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Sequestration of Hg^{2+} by Some Biologically Important Thiols

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Supporting Information

ABSTRACT: A potentiometric and ^1H NMR investigation on the interactions between Hg^{2+} and some biologically important sulfhydryl ligands, such as cysteine (H_2CYS), penicillamine (H_2PSH), and glutathione (H_3GSH), is reported. Equilibria were studied at T = 298.15 K, in the ionic strength range (0.1 to 1) mol·kg^{-1}, using as ionic medium NaCl in the presence of iodide (NaI) as a competitive ligand. Results show the formation of HgL^{2-z}, $HgLH^{3-z}$, $HgLH_2^{4-z}$, HgL_2^{2-2z}, HgL_2H^{3-2z}, and $HgL_2H_2^{4-2z}$ species (L^{z-} = CYS^{2-}, PSH^{2-}, or GSH^{3-}) together with $HgL_2H_3^{-}$ for GSH^{3-} only. Formation constant values are very high with, as an example, log β = 34.54, 34.24, and 32.05 for $Hg(CYS)^0$, $Hg(PSH)^0$, and $Hg(GSH)^-$ species, respectively (at I = 0.25 mol·kg^{-1}, T = 298.15 K). ^1H NMR measurements at I = 0.25 mol·kg^{-1} (NaCl) fully confirm the potentiometric findings. The speciation diagrams show that most of the metal fraction is present as complex species in a wide pH range, and the hydrolysis of the cation is completely suppressed. The sequestering ability of ligands toward Hg^{2+} is very high, and it was analyzed and compared at different pH and ionic strengths.

INTRODUCTION

Mercury and its compounds are highly toxic to living organisms and ecosystems, and their pollution is considered to be a global problem, diffuse and chronic. Mercury toxicity is known to target the central nervous system and the kidneys, although its role in neurodegenerative disorders such as multiple sclerosis, Alzheimer's and Parkinson's diseases, and autism is a controversial subject.[1] Treatment of mercury poisoning in humans generally involves the use of chelation therapy.[2,3] Chelation therapy is the clinical adaptation of chelate chemistry, which is intended to remove heavy metals from the body through binding to a chelation therapeutic drug.

Three basic forms of mercury are known: elemental Hg^0 and inorganic cations, Hg_2^{2+} and Hg^{2+}, organometallic compounds with one or two alkyl- or aryl-substituents covalently bound to mercury atom. The mercury(II) ion has a distinctly "soft" character, showing a strong affinity for ligands with soft donor atoms such as Se, S, and P, and for the halide ions I^-, Br^-, and Cl^-. Thiolates have even been traditionally referred to as mercaptans because of their ability to capture mercury(II).[4] It has been known since at least the early 1970s that 99 % of mercury species circulating in the plasma are bound to protein by thiol groups, and it was speculated that the transport of mercury into organs and resultant organ distribution was determined by the remaining 1 % of mercury bound to "diffusible thiols",[5,6] that is, low molecular weight thiols that are transportable across cell membranes.[7] A recent review[8] reports numerous examples where low molecular weight thiols bound to mercury (and other heavy metals) have facilitated the entry of the mercury into various cell types.

An understanding of the nature and the extent of binding of low molecular weight thiols to Hg^{2+} is, therefore, of crucial importance to predict Hg^{2+} transport and fate and to evaluate methods of removing it from natural and biological systems. In a recent manuscript,[9,10] we have reported an investigation on the interactions between Hg^{2+} and some mercaptocarboxylate ligands (2-mercaptopropanoic, 3-mercaptopropanoic, and 2-mercaptosuccinic acids). Here we extend the study to some biological important sulfhydryl ligands (see Chart 1), such as cysteine (H_2CYS), penicillamine (H_2PSH), and glutathione (H_3GSH). This and a previous manuscript on S-donor ligands[9] represent the continuation of our speciation study on Hg^{2+}–organic ligand systems, started from the investigation on ligands containing O-donor and N-donor groups only, that is, carboxylic acids, amines, and aminoacids such as glycine, histidine, and aspartic acid.[10]

Cysteine and its derivatives aroused particular interest owing to their involvement in many important biological processes and to be an active site both in the catalytic function of the enzyme cysteine proteases and in several peptides and proteins.[11] It is recognized that CYS can coordinate metal cations through sulfur sites in several proteins and metalloenzymes.[12−14] Both CYS and PSH are clinically used as a chelating agent for detoxification. Glutathione is the most abundant nonprotein thiol in biological systems, with intracellular concentrations of between (0.1 and 10) mmol·kg^{-1} present in microorganisms, fungi, and plant and animal tissues.[15,16] It has a large number of vital functions, including the transport of its constituent amino acids and its action as a cofactor in enzymatic transformations and in protecting cellular membranes from toxic heavy metals.

In this paper we report a potentiometric investigation on the interactions between Hg^{2+} and some biologically important

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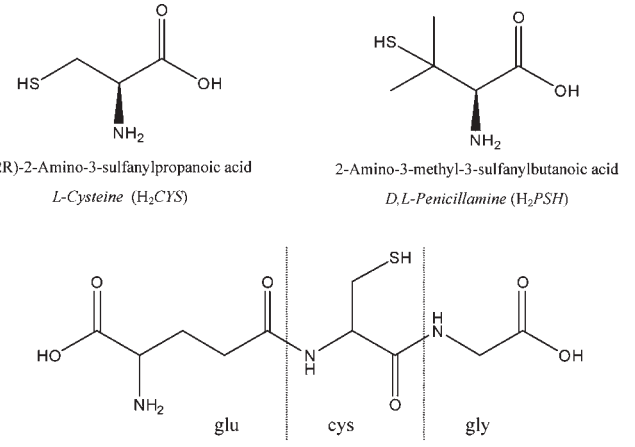
Chart 1



sulfhydryl ligands (Chart 1) such as cysteine (H_2CYS), penicillamine (H_2PSH), and glutathione (H_3GSH). Equilibria were studied at T = 298.15 K, in the ionic strength range (0.1 to 1) mol·kg^{-1}, using as ionic medium NaCl in the presence of iodide (NaI) as a competitive ligand. ^{1}H NMR investigations at I = 0.25 mol·kg^{-1} (NaCl) were carried out to confirm the potentiometric findings and to gain more information about the functional groups involved in the metal−ligand interactions.

EXPERIMENTAL SECTION

Chemicals. Mercury(II) cation was used in the form of chloride salt and was supplied by Riedel-de-Haen Co. The ligands [L-cysteine (H_2CYS), D,L-penicillamine (H_2PSH), and glutathione reduced (H_3GSH)] were supplied by Fluka or Aldrich and used without further purification. Their purity was determined potentiometrically and was always > 99.5 %. Sodium chloride (Aldrich, puriss.) and sodium iodide (Fluka, puriss.) solutions were prepared by weighing the corresponding salts. Sodium chloride was always dried at 110 °C before use. Hydrochloric acid and sodium hydroxide solutions were prepared by diluting concentrated Fluka ampules and standardized against sodium carbonate and potassium hydrogen phthalate, respectively. All solutions were prepared using analytical grade water (R = 18 MΩ), and grade A glassware was always employed.

Apparatus. Potentiometric titrations were carried out at 25.0 ± 0.1 °C using a 809 Metrohm Titrando apparatus (Metrohm Company, Herisau, Switzerland) equipped with a combined Orion glass electrode Ross type 8102. The estimated accuracy was ± 0.20 mV and ± 0.02 mL for emf (electromotive force) and titrant volume readings, respectively. The apparatus was connected to a personal computer, and automatic titrations were performed using the Metrohm TiAMO 1.0 software to control titrant delivery and data acquisition and to check for emf stability. The measurement cells were thermostatted at [298.15(± 0.1) K] by means of water circulation from a thermocryostat (model D1-G Haake).

^{1}H NMR spectra were recorded on a Bruker AMX R-300 spectrometer (Bruker Company, Billerica, MA, U.S.) operating at 300 MHz and T = 298.15 K. The chemical shifts were measured with respect to dioxane, which was used as an internal reference, and converted relative to tetramethylsilane (TMS) using $\delta_{dioxane}$ = 3.70 ppm.

Procedure. For potentiometric investigation on metal−ligand interactions, solutions were prepared dissolving different amounts of ligands [(0.5 to 2) mmol·kg^{-1}] and mercury(II) cation [(0.5 to 1) mmol·kg^{-1}] to obtain a concentration C_{Hg}/C_L (L = CYS^{2-}, PSH^{2-}, GSH^{3-}) ratios ranging from 0.25 to 1. Hydrochloric acid was added to solutions to have the fully protonated form of the ligands. Mixtures of NaCl and NaI (90 % NaCl, 10 % NaI) were used to fix the ionic strength to a predetermined value in the range (0.1 to 1) mol·kg^{-1}. A volume of 25 mL of each solution was titrated with standard sodium hydroxide in the pH range (2.5 to 11). For each experiment, independent titrations of HCl with NaOH standard solutions were performed in the same experimental conditions of temperature and ionic strength as the systems under study to determine the formal electrode potential. The free hydrogen ion concentration scale was used (pH = −log[H$^+$]). Pure nitrogen was bubbled through the solutions in the titration cells to avoid O_2 and CO_2 inside, and the solutions were magnetically stirred.

^{1}H NMR measurements were generally made in a 9:1 H_2O/D_2O solution, at different pH values ranging from 1.5 to 11. The concentrations of Hg^{2+} and of ligands were varied in the range (2 to 6) mmol·kg^{-1}, using different metal ligand ratios [(0.5 to 1) C_{Hg}/C_L]. The individual chemical shifts belonging to the Hg^{2+}−ligand complexes were calculated assuming fast mutual exchange.

Details of potentiometric and ^{1}H NMR measurements are reported in Table 1.

Calculations. The following computer programs were used: (i) BSTAC and STACO[17] to refine all of the parameters of an acid−base titration (such as analytical concentration of reagent and E^0) and to calculate the complex formation constants; (ii) ES4ECI[17] to draw speciation diagrams and to calculate the formation percentage of each species; (iii) LIANA[17] to fit linear and nonlinear equations, for the dependence on ionic strength of formation constants; and (iv) HypNMR[18] to calculate equilibrium constants and the individual chemical shifts of nucleus in each chemical species from the observed chemical shifts measured in the collected NMR spectra.

Formation constant values determined in the ionic strength range (0.1 to 1) mol·kg^{-1} were analyzed by using an extended Debye−Hückel type equation:

$$\log \beta = \log \beta^0 - 0.51 z^* \frac{\sqrt{I}}{1 + 1.5\sqrt{I}} + CI \qquad (1)$$

where $z^* = \Sigma(\text{charges})^2_{\text{reactants}} - \Sigma(\text{charges})^2_{\text{products}}$, β is the formation constant, β^0 is the formation constant at infinite dilution, and C is an empirical parameter.

The sequestering power was evaluated determining the total fraction of metal complexed (X) as a function of pL with pL = −log[L]$_{tot}$ ([L]$_{tot}$ = total ligand concentration).[19] Since this function is a typically sigmoidal curve, which rapidly increases over a relatively small change in concentration, a Boltzmann type equation can be used (with asymptotes of 1 for pL→ ∞ and 0 for pL→ 0):

$$X = \frac{1}{1 + 10^{(pL - pL_{0.5})}} \qquad (2)$$

where $pL_{0.5}$ is an empirical parameter useful for the quantitative evaluation of the sequestering ability of a ligand toward a metal

Table 1. Experimental Details on Potentiometric and ^{1}H NMR Measurements at T = 298.15 K

L	I / mol·kg^{-1}	no. titrations	no. points	C_{Hg} / mmol·kg^{-1}	C_L / mmol·kg^{-1}	pH
			Potentiometric Measurements			
CYS^{2-}	0.25−1	12	960	0.5−1	0.5−2	2.5−11
PSH^{2-}	0.25−1	12	960	0.5−1	0.5−2	2.5−11
GSH^{3-}	0.1−1	12	960	0.5	0.5−2	2.5−11
			^{1}H NMR Measurements			
CYS^{2-}	0.25	3	30	2−2.5	3−5	1.5−11
PSH^{2-}	0.25	3	30	0	5−10	1.5−10
	0.25	4	40	2−2.5	3.5−6	1.5−11
GSH^{3-}	0.25	1	10	0	5	1.5−10
	0.25	3	30	2−2.5	4−6	2−11

cation and numerically represents the ligand concentration necessary to sequester a 0.5 metal ion fraction.

RESULTS AND DISCUSSION

Complexes of Hg^{2+} with Sulfur-Containing Ligands. In some recent papers[9,20] concerning the study of strong metal−ligand interactions, we used mixtures of $NaNO_3$ and NaI (90 % $NaNO_3$ + 10 % NaI) as ionic medium. The choice was due to the necessity to have a competitive ligand (iodide ion) for the metal ion complexation. This method was successfully applied to the study of the interactions between different S-donor ligands and Hg^{2+} or CH_3Hg^+ metal cations, so for typical soft−soft interactions. The same experimental method is here applied to the study of Hg^{2+}−CYS^{2-}, −PSH^{2-}, and −GSH^{3-} interactions, using a mixture of NaCl and NaI (90 % NaCl, 10 % NaI) as the ionic medium. Metal−ligand complexes were determined taking always into account the hydrolysis of Hg^{2+}, the Hg^{2+}−Cl^- and −I^- complex formation, and the ligand protonations under the same ionic strength and temperature conditions. Literature data on these systems are shown in Table 2.

Potentiometric titrations in the conditions reported in the Experimental Section evidenced the formation of HgL^{2-z}, $HgLH^{3-z}$, $HgLH_2^{4-z}$, HgL_2^{2-2z}, HgL_2H^{3-2z}, and $HgL_2H_2^{4-2z}$ species (L^{z-} = CYS^{2-}, PSH^{2-}, or GSH^{3-}), together with $HgL_2H_3^-$ for GSH^{3-} only. Formation constant values determined at different ionic strengths in the range (0.25 to 1) mol·kg^{-1} [or (0.1 to 1) mol·kg^{-1} for GSH^{3-}] are reported in Table 3. As obtained for other Hg^{2+}−S donor ligand systems, formation constant values are very high with, as an example, log β = 34.54, 34.24, and 32.05 for $Hg(CYS)^0$, $Hg(PSH)^0$, and $Hg(GSH)^-$ species, respectively (at I = 0.25 mol·kg^{-1}, T = 298.15 K).

Formation constant values can be converted from molal (m/mol·kg^{-1}) to molar (c/mol·L^{-1}) concentration scales by using the equation: $c/m(\text{mix}) = c/m(\text{NaCl})\ X_{NaCl} + c/m(\text{NaI})\ X_{NaI}$, where X is the mole fraction of salt, $c/m(\text{NaCl}) = 0.99987 - 0.017765c - 0.0006525c^2$, $c/m(\text{NaI}) = 0.99987 - 0.036c - 0.00061c^2$. Data in the molar concentration scale are reported as Supporting Information.

The pH metric determination of formation constants is based on the competition between the proton and the ligand and, therefore, when formation constants are very high, presents some experimental difficulties due to the total displacement of the proton. For this reason, it was necessary to confirm the speciation models using a different procedure or a different experimental technique. In addition to pH metric measurements, ^{1}H NMR titrations of Hg^{2+}/ligand aqueous solutions were carried out. A considerable number of spectra were collected by varying, in the pH range between 1.5 and 11, both the stoichiometric ratio between Hg^{2+} and the ligands, as well as the precursor concentrations. The chemical shifts measured in the collected NMR spectra allowed us to calculate by the HypNMR software

Table 2. Formation Constant Values (log β) for Hg^2−OH^-, −Cl^-, and −I^- Species and for the Ligand Protonation, in NaCl at Different Ionic Strengths and at T = 298.15 K

reaction	I/mol·kg^{-1} 0.10	0.25	0.51	1.02	ref
$Hg^{2+} + H_2O = Hg(OH)^+ + H^+$	−3.60	−3.63	−3.61	−3.55	27
$Hg^{2+} + 2H_2O = Hg(OH)_2^0 + 2H^+$	−6.34	−6.35	−6.32	−6.18	
$Hg^{2+} + 3H_2O = Hg(OH)_3^- + 3H^+$	−21.09	−21.18	−21.27	−21.45	
$2Hg^{2+} + H_2O = Hg_2(OH)^{3+} + H^+$	−3.58	−3.10	−3.09	−3.14	
$Hg^{2+} + Cl^- = HgCl^+$	6.82	6.77	6.77	6.91	27
$Hg^{2+} + 2Cl^- = HgCl_2^0$	13.36	13.29	13.30	13.48	
$Hg^{2+} + 3Cl^- = HgCl_3^-$	14.43	14.35	14.33	14.47	
$Hg^{2+} + 4Cl^- = HgCl_4^{2-}$	15.05	15.01	15.06	15.28	
$Hg^{2+} + Cl^- + H_2O = HgCl(OH)^0 + H^+$	3.68	3.64	3.68	3.88	
$Hg^{2+} + I^- = HgI^+$	13.03	12.92	12.86	12.81	9
$Hg^{2+} + 2I^- = HgI_2^0$	24.07	23.90	23.81	23.72	
$Hg^{2+} + 3I^- = HgI_3^-$	27.84	27.68	27.58	27.49	
$Hg^{2+} + 4I^- = HgI_4^{2-}$	29.98	29.87	29.81	29.74	
$H^+ + CYS^{2-} = H(CYS)^-$	10.46	10.34	10.27	10.25	*a*
$2H^+ + CYS^{2-} = H_2(CYS)^0$	18.78	18.61	18.53	18.54	
$3H^+ + CYS^{2-} = H_3(CYS)^+$	21.03	20.82	20.66	20.51	
$H^+ + PSH^{2-} = H(PSH)^-$	10.40	10.36	10.33	10.41	*b*
$2H^+ + PSH^{2-} = H_2(PSH)^0$	18.21	18.15	18.18	18.37	
$3H^+ + PSH^{2-} = H_3(PSH)^+$	19.99	19.91	20.06	20.41	
$H^+ + GSH^{3-} = H(GSH)^{2-}$	9.50	9.37	9.29	9.29	28
$2H^+ + GSH^{3-} = H_2(GSH)^-$	18.20	17.96	17.84	17.86	
$3H^+ + GSH^{3-} = H_3(GSH)^0$	21.75	21.46	21.29	21.35	
$4H^+ + GSH^{3-} = H_4(GSH)^+$	23.93	23.62	23.47	23.58	

[a] Recalculated from data of ref 29. [b] Unpublished data from this laboratory.

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Table 3. Experimental Values of Formation Constants for $Hg^{2+}-CYS^{2-}$, $-PSH^{2-}$, and $-GSH^{3-}$ Complexes at Different Ionic Strengths (90 % NaCl, 10 % NaI) and at T = 298.15 K

	I	$\log \beta_{pq}{}^{a,b}$						
L	mol·kg^{-1}	10	11	12	20	21	22	23
CYS^{2-}	0.25	34.54(2)	42.47(2)	45.40(2)	43.41(4)	52.35(3)	60.19(2)	
	0.51	34.27(9)	42.24(4)	45.38(4)	43.40(6)	52.24(5)	60.07(3)	
	1.02	33.99(7)	41.63(9)	45.53(4)	43.23(5)	52.15(4)	60.04(2)	
PSH^{2-}	0.25	34.24(2)	41.55(2)	43.79(2)	43.51(3)	51.99(2)	59.08(1)	
	0.51	34.12(2)	41.49(2)	43.77(2)	43.43(2)	51.92(1)	59.05(1)	
	1.02	34.26(3)	41.71(2)	43.91(2)	43.30(3)	51.88(2)	59.20(1)	
GSH^{3-}	0.10	32.31(8)	39.64(6)	43.40(5)	40.67(7)	50.16(7)	59.00(5)	63.11(4)
	0.25	32.05(8)	39.75(6)	43.20(5)	40.36(6)	49.70(6)	58.44(5)	62.48(4)
	0.51	31.97(8)	39.67(6)	43.26(5)	40.07(5)	49.33(5)	58.17(3)	62.25(4)
	1.02	32.05(9)	40.17(9)	43.67(8)	39.85(8)	49.23(8)	58.19(7)	62.38(8)

[a] β_{pq} refer to the reaction: $Hg^{2+} + pL^{z-} + qH^{+} = HgL_pH_q^{(2+q-pz)}$. [b] Least-squares errors on last significant figure are shown in parentheses.

Table 4. Formation Constants[a] of $Hg^{2+}-CYS^{2-}$ and $-PSH^{2-}$ Complexes Obtained by ^{1}H NMR Measurements, at I = 0.25 mol·kg^{-1} (NaCl) and T = 298.15 K

		$\log \beta_{pq}{}^{a}$	
L	pq	ISE-H^+	^{1}H NMR
CYS^{2-}	10	34.54	(34.54)[b]
	11	42.47	41.75(5)[c]
	12	45.40	45.40(1)
	20	43.41	42.8(1)
	21	52.35	52.08(4)
	22	60.19	59.9(5)
PSH^{2-}	10	34.24	(34.24)[b]
	11	41.55	41.3(5)
	12	43.79	43.8(4)
	20	43.51	(43.5)[b]
	21	51.99	52.3(1)
	22	59.08	59.5(2)

[a] β_{pq} refer to the reaction: $Hg^{2+} + pL^{z-} + qH^{+} = HgL_pH_q^{(2+q-pz)}$. [b] Obtained by potentiometry. [c] Least-squares errors on the last significant figure are shown in parentheses.

program[18] the formation constants of the species and the chemical shift values for each individual complex. Moreover, the program can recalculate the average chemical shift at each experimental pH. For $Hg^{2+}-CYS^{2-}$ and $-PSH^{2-}$ systems, results confirmed the speciation model with the formation of HgL^0, $HgLH^+$, $HgLH_2^{2+}$, HgL_2^{2-}, HgL_2H^-, and $HgL_2H_2^0$ species. Quantitative results on $Hg^{2+}-GSH^{3-}$ system were affected by high error values and therefore are not reported. Formation constant values of $Hg^{2+}-CYS^{2-}$ and $-PSH^{2-}$ species obtained by ^{1}H NMR spectroscopy are reported in Table 4, together with those obtained at the same ionic strength conditions by potentiometry. The formation of $Hg(CYS)^0$, $Hg(PSH)^0$, and $Hg(CYS)_2^{2-}$ species was taken into account in the calculations, but using stability values determined by potentiometry. For other species, the two sets of values (Table 4) are comparable, confirming the magnitude of these interactions. Moreover, the excellent agreement between calculated and observed chemical shifts, shown as an example for methyl group

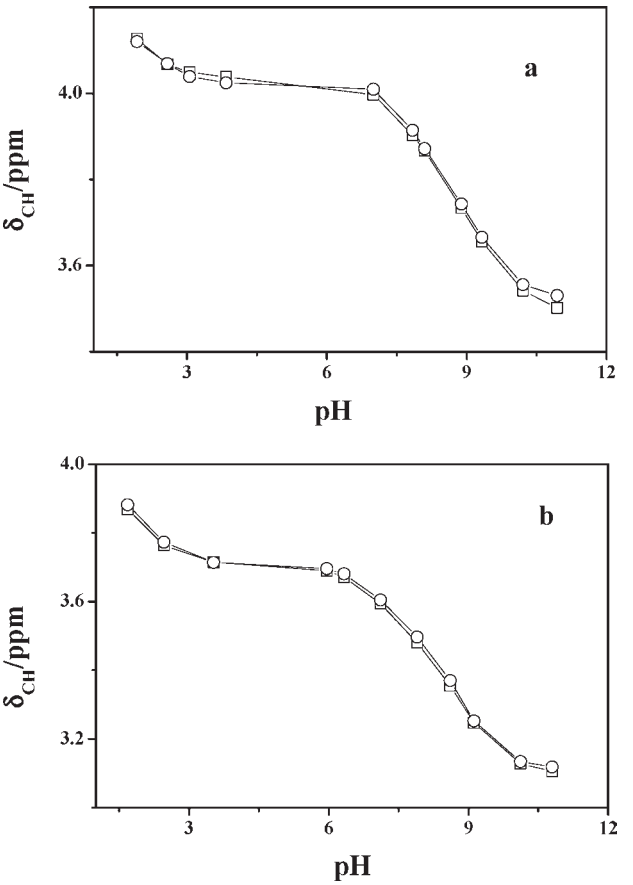

Figure 1. Observed (□) and calculated (○) chemical shifts vs pH of CH in (a) $Hg^{2+}-CYS^{2-}$ and (b) $Hg^{2+}-PSH^{2-}$ mixtures.

of CYS^{2-} and PSH^{2-} reported in Figure 1, allowed us to conclude that spectroscopic findings are fully consistent with the model used for interpretating potentiometric experiments.

The distribution and relevance of species are shown in Figure 2, where speciation diagrams of $Hg^{2+}-CYS^{2-}$, $-PSH^{2-}$, and $-GSH^{3-}$ systems are reported at I = 0.25 mol·kg^{-1} and T = 298.15 K, as an example. The diagrams evidence the competitive action of the iodide in the metal ion complexation, with

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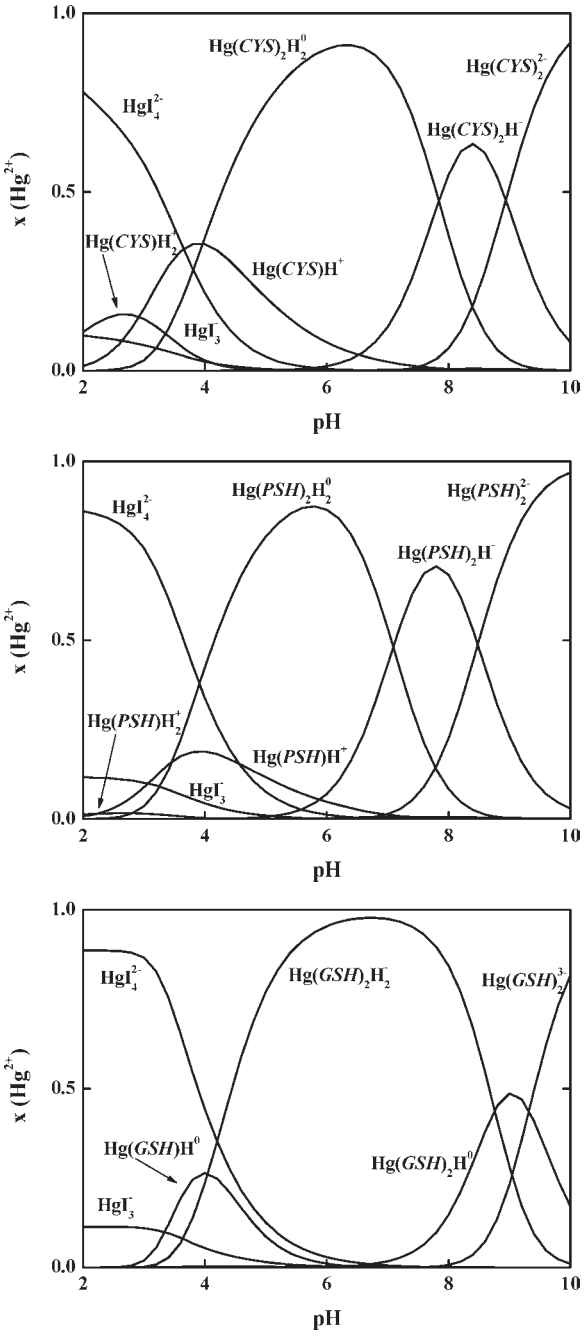

Figure 2. Mole fraction of Hg^{2+} (x) for Hg^{2+}−CYS^{2-}, −PSH^{2-}, and −GSH^{3-} species, at I = 0.25 mol·kg^{-1} (90 % NaCl, 10 % NaI) and at T = 298.15 K. Experimental conditions: C_{Hg} = 1 mmol·kg^{-1}; C_L = 2 mmol·kg^{-1}.

coexistence, at acidic pH values (pH < 4), of Hg^{2+}−I^- and Hg^{2+}−S containing ligand species. At pH > 4, most of the metal fraction is complexed by sulfur donor ligand with a very similar behavior of the three ligands, in particular of CYS^{2-} and PSH^2. At pH ∼ 6, the predominant species is the protonated $HgL_2H_2^{4-2z}$, with ∼0.9 of the metal fraction present under this form. At pH ∼ 8, the HgL_2H^{3-2z} species becomes predominant, while the deprotonated HgL_2^{2-2z} reaches the maximum of metal fraction in the alkaline range (pH = 10). In all systems in the experimental conditions considered (i.e., C_{Hg} = 1 mmol·kg^{-1}, C_L = 2 mmol·kg^{-1}), the formation of HgL^{2-z} species is negligible.

^{1}H NMR Spectra. All of the ^{1}H NMR data collected for the systems CYS^{2-}, PSH^{2-}, and GSH^{3-}, regardless of the presence of Hg^{2+}, show, as a common feature, the presence of single resonances, thus indicating that all of the species at equilibria are involved in a rapid exchange on the NMR time scale; as a consequence the signals of bound and free ligands cannot be directly detected from the spectra. Furthermore, for CYS^{2-} and PSH^{2-} containing solutions, upon pH increasing, an upfield chemical shift of all of the peaks occurred.

In more detail, both in CYS^{2-} and PSH^{2-} ^{1}H NMR solution spectra, the methyl group appears as a single sharp signal shifting from (3.9 to 3.2) ppm, and from (4.1 to 3.2) ppm, respectively, depending on the pH of the solution under study. While the presence of Hg^{2+} does not change to a great extent the chemical shift of CH in PSH^{2-} solutions in the whole pH range, for CYS^{2-} solutions Hg^{2+} imparts a higher shift of the above signal at pH < 8. From the comparison of the spectra of CYS^{2-} and Hg^{2+}/CYS^{2-} solutions it can be observed that the presence of Hg^{2+} causes a significant shift of the resonance the methylene group of CYS^{2-} in the whole investigated pH range, suggesting that this group, near to −SH, is much more influenced by the metal. Furthermore, the spectra of all of the Hg^{2+}/CYS^{2-} solutions, regardless of the ratio and the concentrations employed, show that the CH_2 signal does not change significantly up to pH = 6; then it steadily upfield shifts up to pH = 9.

By comparing the methyl groups in the spectra of PSH^{2-} and Hg^{2+}/PSH^{2-} samples (see Figure 3), it appears that the presence of Hg^{2+} influences the two CH_3 chemical shifts quite differently. In particular, one of the CH_3 groups, regardless of the Hg^{2+}/PSH^{2-} ratio employed, is featured by a chemical shift considerably higher than the corresponding signal in the Hg^{2+}−free solution up to pH = 8. From pH = 8 up to ∼10 all of the resonances due to this methyl are almost equal, that is, both in the PSH^{2-} and Hg^{2+}/PSH^{2-} solutions. On the contrary, the signal due to the other methyl group in the presence of Hg^{2+} is very different with respect to the one observed for the solutions with PSH^{2-} only, in the whole pH range, thus indicating that mercury somewhat exerts a significant influence on this CH_3.

As already stated, the spectra collected for GSH^{3-} and Hg^{2+}/GSH^{3-} solutions show a single peak for each kind of proton so that, although different species are present in solution, as pointed out by the speciation model, direct measurements of individual NMR parameters cannot be carried out. Chart 1 shows the glutathione fragments, that is, glutamic acid (Glu), cysteine (Cys), and glycine (Gly). As far as the resonances of the Glu fragment are concerned, both GluHα and GluHβ signals display a comparable chemical shift regardless of the presence of Hg^{2+} up to pH ∼ 6. As an example, ^{1}H NMR spectra at pH ∼ 2 are reported in Figure 4. The situation dramatically changes starting from pH ∼ 6 as for GSH^{3-} solutions the peaks due to the above groups shift upfield, indicating the deprotonation of ammonium moiety; on the contrary in the Hg^{2+}-containing samples GluHα and GluHβ resonances steadily downfield shift, strongly suggesting the Glu fragment coordination toward metal. The comparison of the GluHγ signals of GSH^{3-} and Hg^{2+}/GSH^{3-} solutions shows that the presence of Hg^{2+} influences the shift of the resonance the methylene group of GSH^{3-} in the whole investigated pH range; this evidence may be ascribed to the proximity of this CH_2 to the amide moiety of the adjacent Cys fragment. For GlyHα resonance all of the spectra, that is, GSH^{3-} and

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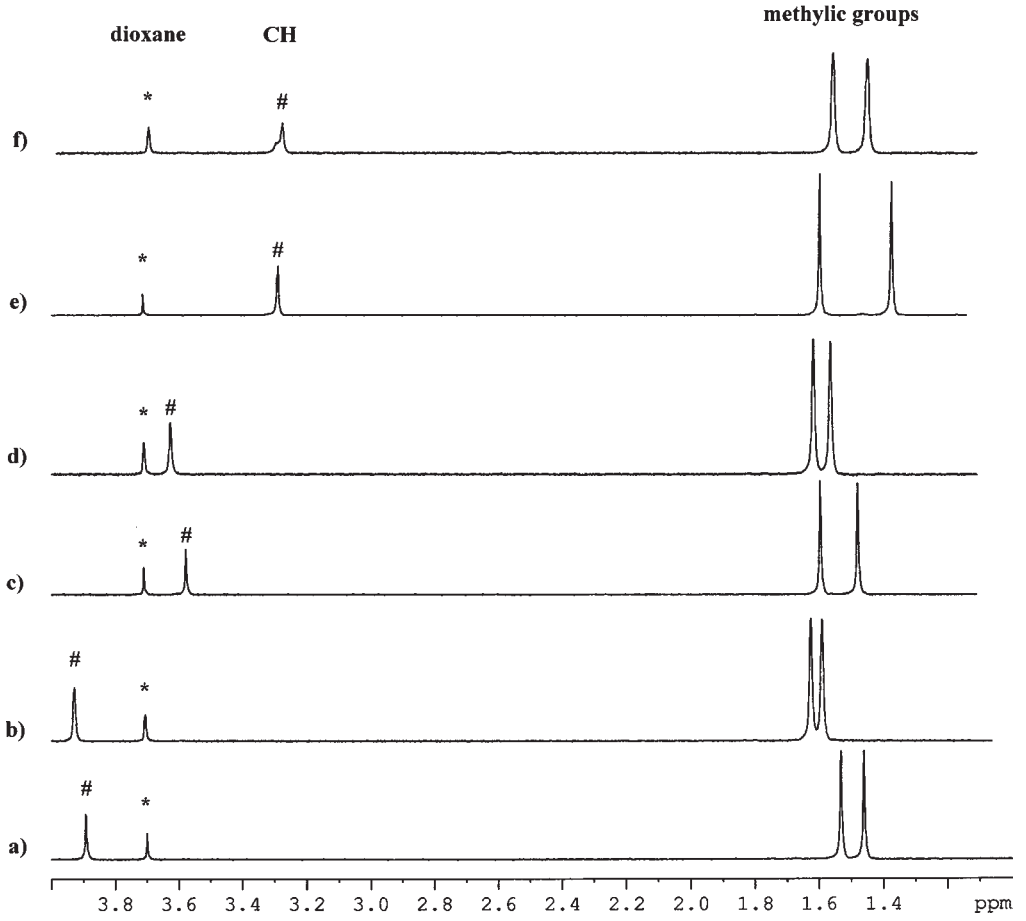

Figure 3. ^{1}H NMR spectra of PSH^{2-} and Hg^{2+}−PSH^{2-} solutions at selected pH: (a) C_{PSH} = 5 mmol·kg^{-1}, pH = 1.42; (b) C_{PSH} = 5 mmol·kg^{-1}, C_{Hg} = 2.5 mmol·kg^{-1}, pH = 1.43; (c) C_{PSH} = 5 mmol·kg^{-1}, pH = 7.62; (d) C_{PSH} = 5 mmol·kg^{-1}, C_{Hg} = 2.5 mmol·kg^{-1}, pH = 7.67; (e) C_{PSH} = 5 mmol·kg^{-1}, pH = 9.76; (f) C_{PSH} = 5 mmol·kg^{-1}, C_{Hg} = 2.5 mmol·kg^{-1}, pH = 9.75.

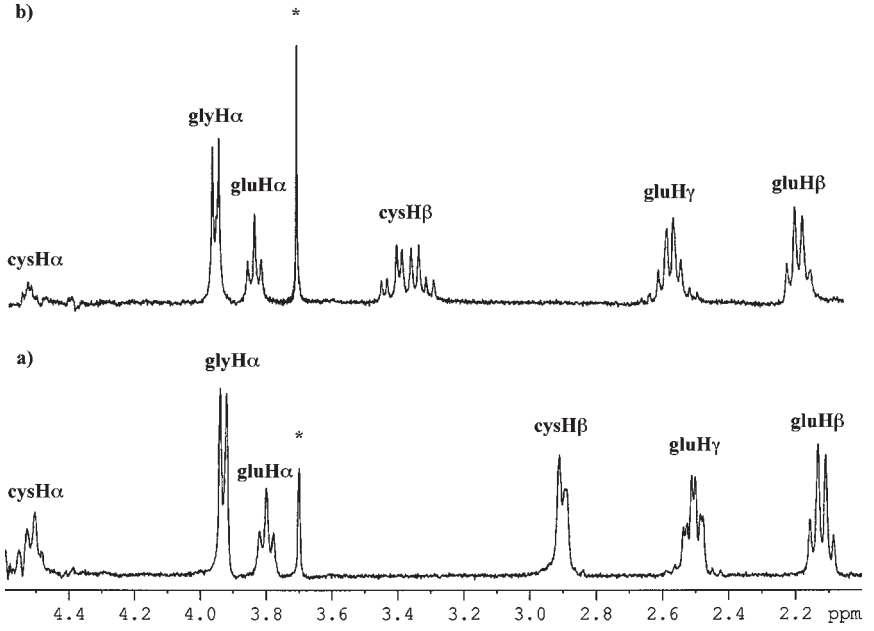

Figure 4. ^{1}H NMR spectra of GSH^{3-} and Hg^{2+}−GSH^{3-} solutions: (a) C_{GSH} = 5 mmol·kg^{-1}, pH = 2.27; (b) C_{GSH} = 5 mmol·kg^{-1}, C_{Hg} = 2.5 mmol·kg^{-1}, pH = 2.24 (* = dioxane signal).

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Table 5. Formation Constants for Hg^{2+}−CYS^{2-}, −PSH^{2-}, and −GSH^{3-} Complexes at T = 298.15 K and I = 0 mol·kg^{-1}, together with the Empirical Parameter C (eq 1) for the Dependence on Ionic Strength

reaction	log β^0	C
$H^+ + CYS^{2-} = H(CYS)^-$	10.87(3)[a]	0.20(5)[a]
$2H^+ + CYS^{2-} = H_2(CYS)^0$	19.38(5)	0.39(16)
$3H^+ + CYS^{2-} = H_3(CYS)^+$	21.67(8)	0.07(15)
$Hg^{2+} + CYS^{2-} = Hg(CYS)^0$	35.73(3)	−0.10(3)
$Hg^{2+} + CYS^{2-} + H^+ = Hg(CYS)H^+$	43.83(8)	−0.53(11)
$Hg^{2+} + CYS^{2-} + 2H^+ = Hg(CYS)H_2^{2+}$	46.12(4)	0.62(3)
$Hg^{2+} + 2CYS^{2-} = Hg(CYS)_2^{2-}$	44.55(9)	0.33(11)
$Hg^{2+} + 2CYS^{2-} + H^+ = Hg(CYS)_2H^-$	53.97(8)	0.64(9)
$Hg^{2+} + 2CYS^{2-} + 2H^+ = Hg(CYS)_2H_2^0$	62.04(8)	0.86(9)
$H^+ + PSH^{2-} = H(PSH)^-$	10.89[b]	0.31(8)
$2H^+ + PSH^{2-} = H_2(PSH)^0$	18.93[b]	0.63(9)
$3H^+ + PSH^{2-} = H_3(PSH)^+$	20.69[b]	0.88(9)
$Hg^{2+} + PSH^{2-} = Hg(PSH)^0$	35.22(5)	0.66(6)
$Hg^{2+} + PSH^{2-} + H^+ = Hg(PSH)H^+$	42.50(5)	0.83(6)
$Hg^{2+} + PSH^{2-} + 2H^+ = Hg(PSH)H_2^{2+}$	44.51(2)	0.62(3)
$Hg^{2+} + 2PSH^{2-} = Hg(PSH)_2^{2-}$	44.63(5)	0.31(7)
$Hg^{2+} + 2PSH^{2-} + H^+ = Hg(PSH)_2H^-$	53.59(6)	0.74(8)
$Hg^{2+} + 2PSH^{2-} + 2H^+ = Hg(PSH)_2H_2^0$	60.84(5)	1.21(7)
$Hg^{2+} + GSH^{3-} = Hg(GSH)^-$	33.54(5)	0.97(8)
$Hg^{2+} + GSH^{3-} + H^+ = Hg(GSH)H^0$	41.00(6)	2.07(9)
$Hg^{2+} + GSH^{3-} + 2H^+ = Hg(GSH)H_2^+$	44.78(6)	1.75(8)
$Hg^{2+} + 2GSH^{3-} = Hg(GSH)_2^{4-}$	41.33(6)	−0.27(9)
$Hg^{2+} + 2GSH^{3-} + H^+ = Hg(GSH)_2H^{3-}$	51.63(6)	0.44(8)
$Hg^{2+} + 2GSH^{3-} + 2H^+ = Hg(GSH)_2H_2^{2-}$	61.07(2)	1.19(3)
$Hg^{2+} + 2GSH^{3-} + 3H^+ = Hg(GSH)_2H_3^-$	65.58(4)	1.69(6)

[a] Least-squares errors on the last significant figure are shown in parentheses. [b] Unpublished data from this laboratory.

Hg^{2+}/GSH^{3-} solutions, show peaks characterized by a comparable chemical shift, suggesting that Hg^{2+} in solution does not interact with −COOH of the Gly fragment. As far as the two Cys resonances of GSH^{3-} are concerned, namely, CysHα and CysHβ, both series of signals are deshielded by the presence of Hg^{2+}. In particular, from the spectra it can be observed that a considerable shift of CysHβ peaks at higher parts per million occurred in the metal-containing solutions in the entire pH range investigated. On the basis of the spectra collected for the Hg^{2+}/GSH^{3-} system, some conclusions can be suggested: the functional group of GSH^{3-} much more involved in the Hg^{2+} coordination, as expected, on the whole pH range studied is Cys, and the hypothesis of the coordination both via peptide and thiolate groups of Cys moiety is supported by the evidence that the resonances CysHα, CysHβ, and GluHγ are those more shifted by the presence of the metal.[21] Furthermore, from pH ∼ 6 on, probably Glu moiety is involved in coordination as well; that is, starting from pH = 6 the GluHα and GluHβ signals shift with respect to the resonances of Hg^{2+}-free solutions.

Dependence on Ionic Strength. By using eq 1 and formation constants determined in the ionic strength range (0.1 to 1) mol·kg^{-1}, formation constants extrapolated at infinite dilution were calculated. The values are reported in Table 5, together with the empirical parameter C. The effect of ionic strength on metal speciation is shown in Figure 5, where as an example the distribution of Hg^{2+}−CYS^{2-} species in NaCl at two different ionic strength values, I = 0.25 and I = 1 mol·kg^{-1}, is reported. The variation of ionic strength affects the distribution of metal in the acidic (2 to 6) pH range. In particular at pH ∼ 4 the fraction of Hg^{2+} present as $Hg(CYS)H^+$ species varies from 0.6 to 0.2, increasing the ionic strength from (0.25 to 1) mol·kg^{-1}.

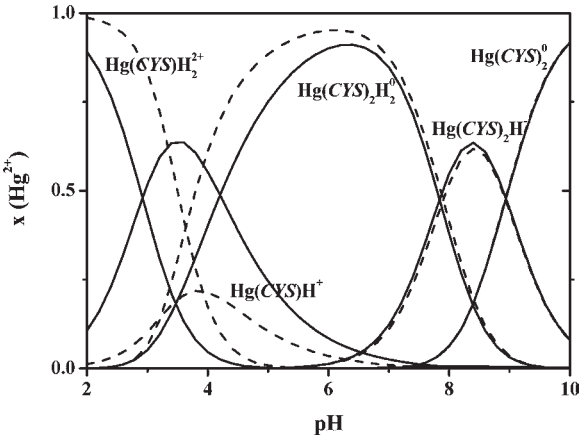

Figure 5. Mole fraction of Hg^{2+} (x) for the Hg^{2+}−CYS^{2-} species in NaCl at I = 0.25 mol·kg^{-1} (full lines) and at I = 1 mol·kg^{-1} (dotted lines) and at T = 298.15 K. Experimental conditions: C_{Hg} = 1 mmol·kg^{-1}; C_{CYS} = 2 mmol·kg^{-1}.

Table 6. $pL_{0.5}$ Values for Hg^{2+}−CYS^{2-}, −PSH^{2-}, and −GSH^{3-} Systems, in Different Experimental Conditions at T = 298.15 K

		I	
L	pH	mol·kg^{-1}	$pL_{0.5}$
CYS^{2-}	5	0.25	24.32
	5	1	24.02
	8	0.25	23.11
	8	1	21.62
PSH^{2-}	5	0.25	24.20
	5	1	24.12
	8	0.25	22.11
	8	1	21.87
GSH^{3-}	5	0.25	22.89
	5	1	23.44
	8	0.25	20.46
	8	1	20.64

Sequestering Ability. The knowledge of Hg^{2+}−CYS^{2-}, −PSH^{2-}, and −GSH^{3-} complex formation constants is essential to evaluate the sequestering power of these ligands toward Hg^{2+} and therefore their use for removing Hg^{2+} from biological or natural systems. The sequestering power was evaluated by using eq 2 and complex formation constants for Hg^{2+}−CYS^{2-}, −PSH^{2-}, and −GSH^{3-} systems. Calculated values of $pL_{0.5}$, that is, the ligand concentration necessary to sequester 0.5 metal ion fraction, are reported in Table 6 in different conditions. We choose, as an example, two different pH values (pH = 5 and 8) and two different ionic strength values [I = (0.25 and 1) mol·kg^{-1}] to evaluate the effect of these parameters on sequestering ability. In all experimental conditions, sequestering power is very high with $pL_{0.5}$ that varies from 20.46 for GSH^{3-} (at pH = 8

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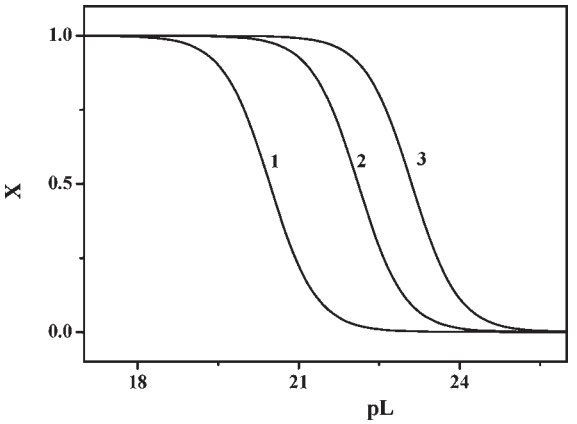

Figure 6. Sequestration diagram for the Hg^{2+}−sulfur containing ligand systems vs pL in NaCl at I = 0.25 mol·kg^{-1}, pH = 8, and T = 298.15 K. (1) GSH^{3-}; (2) PSH^{2-}; (3) CYS^{2-}. X = total fraction of metal complexed (eq 2).

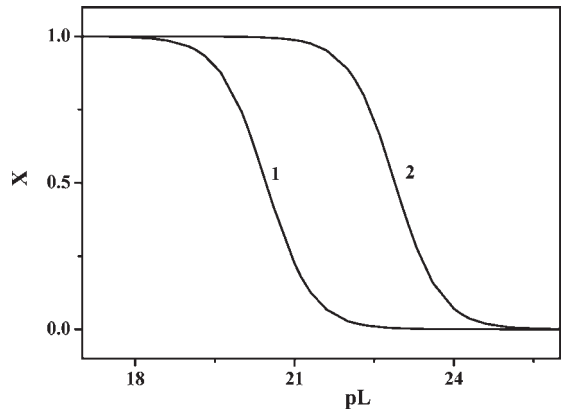

Figure 7. Sequestration diagram for the Hg^{2+}−GSH^{3-} system vs pL in NaCl at (1) pH = 8 and (2) pH = 5, I = 0.25 mol·kg^{-1}, and T = 298.15 K. X = total fraction of metal complexed (eq 2).

and I = 0.25 mol·kg^{-1}) to 24.32 for CYS^{2-} at pH = 5 and I = 0.25 mol·kg^{-1}. Figure 6 shows the total fraction of Hg^{2+} complexed by CYS^{2-}, PSH^{2-}, and $-GSH^{3-}$ ligands at pH = 8, I = 0.25 mol·kg^{-1}, and T = 298.15 K. In these conditions, the sequestering power follows the trend CYS^{2-} > PSH^{2-} > GSH^{3-} with $pL_{0.5}$ = 23.11, 22.11, and 20.46, respectively. By decreasing pH values from 8 to 5, consistent variations were obtained. As an example, in Figure 7 the total metal fraction complexed versus pL for the Hg^{2+}−GSH^{3-} system is reported, at the two different pH values. As can be observed, by decreasing the pH from 8 to 5, an higher sequestering ability was obtained with $pL_{0.5}$ values that varies from 20.64 (at pH = 8) to 22.89 (at pH = 5). The same trend was obtained for Hg^{2+}−CYS^{2-} and Hg^{2+}−PSH^{2-} systems (see Table 6). Less significant is the effect of ionic strength on sequestering ability, with different trends for Hg^{2+}−CYS^{2-} and $-PSH^{2-}$ with respect to Hg^{2+}−GSH^{3-} systems. In Figure 8 the different sequestering power of GSH^{3-} at two different ionic strengths [I = (0.25 and 1) mol·kg^{-1}] is evidenced, with $pL_{0.5}$ values that weakly increase from 20.46 to 20.64 increasing ionic strength. The opposite trend can be observed for CYS^{2-} and PSH^{2-} ligands, whose sequestering power decreases when ionic strength increases.

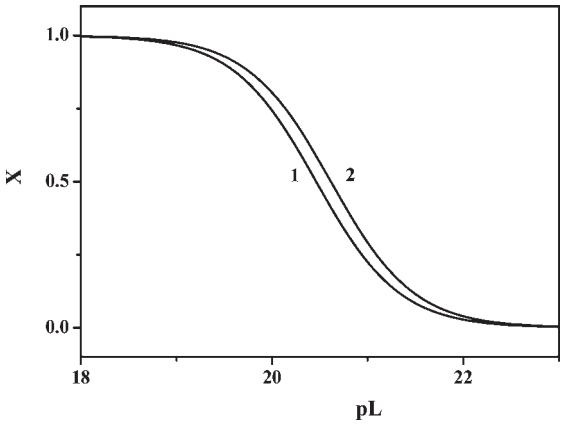

Figure 8. Sequestration diagram for the Hg^{2+}−GSH^{3-} system vs pL in NaCl at (1) I = 0.25 mol·kg^{-1} and (2) I = 1 mol·kg^{-1}, pH = 8, and T = 298.15 K. X = total fraction of metal complexed (eq 2).

Literature Comparisons. Despite the biological and environmental importance of the Hg^{2+}−thiol containing compound interactions, very few studies are reported in the literature regarding the nature and magnitude of the complexes so formed. Literature formation constant values on Hg^{2+}−CYS^{2-}, $-PSH^{2-}$, and $-GSH^{3-}$ systems are resumed in Table 7. The first comment in Table 7 concerns the scarceness of data for all systems, in particular for glutathione. In addition, enormous discrepancies can be observed among different speciation models proposed. As an example, by considering the Hg^{2+}−CYS^{2-} system, some authors report the formation of $Hg(CYS)$[22,23] or $Hg(CYS)_2$ species[24] only, others the formation of $Hg(CYS)_2$, $Hg(CYS)_2H$, and $Hg(CYS)_2H_2$,[25] and others the formation of $Hg(CYS)_2H$ and $Hg(CYS)_2H_2$,[26] with very different formation constant values: that is, for $Hg(CYS)^0$ species log β ranges from 14.21 to 20.5. Differences are even bigger for $Hg(PSH)^0$ species with log β values that range from 17.5 to 38.3. The extreme variability of data can be certainly attributable to the differences in the procedure followed for the determination of too high formation constant values.

A comparison can be made with stability of Hg^{2+}−mercaptocarboxylate species.[9] Formation constant values are very similar with, as an example, log β (at I = 0.1 mol·L^{-1}, T = 298.15 K) for the HgL^{2-z} species = 34.17, 32.10, and 35.10, for 2-mercaptopropanoic, 3-mercaptopropanoic, and 2-mercaptosuccinic acids, respectively.

FINAL REMARKS

A study on Hg^{2+}−thiolic ligand interactions evidenced for different systems quite similar speciation models, with the formation of HgL^{2-z}, $HgLH^{3-z}$, $HgLH_2^{4-z}$, HgL_2^{2-2z}, HgL_2H^{3-2z}, and $HgL_2H_2^{4-2z}$ species (L^{z-} = CYS^{2-}, PSH^{2-}, GSH^{3-}) and of $HgL_2H_3^-$ for GSH^{3-} only. High values of formation constant were obtained, and all ligands showed a high sequestering ability toward Hg^{2+}; that is, less than a picomolar ligand concentration is sufficient to sequestrate 0.5 metal fraction, at the different pH and ionic strength conditions considered. The sequestering ability is comparable with those obtained for mercaptocarboxylic acids,[9] while it is much higher with respect to those obtained for carboxylic acids, amines, and aminoacids such as glycine.[10] As an example, for 2-mercaptosuccinic acid we obtained $pL_{0.5}$ = 23.03,[9] and for succinic acid,

Table 7. Literature Values of $Hg^{2+}-CYS^{2-}$, $-PSH^{2-}$, and $-GSH^{3-}$ Complex Formation Constants, at T = 298.15 K

	I	log β							
	mol·L^{-1}	ML	MLH	ML_2H	ML_2	ML_2H	ML_2H_2	other species	ref
PSH^{2-}	0.1	18.8			24.9	27.02			
	0.5 $NaClO_4$						52.03	ML_3H_3: 72.43	30
	0.1 $NaClO_4$	38.3			44.4				31
	0.1 KNO_3	16.15							22
	0.15 KNO_3	17.5			23.50				32
CYS^2	0.025[a]	20.5							23
	0.1 KNO_3				43.57	54.37	61.79		25
	0.1 KNO_3	14.21							22
	1.0 KNO_3				41.80				24
	0.1 $NaNO_3$					8.94	16.31		26
GSH^{3-}	0.1 NaCl	26.04	32.49	35.68	33.40	42.40	52.29	ML_2H_3: 55.28; ML(OH): 15.80	33
	1 KNO_3				41.58				25

[a] T = 293.15 K.

ethylenediamine, and glycine we obtained $pL_{0.5}$ = 1.56, 6.16, and 3.88, respectively (at pH = 8, I = 0.1 mol·kg^{-1}, and T = 298.15 K).[10]

The difficulty associated with the experimental determination of very high values of formation constants is probably the cause of the extreme variability of literature data. Therefore, to validate our speciation models, two independent techniques (potentiometry and ^{1}H NMR spectroscopy) were employed. Potentiometric measurements were performed in the presence of a competitive ligand (iodide) able to compete in the metal ion complexation. ^{1}H NMR spectroscopy fully supported the chemical model proposed for $Hg^{2+}-CYS^{2-}$ and $-PSH^{2-}$ systems, providing fairly similar formation constant values to those obtained by potentiometry.

The contribution to free energy for the different donor groups (carboxylic, amino, and thiolic) is in agreement with mean values reported in previous work on Hg^{2+}−mercaptocarboxylate interactions.[9] By considering HgL^{2-z} species for CYS^{2-}, PSH^{2-}, and GSH^{3-} altogether, the resulting mean value of free energy is -193 ± 8 (at I = 0.1 mol·kg^{-1}). By subtracting the carboxylate and the amino groups contributions [approximately equal to (-22 and -57) kJ·mol^{-1}, respectively],[9] the contribution of thiolate group is -114 ± 10 kJ·mol^{-1}. This value is of the same order of magnitude with respect to -164 ± 9 kJ·mol^{-1} previously reported.[9]

■ ASSOCIATED CONTENT

Supporting Information. Formation constant values (Tables 1S, 2S, and 3S). This material is available free of charge via the Internet at http://pubs.acs.org.

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