2−8 (i.e., log K^{H}_{mi}) were fitted to eq 3 to determine simplified SIT interaction coefficients [i.e., $\Delta\epsilon_i$, as true constants or as expressed by eq 9], reported in Table 11. Also in this case, with regard to the fit of the fourth protonation constant values, refinement of just the $\Delta\epsilon_4$ coefficient was sufficient, whereas we used eq 10 for the fitting of log K^{H}_{m1} values in tetraalkylammonium salts. Standard deviations of total fits are: σ_{fit} (log K^{H}_{m1}) = 0.009, σ_{fit} (log K^{H}_{m2}) = 0.011, σ_{fit} (log K^{H}_{m3}) = 0.012, σ_{fit} (log K^{H}_{m4}) = 0.032.

The dependence on medium and ionic strength of glutathione protonation constants was also modeled by Pitzer equations, as reported above. Refined simplified Pitzer interaction parameters, obtained by fitting GSH protonation constants (in the molal scale) to eq 15, are reported in Table 12. To simplify calculations and to avoid eventual systematic errors, a different (opposite) procedure was adopted: fits were performed independently for

Table 13. Selection of Literature Data for Glutathione Protonation Constants at $T = 298$ K

I_c − medium	log K^{H}_{c1}	log K^{H}_{c2}	log K^{H}_{c3}	log K^{H}_{c4}	ref
0.10	9.51	8.64	3.49	2.08	44
0.10 − $NaClO_{4aq}$	9.52	8.65	3.53	2.10	17
0.10 − $NaCl_{aq}$	**9.52**	**8.68**	**3.52**	**2.13**	**t.w.**[b]
0.10 − KNO_{3aq} or KCl_{aq}[a]	9.54	8.68	3.50	2.08	1
0.10 − KNO_{3aq} or KCl_{aq}[a]	9.56	8.65	3.55	2.16	1
0.10 − KNO_{3aq}	9.66	8.74	3.51	2.13	4
0.15 − KNO_{3aq}	9.65	8.75	3.59	−	7
0.15 − KNO_{3aq}	9.44	8.63	3.48	2.09	16
0.15 − KCl_{aq}	**9.48**	**8.63**	**3.48**	**2.14**	**t.w.**[b]
0.30 − $NaClO_{4aq}$	9.78	8.82	3.58	−	13
0.30 − $NaCl_{aq}$	**9.35**	**8.58**	**3.47**	**2.15**	**t.w.**[b]
0.40 − $NaNO_{3aq}$	9.48	8.67	−	−	15
0.40 − $NaCl_{aq}$	**9.31**	**8.56**	**3.46**	**2.16**	**t.w.**[b]
0.50	9.37	8.58	3.48	2.1	44
0.50 − $NaCl_{aq}$	**9.29**	**8.55**	**3.46**	**2.17**	**t.w.**[b]
1.00 − KNO_{3aq}	9.69	8.75	3.49	1.98	12
1.00 − KCl_{aq}	**9.38**	**8.58**	**3.46**	**2.26**	**t.w.**[b]
1.50 − KCl_{aq}	9.44	8.63	3.49	2.11	16
1.50 − KCl_{aq}	**9.46**	**8.66**	**3.51**	**2.33**	**t.w.**[b]
2.00 − KNO_{3aq}	8.93	8.27	3.47	2.18	4
2.00 − KCl_{aq}	**9.56**	**8.74**	**3.58**	**2.39**	**t.w.**[b]
3.00 − $NaClO_{4aq}$	9.88	9.16	3.82	2.60	11
3.00 − $NaCl_{aq}$	**9.69**	**8.92**	**3.73**	**2.43**	**t.w.**[b]

[a] KCl_{aq} or KNO_{3aq} used as background salts but not specified. [b] This work. Values calculated by the EDH model.

each ionic medium but considering simultaneously all four protonation steps. Standard deviations of total fits, obtained in this way, are: σ_{fit} ($\mathrm{LiCl_{aq}}$) = 0.009, σ_{fit} ($\mathrm{NaCl_{aq}}$) = 0.018, σ_{fit} ($\mathrm{KCl_{aq}}$) = 0.028, σ_{fit} ($\mathrm{RbCl_{aq}}$) = 0.015, σ_{fit} ($\mathrm{CsCl_{aq}}$) = 0.022, σ_{fit} ($\mathrm{(CH_3)_4NCl_{aq}}$) = 0.010, σ_{fit} ($\mathrm{(C_2H_5)_4NI_{aq}}$) = 0.005.

Literature Comparisons. Much has been published on the thermodynamic properties of glutathione. Nevertheless, despite many papers that can be found in the literature on its acid−base properties, including the determination of macroscopic and microscopic protonation constants in different experimental conditions,[1,3−17,39] to our knowledge and at the present time no data were published on the modeling of the dependence of these constants on medium and ionic strength. Moreover, most of the data reported by different databases[40−44] were obtained at T = 298 K or 310 K and in “commonly used” ionic media, such as $\mathrm{KCl_{aq}}$, $\mathrm{KNO_{3aq}}$, $\mathrm{NaNO_{3aq}}$, and $\mathrm{NaClO_{4aq}}$. Therefore, for the first time in the present paper, glutathione protonation constants were determined not only in $\mathrm{LiCl_{aq}}$, $\mathrm{RbCl_{aq}}$, and $\mathrm{CsCl_{aq}}$ but also in tetraalkylammonium halides. In Table 13, a selection of these literature data at T = 298 K are reported together with some protonation constant values determined in this work. From a rapid analysis of this table, we can affirm that our data are in good agreement with most of those reported in the literature: some small discrepancies can be attributed to the different behavior shown by glutathione (and, usually, by other ligands) in chloride media with respect to nitrate and perchlorate. With regard to $\mathrm{NaCl_{aq}}$, the most important dissolved inorganic salt in natural waters and biological fluids, only in one paper by Garcia Bugarin and Filella[3] were GSH protonation constants determined in this medium at $I = 0.15\ \mathrm{mol{\cdot}L^{-1}}$, but at T = 310 K. However, by taking into account this difference, we can affirm that values obtained by these authors ($\log K_{c1}^{\mathrm{H}} = 9.12$, $\log K_{c2}^{\mathrm{H}} = 8.30$, $\log K_{c3}^{\mathrm{H}} = 3.36$, and $\log K_{c4}^{\mathrm{H}} = 2.15$) are in agreement with those determined in this work in the same medium and ionic strength conditions, but at T = 298 K ($\log K_{c1}^{\mathrm{H}} = 9.45$, $\log K_{c2}^{\mathrm{H}} = 8.64$, $\log K_{c3}^{\mathrm{H}} = 3.50$, and $\log K_{c4}^{\mathrm{H}} = 2.14$).

Literature Cited

(1) Oram, P. D.; Fang, X.; Fernando, Q.; Letkeman, P.; Letkeman, D. The formation constants of mercury(II)−glutathione complexes. *Chem. Res. Toxicol.* **1996**, *9*, 709−712.

(2) Prasad, M. N. V. Metal−biomolecule complexes in plants: Occurrence, functions, and applications. *Analusis Magazine* **1998**, *26* (6), M25−M28.

(3) Garcia Bugarin, M.; Filella, M. The formation constants of dimethylthallium(III)−glutathione complexes in aqueous solution. *J. Inorg. Biochem.* **1999**, *73*, 17−29.

(4) Krezel, A.; Bal, W. Structure−function relationships in glutathione and its analogues. *Org. Biomol. Chem.* **2003**, *1*, 3885−3890.

(5) Noszal, B.; Szakacs, Z. Microscopic protonation equilibria of oxidized glutathione. *J. Phys. Chem. B* **2003**, *107*, 5074−5080.

(6) Stricks, W.; Kolthoff, I. M. Reactions between mercuric mercury and cysteine and glutathione. Apparent dissociation constants, heats and entropies of formation of various forms of mercuric mercapto-cysteine and -glutathione. *J. Am. Chem. Soc.* **1953**, *75*, 5673−5681.

(7) Li, N. C.; Gawron, O.; Bascuas, G. Stability of zinc complexes with glutathione and oxidized glutathione. *J. Am. Chem. Soc.* **1954**, *76*, 225−229.

(8) Benesch, R. E.; Benesch, R. The acid strength of the -SH group in cysteine and related compounds. *J. Am. Chem. Soc.* **1955**, *77*, 5877−5881.

(9) Simpson, R. B. Association constants of methylmercury with sulfhydryl and other bases. *J. Am. Chem. Soc.* **1961**, *83*, 4711−4717.

(10) Rabenstein, D. L. Nuclear magnetic resonance studies of the acid−base chemistry of amino acids and peptides. I. Microscopic ionization constants of glutathione and methylmercury-complexed glutathione. *J. Am. Chem. Soc.* **1973**, *95* (9), 2797−2803.

(11) Corrie, A. M.; Walker, M. D.; Williams, D. R. Thermodynamic considerations in co-ordination. Part XXII. Sequestering ligands for improving the treatment of plumbism and cdmiumism. *J. Chem. Soc., Dalton Trans.* **1976**, 1012−1015.

(12) Arnold, A. P.; Canty, A. Methylmercury(II) sulfhydryl interactions. Potentiometric determination of the formation constants for complexation of methylmercury(II) by sulfhydryl containing amino acids and related molecules, including glutathione. *Can. J. Chem.* **1983**, *61* (7), 1428−1434.

(13) Hynes, M.; O’Dowd, M. Interactions of the trimethyltin(IV) cation with carboxylic acids, amino acids, and related ligands. *J. Chem. Soc., Dalton Trans.* **1987**, 563−566.

(14) Cheesman, B. V.; Arnold, A. P.; Rabenstein, D. L. Nuclear magnetic resonance studies of the solution chemistry of metal complexes. 25. $\mathrm{Hg(thiol)_3}$ complexes and Hg(II)−thiol ligand exchange kinetics. *J. Am. Chem. Soc.* **1988**, *110*, 6359−6364.

(15) Kadima, W.; Rabenstein, D. L. Nuclear magnetic resonance studies of the solution chemistry of metal complexes. 26. Mixed ligand complexes of cadmium, nitrilotriacetic acid, glutathione, and related ligands. *J. Inorg. Biochem.* **1990**, *38* (4), 277−288.

(16) Armas, M. T.; Mederos, A.; Gili, P.; Dominguez, S.; Hernandez-Molina, R.; Lorenzo, P.; Baran, E. J.; Araujo, M. L.; Brito, F. Speciation in the oxovanadium(IV)/glutathione system. *Polyhedron* **2001**, *20*, 799−804.

(17) Rey, N. A.; Howarth, O. W.; Pereira-Maia, E. C. Equilibrium characterization of the As(III)−cysteine and the As(III)−glutathione systems in aqueous soultion. *J. Inorg. Biochem.* **2004**, *98*, 1151−1159.

(18) Biederman, G. Ionic Media. In *Dahlem Workshop on the Nature of Seawater*; Dahlem Konferenzen: Berlin, 1975; pp 339−362.

(19) Biederman, G. Introduction to the specific interaction theory with emphasis on chemical equilibria. In *Metal complexes in solution*; Jenne, E.A., Rizzarelli, E., Romano, V., Sammartano, S., Eds.; Piccin: Padua, Italy, 1986; pp 303−314.

(20) Grenthe, I.; Puigdomenech, I. *Modelling in Aquatic Chemistry*; OECD: Paris, 1997.

(21) Pitzer, K. S. Thermodynamics of Electrolytes. I. Theoretical Basis and General Equations. *J. Phys. Chem.* **1973**, *77* (2), 268−277.

(22) Pitzer, K. S. *Activity Coefficients in Electrolyte Solutions*, 2nd ed.; CRC Press, Inc.: Boca Raton, FL, 1991.

(23) Millero, F. J. Use of models to determine ionic interactions in natural waters. *Thalassia Jugoslavica* **1982**, *18* (1−4), 253−291.

(24) Millero, F. J. *Physical Chemistry of Natural Waters*; Wiley-Interscience Series in Geochemistry; John Wiley & Sons, Inc.: New York, 2001.

(25) Grenthe, I.; Wanner, H. *Guidelines for the extrapolation to zero ionic strength*; AEN-NEA: Issy-les-Moulineaux, France, 2000.

(26) Osthols, E.; Wanner, H. *The NEA thermochemical data base project*; AEN-NEA: Yssy-les-Moulineaux, France, 2000.

(27) De Stefano, C.; Princi, P.; Rigano, C.; Sammartano, S. Computer Analysis of Equilibrium Data in Solution. ESAB2M: An Improved Version of the ESAB Program. *Ann. Chim. (Rome)* **1987**, *77*, 643−675.

(28) De Stefano, C.; Mineo, P.; Rigano, C.; Sammartano, S. Ionic Strength Dependence of Formation Constants. XVII. The Calculation of Equilibrium Concentrations and Formation Constants. *Ann. Chim. (Rome)* **1993**, *83*, 243−277.

(29) De Stefano, C.; Foti, C.; Giuffrè, O.; Mineo, P.; Rigano, C.; Sammartano, S. Binding of Tripolyphosphate by Aliphatic Amines: Formation, Stability and Calculation Problems. *Ann. Chim. (Rome)* **1996**, *86*, 257−280.

(30) De Stefano, C.; Sammartano, S.; Mineo, P.; Rigano, C. Computer Tools for the Speciation of Natural Fluids. In *Marine Chemistry - An Environmental Analytical Chemistry Approach*; Gianguzza, A., Pelizzetti, E., Sammartano, S., Eds.; Kluwer Academic Publishers: Amsterdam, 1997; pp 71−83.

(31) De Stefano, C.; Milea, D.; Pettignano, A.; Sammartano, S. Modeling ATP protonation and activity coefficients in $\mathrm{NaCl_{aq}}$ and $\mathrm{KCl_{aq}}$ by SIT and Pitzer equations. *Biophys. Chem.* **2006**, *121*, 121−130.

(32) Bretti, C.; Foti, C.; Sammartano, S. Calculation of SIT parametres. Part I. A new approach in the use of SIT in determining the dependence on ionic strength of activity coefficients. Application to some chloride salts of interest in the speciation of natural fluids. *Chem. Spec. Bioavailability* **2004**, *16* (3), 105−110.

(33) Bretti, C.; Giacalone, A.; Gianguzza, A.; Milea, D.; Sammartano, S. Modeling *S*-carboxymethyl-L-cysteine protonation and activity coefficients in sodium and tetramethylammonium chloride aqueous solutions by SIT and Pitzer equations. *Fluid Phase Equilib.* **2007**, *252* (1−2), 119−129.

(34) Bretti, C.; Crea, F.; Foti, C.; Sammartano, S. Solubility and activity coefficients of *o*-phthalic acid and cystine in $\mathrm{NaCl_{aq}}$, $\mathrm{(CH_3)_4NCl_{aq}}$, and $\mathrm{(C_2H_5)_4NI_{aq}}$ at different ionic strengths, at t = 25 °C. *J. Chem. Eng. Data* **2005**, *50*, 1761−1767.

(35) Bretti, C.; Crea, F.; Foti, C.; Sammartano, S. Solubility and Activity Coefficients of Acidic and Basic Nonelectrolytes in Aqueous Salt Solutions. 2. Solubility and Activity Coefficients of Suberic, Azelaic and Sebacic Acids in $\mathrm{NaCl_{(aq)}}$, $\mathrm{(CH_3)_4NCl_{(aq)}}$ and $\mathrm{(C_2H_5)_4NI_{(aq)}}$ at

Different Ionic Strengths and at $t = 25$ °C. *J. Chem. Eng. Data* **2006**, *51* (5), 1660−1667.
(36) Brandariz, I. Salting coefficient of triethanolamine in major seawater salts. *Fluid Phase Equilib.* **2006**, *239* (1), 12−15.
(37) Crea, F.; De Robertis, A.; Sammartano, S. Medium and alkyl chain effects on the protonation of dicarboxylates in $NaCl_{(aq)}$ and $Et_4NI_{(aq)}$ at 25 °C. *J. Solution Chem.* **2004**, *33*, 497−526.
(38) De Stefano, C.; Giuffrè, O.; Sammartano, S. Protonation constants of ethylenediamine, diethylenetriamine and spermine in $NaCl_{aq}$, NaI_{aq}, $(CH_3)_4NCl_{aq}$ and $(C_2H_5)_4NI_{aq}$, at different ionic strengths and $t = 25$ °C. *J. Chem. Eng. Data* **2005**, *50*, 1917−1923.
(39) Reid, R. S.; Rabenstein, D. L. Nuclear magnetic resonance studies of the solution chemistry of metal complexes. 19. Formation constants for the complexation of methylmercury by glutathione, ergothioneine, and hemoglobin. *J. Am. Chem. Soc.* **1982**, *104*, 6733−6737.
(40) Sillén, L.G.; Martell, A. E. *Stability Constants of Metal Ion Complexes. Special Publ. 17.*; The Chemical Society, Wiley: London, 1964.
(41) Sillén, L. G.; Martell, A. E. *Stability Constants of Metal Ion Complexes. Supplement Special Publ. 25.*; The Chemical Society, Wiley: London, 1964.
(42) Pettit, D.; Powell, K. *IUPAC Stability Constants Database*; Academic Software: Otley, UK, 1997.
(43) May, P. M.; Murray, K. Database of Chemical Reactions Designed to Achieve Thermodynamic Consistency Automatically. *J. Chem. Eng. Data* **2001**, *46*, 1035−1040.
(44) Martell, A. E.; Smith, R. M.; Motekaitis, R. J. *NIST Standard Reference Database 46*, version 8; Gaithersburg, 2004.

Received for review December 28, 2006. Accepted March 12, 2007. We thank the University of Messina (PRA) and the Italian Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR, FIRB project n. RBAUO1HLFX-004), for financial support.

JE6005899