JOURNAL OF
CHEMICAL &
ENGINEERING
DATA

ARTICLE

pubs.acs.org/jced

Potentiometric, Calorimetric, and ^{1}H NMR Investigation on Hg^{2+}-Mercaptocarboxylate Interaction in Aqueous Solution

Paola Cardiano, Daniela Cucinotta, Claudia Foti, Ottavia Giuffrè,* and Silvio Sammartano

Dipartimento di Chimica Inorganica, Chimica Analitica e Chimica Fisica, Università di Messina, Viale Ferdinando Stagno D'Alcontres 31, 98166 Messina, Italy

S Supporting Information

ABSTRACT: The interaction between Hg^{2+} and three different mercaptocarboxylic acids (thiolactic, 3-mercaptopropanoic, and thiomalic acids) was studied in aqueous solution by potentiometry, calorimetry, and for thiolactic acid ^{1}H NMR spectroscopy. Potentiometric measurements were performed in $NaNO_3$ in the presence of a small amount of iodide (NaI) as a competitive ligand. For all systems, the formation of the MLH, ML, and ML_2 species was found, with very high values of formation constants (for the ML species, log β ranges from 32.10 to 35.10 at I = 0.1 $mol \cdot L^{-1}$). For the system containing thiolactic acid, the ^{1}H NMR spectroscopy fully supported the chemical model proposed, providing fairly similar formation constant values to those obtained by potentiometry. In confirmation of the very high stabilities of Hg^{2+}-mercaptocarboxylate species, the speciation diagrams show that the metal fractions of the complex species are very high over a wide pH range, suppressing almost completely the hydrolysis of the cation. All enthalpy values are strongly exothermic, as typical for soft−soft interactions, where the contribution to the Gibbs energy of complexation is mainly enthalpic in nature. The sequestering ability of Hg^{2+} is very high even at physiological pH, and it was analyzed and compared at different ionic strengths, pH, and temperatures.

INTRODUCTION

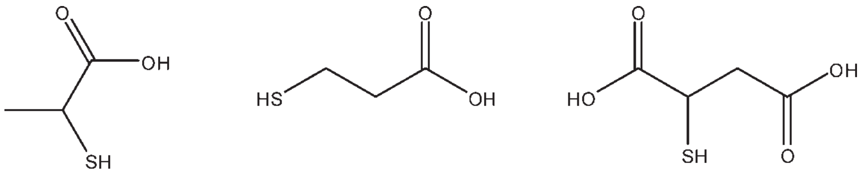
Sustained interest in the coordination chemistry of Hg^{2+} arises from its inherent toxicity. Mercury is extremely toxic to living organisms and, owing to its bioaccumulation in the food chain, is dangerous to higher organisms.[1] For humans its toxicity is mainly directed toward the central nervous system and the kidney[2,3] and derives from the affinity of Hg^{2+} compounds for cysteinyl sulfur residues. Clinical chelation therapy of mercury poisoning generally uses thiol compounds such as dimercaptosuccinic acid and dimercaptopropane sulfonic acid.[4,5] These ligands are of special interest as sequestering agents for metal ions, since they have two mercapto groups available for chelate ring formation.[6] The very high affinity of Hg^{2+} for sulfur donors is well-known, also suggesting the use of sulfur-containing ligands for Hg removal from wastewaters. Usually, in natural waters, mercury has a low abundance in geological formations, and its concentration is less than 0.1 $\mu g \cdot L^{-1}$. Ocean waters have an average mercury concentration of 0.03 $\mu g \cdot L^{-1}$. Most waters with greater than 0.1 $\mu g \cdot L^{-1}$ mercury have mercury pollution.[7] One promising technique for the mercuric ion remediation from groundwater is to trap it using complexing ligands, such as thiols. As coordinating agents for mercury, they are covalently linked to a high surface area support.[8,9] Thiols are naturally occurring ligands because they can be formed via a variety of pathways. Their concentration in seawater and marine sediment interstitial waters is commonly reported to range from nanomolar to millimolar.[10] The main contribution of analytical chemistry to the fields of heavy metal intoxication treatment and mercury remediation of natural waters is the determination of the formation constants of the species between the metal ions and chelating agents and the definition of speciation models of these systems, to compare the strength and characteristics of the complexes formed.[1]

The literature reports only a few data concerning Hg^{2+}-mercaptocarboxylate systems. This shortage of data is certainly attributable to the considerable difficulty in determining experimentally too high values of formation constants. Because of the very high stability of species, potentiometric measurements require the presence of an auxiliary ligand able to compete for the metal ion. In this paper we tested the use of iodide as the competitive ligand, and for one system we used two different instrumental techniques to confirm the speciation model, the magnitude of the formation constants obtained by potentiometry, and therefore the method of the use of iodide as a competitive ligand in the potentiometric measurements.

This work represents the continuation of our speciation study on Hg^{2+}-organic ligand systems. In the first part of this short series the ligand classes considered were: (i) O-donor ligands, such as polycarboxylates and polyelectrolytes; (ii) N-donor ligands, such as polyamines and polyelectrolytes; and (iii) amino acids.[11] The missing link is constituted by S-donor ligands. 2-Mercaptopropanoic, 3-mercaptopropanoic, and 2-mercaptosuccinic acids (Chart 1) were chosen because they are S-donor ligands, also containing O-donor groups. For this reason, in this work thermodynamic formation parameters and speciation models in aqueous solution over a wide pH range, between Hg^{2+} and three mercaptocarboxylic acids, are discussed on the basis of potentiometric and calorimetric results. Equilibria are studied at I = 0.1 $mol \cdot L^{-1}$, using as ionic medium $NaNO_3$ in the

Received: October 7, 2010
Accepted: March 7, 2011
Published: March 22, 2011

Chart 1. Structures of TLA, MPA, and TMA



2-mercaptopropanoic acid or thiolactic acid (*TLA*)

3-mercaptopropanoic acid (*MPA*)

2-mercaptosuccinic acid or thiomalic acid (*TMA*)

presence of iodide (NaI) as a competitive ligand and $T = 298.15$ K. Because of the absence of literature values for the Hg^{2+}-thiolactate system, ^1H NMR investigations are carried out to confirm the potentiometric findings. For the Hg^{2+}-thiolactate and -thiomalate systems the dependence of formation constants on ionic strength over the range $0.1 \leq I \leq 1$ mol·L^{-1} is reported.

EXPERIMENTAL SECTION

Chemicals. Solutions of mercury(II) chloride from corresponding Riedel-de-Haen products were prepared. Mercaptocarboxylate ligands (Fluka or Aldrich) were used without further purification. Their purity (always > 99.5 %) was checked by potentiometric titration. Hydrochloric acid and sodium hydroxide solutions were prepared by diluting concentrated Fluka ampules and standardized against sodium carbonate and potassium hydrogen phthalate, respectively. NaI and $NaNO_3$ solutions were prepared by weighing the corresponding salt (Fluka, puriss.). $NaNO_3$ was used after stove drying at 413.15 K. All solutions were prepared by using ultrapure water (conductivity < 0.1 μS·cm^{-1}) and grade A glassware.

Apparatus. Potentiometric measurements were performed using a Metrohm model 809 titrando coupled with a Metrohm 800 Dosino dispenser and equipped with an Orion (Ross model 8101) glass electrode and an Ag/AgCl reference electrode. Data were automatically acquired by the software Metrohm TiAMO 1.0. For all of the potentiometric measurements the electrode couple was standardized, in terms of pH = $-\log[H^+]$ (free concentration of hydrogen ion), by titrating a 10 mmol·L^{-1} HCl solution (at the same ionic strength value of the solution under study) with standard NaOH to determine the standard potential E^0 before each experiment. The potentiometric titrations were carried out in a stream of purified nitrogen gently bubbled in the titration cell to avoid O_2 and CO_2 contamination. The measurement cells were thermostatted at $[(298.15 \pm 0.1)$ K$]$ by means of water circulation from a thermocryostat (mod. D1-G Haake).

Calorimetric measurements were performed at (298.150 ± 0.001) K, by means of a CSC (Calorimetry Science Corporation) 4300 Isoperibol Titration calorimeter. The titrant was delivered by a 2.5 mL capacity Hamilton syringe, model 1002TLL. A computer program was used for the acquisition of the calorimetric data. The system accuracy was checked by titrating a TRIS [tris-(hydroxymethyl)amino-methane] buffer with HCl. The heat of dilution was measured before each experiment. The precision of the calorimetric apparatus was $(Q \pm 0.015)$ J and $(v \pm 0.001)$ cm^3.

^1H NMR spectra were recorded on a Bruker AMX R-300 spectrometer 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 $\delta_{\text{dioxane}} = 3.70$ ppm. The individual chemical shifts belonging to the Hg^{2+}-TLA (where TLA is 2-mercaptopropanoic acid) complexes were calculated assuming fast mutual exchange.

Procedure. For the investigation of ligand protonation constants, 25 mL of solution containing the ligand under study $[(1 \leq C_L \leq 4)$ mmol·L$^{-1}]$ and $NaNO_3$, to reach the prefixed ionic strength values $[(0 < I \leq 1)$ mol·L$^{-1}]$, was titrated with standard NaOH. For ligand protonation enthalpies, 25 mL of solution containing the ligand salt $[(0.5 \leq C_L \leq 2)$ mmol·L$^{-1}]$ and $NaNO_3$, to reach the prefixed ionic strength values $[(I = 0.1)$ mol·L$^{-1}]$, was titrated with standard HCl.

For the investigation of Hg^{2+}-mercaptocarboxylate ligands, 25 mL of solution containing Hg^{2+} $[(0.5 \leq C_{Hg} \leq 1.5)$ mmol·L$^{-1}]$, the ligand under study at different metal ligand ratios (from $C_{Hg}/C_L = 0.2$ to $C_{Hg}/C_L = 1$) and $NaNO_3$/NaI mixtures (90 % $NaNO_3$, 10 % NaI), to reach the prefixed ionic strength values $[(0 < I \leq 1)$ mol·L$^{-1}]$, was titrated with standard NaOH.

The formation enthalpies of complex species of Hg^{2+} with 2-mercaptopropanoic acid (TLA), 3-mercaptopropanoic acid (MPA), and 2-mercaptosuccinic acid (TMA) were obtained by adding to 25 mL of the solution containing Hg^{2+} $[(0.5 \leq C_{Hg} \leq 2)$ mmol·L$^{-1}]$ and $NaNO_3$ (to reach ionic strength value of 0.1 mol·L^{-1}), the ligand under study as the sodium salt. The investigated metal/ligand ratios C_{Hg}/C_L were from 0.35 to 1.

^1H NMR measurements were generally made in a 9:1 H_2O/D_2O solution at different pH values , ranging from 2 to 10, using different metal—ligand ratios.

Details of potentiometric, calorimetric, and ^1H NMR measurements, for complex species determination, are reported in Table 1.

Calculations. The following computer programs were used: (i) BSTAC and STACO[12] to refine all of the parameters of an acid—base titration (such as analytical concentration of reagent and E^0) and to calculate ligand protonation and complex formation constants; (ii) ES4ECI[12] to draw speciation diagrams and to calculate the formation percentage of each species; (iii) ES5CM[13] to calculate enthalpy values of ligand protonation and complex formation from calorimetric titration data; (iv) LIANA[12] to fit linear and nonlinear equations, for the dependence on ionic strength of formation constants; (v) HypNMR[14] to calculate equilibrium constants and the individual chemical shifts of each nucleus in each chemical species from the observed chemical shifts measured in the collected NMR spectra.

RESULTS AND DISCUSSION

Thermodynamic Parameters for Ligand Protonation and Hg^{2+} Hydrolysis. For the study of the interaction between Hg^{2+}

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

ligand	I^a/mol·L^{-1}	no. titr.^{b}	no. points^c	$C_H{}^d$	$C_{Hg}{}^d$	$C_L{}^d$	pH
			Potentiometric Measurements				
TLA	0.1−1	32	100	1−12	0.5−1.5	0.5−6	2.5−11.0
MPA^e	0.1	6	75	—	0.5	1−3^e	10.0−4.1
TMA	0.1−1	24	100	1.5−12	0.5−1	0.5−4	3.0−10.0
			Calorimetric Measurements				
TLA	0.1	4	30	—	1.5−2	56	4.4−9.6
MPA	0.1	4	30	—	0.5	57	4.5−10.3
TMA	0.1	4	30	—	1.5−2	75	3.6−10.2
			^{1}H NMR Measurements				
TLA	0.005−0.065	4	7−10	—	1−5	1−30	2.0−10.0

[a] 90 % $NaNO_3$, 10 % NaI. [b] Number of titrations. [c] Number of points for titration. [d] In mmol·L^{-1}. [e] MPA ligand was used as salt (Na_2MPA), and the titrant was HCl.

Table 2. Formation Constants for Hg^{2+}-Iodide Complexes at I = 0.5 mol·L^{-1} in $NaClO_4$ and T = 298.15 K

reaction	log β^a
$Hg^{2+} + I^- = HgI^+$	12.87
$Hg^{2+} + 2I^- = HgI_2{}^0$	23.82
$Hg^{2+} + 3I^- = HgI_3{}^-$	27.60
$Hg^{2+} + 4I^- = HgI_4{}^{2-}$	29.83

[a] Ref 16.

Table 3. Thermodynamic Parameters^a of Ligand Protonation in $NaNO_3$ at T = 298.15 K

ligand	I^b	log $\beta_1{}^c$	$\Delta H_1{}^{c,d}$	log $\beta_2{}^c$	$\Delta H_2{}^{c,d}$	log $\beta_3{}^c$	$\Delta H_3{}^{c,d}$
TLA	0.10	10.020(4)	−17.5(4)	13.541(3)	−12.5(4)		
	0.25	9.982(2)		13.507(2)			
	0.50	9.857(5)		13.367(4)			
	1.00	9.829(4)		13.313(3)			
MPA	0.10	10.106(2)	−20.5(4)	14.281(2)	−18.7(4)		
	0.25	10.084(3)		14.249(3)			
	0.50	10.057(7)		14.214(7)			
	1.00	10.08(2)		14.20(2)			
TMA	0.10	10.34(3)	−20.5(2)	14.93(2)	−19.3(3)	18.15(6)	−19.1(3)
	0.25	10.15(2)		14.63(1)		17.78(4)	
	0.50	9.99(2)		14.39(4)		17.51(4)	
	1.00	9.82(2)		14.26(2)		17.28(7)	

[a] Referred to reaction: $iH + L = H_iL$ (charges omitted for simplicity). [b] In mol·L^{-1}. [c] Least-squares errors on last significant figure are shown in parentheses. [d] In kJ mol^{-1}.

and several ligands it is necessary to have knowledge of the ligand protonation, the hydrolysis of Hg^{2+}, the Hg^{2+}-Cl^-, and Hg^{2+}-I^- complex formation parameters under the same ionic strength conditions. For hydrolysis and Hg^{2+}-Cl^- complex formation parameters, literature data were used.[11,15] Literature values of formation constants of Hg^{2+}-I^- species at (I = 0.5) mol·L^{-1} are shown in Table 2.[16] From these values, those at the various ionic strengths used in this investigation were recalculated by the following equation:

$$\log \beta_I = \log \beta_{I=0.5} - 0.51 \cdot z^* \left(\frac{\sqrt{I}}{1 + 1.5\sqrt{I}} - \frac{\sqrt{0.5}}{1 + 1.5\sqrt{0.5}} \right) + 0.067 \cdot z^* (I - 0.5) - 0.04 \cdot z^* \cdot (I - 0.5)^{3/2} \quad (1)$$

where $z^* = \Sigma(\text{charges})^2_{\text{reactants}} - \Sigma(\text{charges})^2_{\text{products}}$. The empirical parameters present in this equation were calculated by analysis of literature formation data for several metal ligand systems (unpublished data from this laboratory), by using a similar approach reported in ref 17.

Protonation constants of TLA, MPA, and TMA, previously reported at different ionic strengths in NaCl and $(CH_3)_4NCl$,[18] were determined here in $NaNO_3$ in the range ($0.1 \le I \le 1$) mol·L^{-1} together with enthalpy protonation values at I = 0.1 mol·L^{-1}. Results are shown in Table 3.

Complexes of Hg^{2+} with Mercaptocarboxylates. Since sulfur containing ligands form very stable Hg^{2+} complexes, the potentiometric investigation on metal complex formation was carried out adding a known amount of sodium iodide to the solution: the iodide ion acts as an auxiliary ligand that can compete for the metal ion.[19,20] Complex formation constants of Hg^{2+} (M^{2+}) with mercaptocarboxylates (L^{z-}) are expressed as β_{pqr}, according to the equilibrium reaction:

$$pM^{2+} + qL^{z-} + rH^+ \rightleftarrows M_pL_qH_r{}^{(2p - qz + r)} \quad (2)$$

After several trials for each system, the speciation model which had the best fit was chosen by considering the variance ratio, $\sigma^2/\sigma_0{}^2$ ($\sigma_0{}^2$ = variance for the best fit, σ^2 = variance of the fit). The formation constants in the $NaNO_3$ ionic medium, at T = 298.15 K, (I = 0.1) mol·L^{-1} for MPA, and ($0.1 \le I \le 1$) mol·L^{-1} for TLA and TMA, are reported in Table 4. For all of the systems investigated, the results show the same speciation model with the formation of three complex species between Hg^{2+} and the ligand, namely, $MLH^{(3-z)}$, $ML^{(2-z)}$, and $ML_2{}^{(2-2z)}$. Formation constant values of the species, referring to these three systems, show a very high stability.

To confirm the speciation model and the values of the formation constants of the Hg^{2+}-TLA system, ^{1}H NMR investigations in aqueous solution were carried out. In our previous papers on different metal−ligand species, ^{1}H NMR investigations

Table 4. Experimental and Calculated Formation Constants[a] for Hg^{2+}-Mercaptocarboxylate Systems in $NaNO_3$ at Different Ionic Strengths (in $mol \cdot L^{-1}$) and T = 298.15 K

ligand	species	I	$\log \beta_{exp}$[b,c]	$\log \beta_{calc}$[d]
TLA	MLH^+	0.1	36.28(6)	36.33
		0.1	36.37(6)	–
		0.25	36.02(4)	35.96
		0.5	35.37(9)	35.60
		0.5	35.65(8)	–
		1	35.11(6)	35.11
	ML^0	0.1	33.97(5)	34.17
		0.1	34.18(5)	–
		0.25	33.86(3)	33.84
		0.5	33.58(3)	33.54
		0.5	33.56(4)	–
		1	33.14(5)	33.17
	ML_2^{2-}	0.1	42.54(5)	42.68
		0.1	42.68(4)	–
		0.25	42.32(3)	42.32
		0.5	42.26(3)	41.96
		0.5	41.96(3)	–
		1	41.42(5)	41.49
MPA	MLH^+	0.1	35.33(5)	–
	ML^0	0.1	32.10(4)	–
	ML_2^{2-}	0.1	39.54(4)	–
TMA	MLH^0	0.1	39.02(5)	39.00
		0.1	38.93(6)	–
		0.25	38.59(3)	38.51
		0.25	38.44(3)	–
		0.5	38.29(6)	38.13
		0.5	38.05(6)	–
		1	37.87(5)	37.79
		1	37.68(4)	–
	ML^-	0.1	35.50(5)	35.10
		0.1	35.07(5)	–
		0.25	34.65(3)	34.68
		0.25	34.66(4)	–
		0.5	34.33(6)	34.36
		0.5	34.35(6)	–
		1	34.16(4)	34.07
		1	34.07(5)	–
	ML_2^{4-}	0.1	43.47(6)	42.92
		0.1	42.86(7)	–
		0.25	42.59(4)	42.66
		0.25	42.62(5)	–
		0.5	42.42(6)	42.42
		0.5	42.40(6)	–
		1	42.12(5)	42.12
		1	42.13(4)	–

[a] Referred to the reaction 2. [b] Experimental formation constants. [c] Least-squares errors on last significant figure are shown in parentheses. [d] Formation constants calculated by using eq 3.

were also used to confirm the speciation model and to give structural information on the complexes.[21−23] In this study a wide number of spectra were collected in the pH range 2 to 10, by varying the metal/ligand ratio and the concentrations of metal and ligand as well. Depending on the metal/ligand ratio employed and on the chosen concentration, in a few cases it was possible to discriminate the methylic and methyne signals due to bound and free TLA. As an example, Figure 1 shows the spectrum recorded at pH = 2.3 for a solution containing Hg^{2+} and TLA at C_M = 1.5 $mmol \cdot L^{-1}$ and C_L = 30 $mmol \cdot L^{-1}$. More generally the spectra showed a single set of peaks, suggesting that, although several species may be present in solution, they are involved in fast mutual exchange; therefore, in most cases the resonances due to the bound and the free ligand could not be directly observed from the spectra. The HypNMR software[14] is able to give, from the experimental ^1H NMR chemical shift, the formation constants and the chemical shift values for each individual complex and to recalculate chemical shift values at each experimental pH. The excellent agreement between calculated and observed NMR parameters both for CH_3 and CH of TLA (Figure 2) allowed us to conclude that the spectroscopic findings are fully consistent with the model used for interpretating potentiometric experiments. Moreover, from ^1H NMR data, we managed to get the values of formation constants of all three species of the Hg-TLA system. These values are shown in Table 5. For comparison, in the same table are reported values calculated at I = 0.02 $mol \cdot L^{-1}$, considering the dependence on ionic strength of the potentiometric data (see after eq 3, Table 7). Although the agreement with potentiometric results cannot be considered excellent, the aim to confirm the magnitude of the values of stability constants obtained by potentiometry can be considered achieved.

Speciation Diagrams. In confirmation of the high stabilities of Hg^{2+}-mercaptocarboxylate species, the speciation diagrams, calculated by considering lower concentrations of both metal and ligand than those of experimental measurements, show that the metal fractions of the complex species are very high over the entire pH range investigated, 2 ≤ pH ≤ 9 to 10, suppressing completely the hydrolysis of the cation; that is, the formation percentages of hydrolytic species are always lower than 0.01, for all three systems (Figure 3) .

For the TLA system, at C_M = 0.1 $mmol \cdot L^{-1}$ and C_L = 0.2 $mmol \cdot L^{-1}$ (Figure 3a) over a wide pH range 2.5 ≤ pH ≤ 5.5, ML^0 is the predominant species with over 0.95 of the metal fraction at pH = 4.0 and is still significant up to pH = 7.0. In the range 6.5 ≤ pH ≤ 9.0, ML_2^{2-} is the predominant species with over 0.90 of the metal fraction at pH = 8.0.

For the MPA system, at C_M = 0.1 $mmol \cdot L^{-1}$ and C_L = 0.2 $mmol \cdot L^{-1}$ (Figure 3b), the formation of MLH^+ species is significant up to pH = 4.0. In the range 4.0 ≤ pH ≤ 6.5, the ML^0 species yields high metal fractions of about 0.90, at pH = 5.0. Starting from pH = 7.5, the predominant species ML_2^{2-} suppresses the hydrolysis.

For the TMA containing system, at C_M = 0.1 $mmol \cdot L^{-1}$ and C_L = 0.2 $mmol \cdot L^{-1}$ (Figure 3c), the distribution of the species shows some differences in the acidic pH range, mainly with respect to the system containing TLA. The formation of the MLH^0 species is significant up to pH = 4.5. In the range 4.5 ≤ pH ≤ 6.5, the ML^- species yields high metal fractions of about 0.90, at pH = 5.2. Starting from pH = 7.0, ML_2^{4-} is the predominant species suppressing the hydrolysis.

Enthalpy Changes and Temperature Dependence. The overall thermodynamic parameters regarding the formation of Hg^{2+}-TLA, -MPA, and -TMA species are reported in Table 6 at I = 0.1 $mol \cdot L^{-1}$ and T = 298.15 K. All enthalpy values calculated

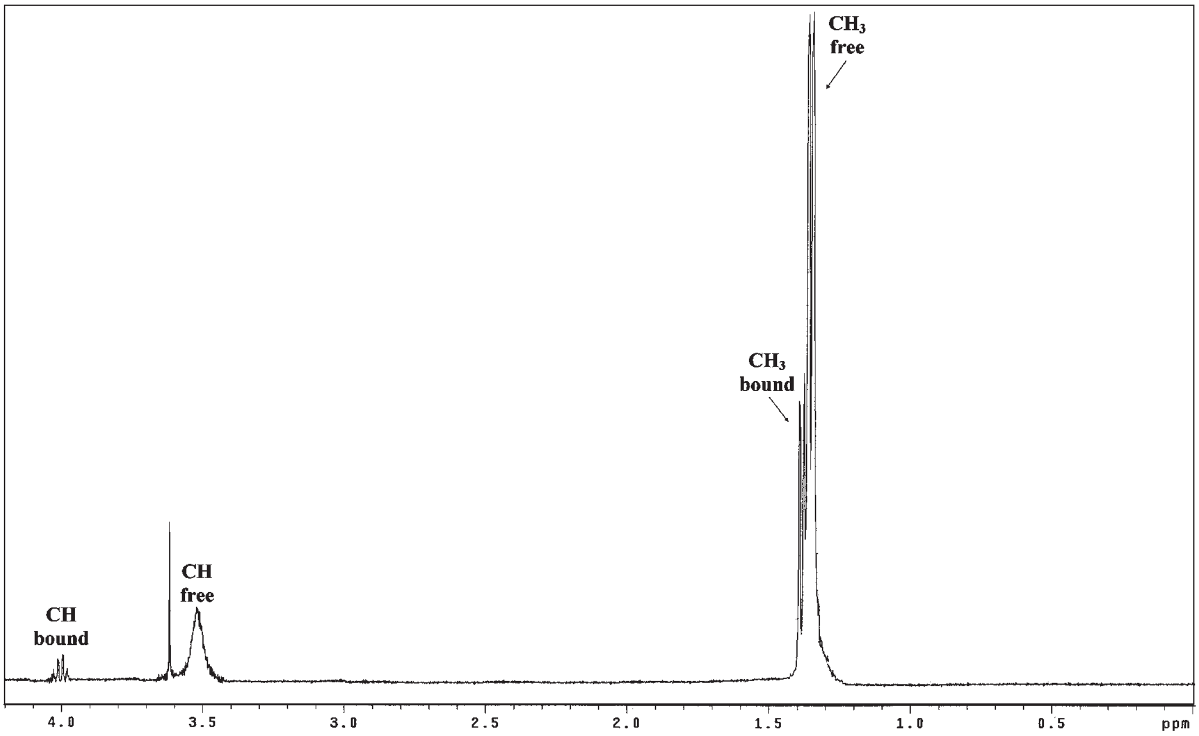


Figure 1. ^{1}H NMR spectrum of Hg^{2+}(M)-TLA(L) in 9:1 H_2O/D_2O solution, at C_M = 1.5 mmol·L^{-1}, C_L = 30 mmol·L^{-1}, and pH = 2.3.

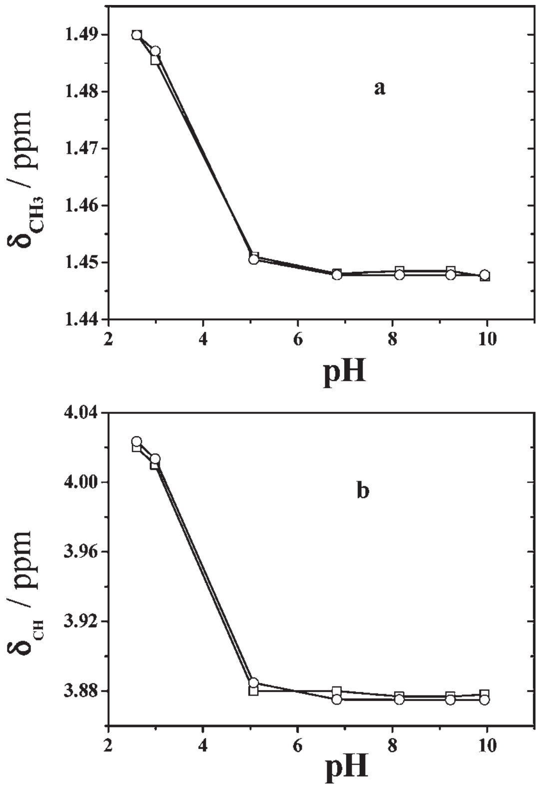


Figure 2. Chemical shifts vs pH for Hg^{2+}(M)-TLA(L) 1:1 mixtures (C_M = C_L = 1 mmol·L^{-1}), of: (a) CH_3; (b) CH. —□—, observed values; —○—, calculated values by HypNMR.

in this investigation are strongly exothermic and as expected from an interaction between a typical soft metal, such as Hg^{2+}, and a soft ligand, such as a S-donor ligand; the contribution to the Gibbs energy of complexation is mainly enthalpic in nature.[24] For the ML species, the enthalpy values are very similar and within the experimental errors, whereas entropy values are more discriminating. As an example, ΔH = (−136 ± 1, −128 ± 3, and −128 ± 1) kJ·mol^{-1}, and $T\Delta S$ = (59 ± 1, 55 ± 3, and 72 ± 1) kJ·mol^{-1} for TLA, MPA, and TMA, respectively. This indicates that the entropy value referring to a ML species of the mercaptocarboxylate containing two carboxylic groups (TMA) is significantly different with respect to entropy values referring to the ML species of mercaptocarboxylates containing one carboxylic group (TLA and MPA).

Table 5. Formation Constants[a] of Hg^{2+}-TLA Species at I = 0.02 mol·L^{-1}[b] and T = 298.15 K

	log β_{pqr}	
pqr	^{1}H NMR	potentiometry[c]
111	37.9(2)[d]	36.7(1)[d]
110	34.0(2)	34.5(1)
120	44.6(3)	43.1(1)

[a] Referred to the reaction 2. [b] Mean value of ionic strength in the ^{1}H NMR measurements. [c] Calculated from the dependence on ionic strength of the potentiometric data (see after Table 7). [d] Least-squares errors on last significant figure are shown in parentheses.

From the van't Hoff equation, formation constant values of Hg^{2+}-mercaptocarboxylate species are also calculated at T = 310.15 K and are collected in the Supporting Information. The resulting speciation diagrams at T = 310.15 K showed very little difference with respect to those at T = 298.15 K.

Ionic Strength Dependence. Formation constants of the Hg^{2+}-TLA and -TMA species were determined in the ionic

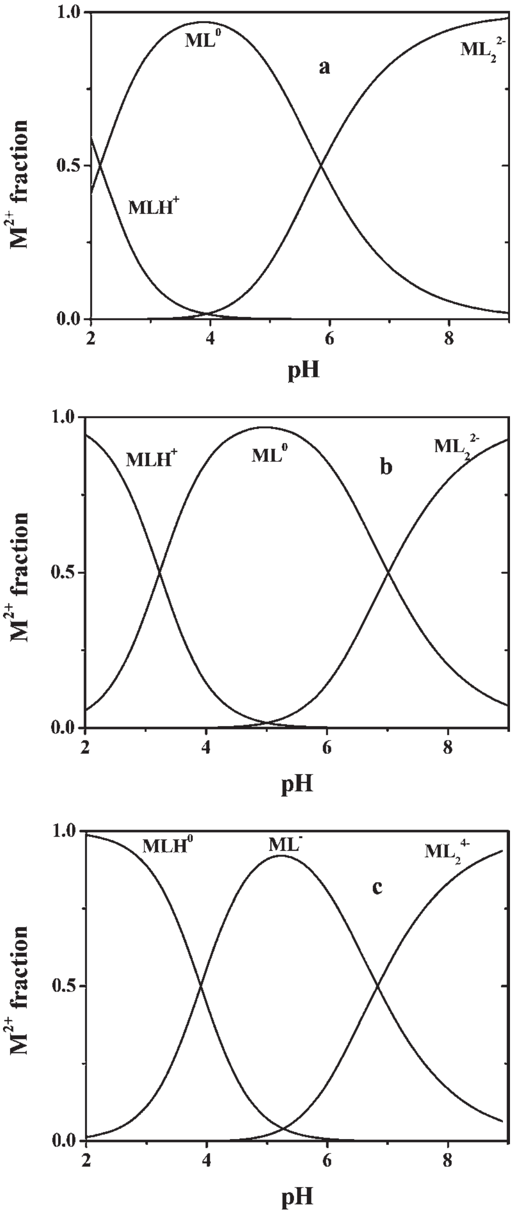


Figure 3. Speciation diagram of $Hg^{2+}(M^{2+})$-mercaptocarboxylate (L^{z-}) system vs pH. Conditions: $I = 0.1$ mol·L^{-1} ($NaNO_3$), C_M = 0.1 mmol·L^{-1}, C_L = 0.2 mmol·L^{-1}, T = 298.15 K. (a) L = TLA; (b) L = MPA; (c) L = TMA.

Table 6. Overall Thermodynamic Formation Parameters[a,b] for Hg^{2+}-Mercaptocarboxylate Systems at I = 0.1 mol·L^{-1} ($NaNO_3$) and T = 298.15 K

ligand	*pqr*	$-\Delta G^c$	ΔH^c	$T\Delta S^c$
TLA	111	207.3(3)	−134(2)	73(2)
	110	195.0(3)	−136(1)	59(1)
	120	243.6(3)	−172(1)	72(1)
MPA	111	201.7(3)	−130(2)	72(2)
	110	183.2(2)	−128(3)	55(3)
	120	225.7(2)	−164(5)	62(5)
TMA	111	222.6(3)	−144(2)	79(2)
	110	200.4(3)	−128(1)	72(1)
	120	245.0(3)	−167(2)	78(2)

[a] Referred to the reaction 2. [b] In kJ·mol^{-1}; [c] Least-squares errors on last significant figure are shown in parentheses.

Table 7. Complex Formation Constants[a] Calculated at I = 0 mol·L^{-1}, together with Empirical Parameter for the Dependence on Ionic Strength (eq 3), at T = 298.15 K

ligand	*pqr*	$\log \beta_{pqr}$ [b]	C [b]
TLA	111	37.3(1)	−0.5(1)
	110	35.1(1)	−0.3(2)
	120	43.6(1)	−0.5(1)
TMA	111	40.5(1)	0.1(1)
	110	36.4(1)	0.12(9)
	120	43.6(2)	−0.3(1)

[a] Referred to the reaction 2. [b] Least-squares errors on last significant figure are shown in parentheses.

strength range ($0 < I \leq 1$) mol·L^{-1} ($NaNO_3$). The dependence on ionic strength of formation constants was taken into account by a Debye−Hückel type equation equivalent to eq 1, with I = 0 mol·L^{-1} as the reference ionic strength and without the term $I^{3/2}$:

$$\log \beta = \log {}^{T}\beta - 0.51 \cdot z^* \frac{\sqrt{I}}{1 + 1.5\sqrt{I}} + CI \qquad (3)$$

where $^{T}\beta$ is the formation constant at infinite dilution, C is an empirical parameter, $z^* = \Sigma(\text{charge})^2_{\text{reactants}} - \Sigma(\text{charge})^2_{\text{products}}$. The empirical parameter C for each equilibrium constant, together with formation constant value at I = 0 mol·L^{-1}, is reported in Table 7. In previous papers,[17,25] the C parameter was correlated to the stoichiometry of the formation reaction. Analogously, C parameters of the Table 7 can be expressed by the equation:

$$C = -0.35(7)p^* + 0.040(7)z^* \qquad (4)$$

where $p^* = \Sigma(\text{stoichiometric coefficients})_{\text{reactants}} - \Sigma(\text{stoichiometric coefficients})_{\text{products}}$. Calculated $\log \beta$ values at different ionic strengths are reported in Table 4.

Sequestering Ability of Mercaptocarboxylate toward Hg^{2+}. The sequestering power of a ligand toward a metal can be expressed by the function Σ versus pL, where Σ is the total fraction of metal complexed and pL = $-\log[L]_{tot}$. Since this function is a typically sigmoidal curve (or a dose response curve), which rapidly increases over a relatively small change in concentration, we can use the Boltzmann type equation (with asymptotes of 1 for pL → −∞ and 0 for pL → +∞):

$$\Sigma = \frac{1}{1 + e^{(\mathrm{pL} - \mathrm{pL}_{50})/s}} \qquad (5)$$

where $s = 1/\ln 10$ and pL_{50} is an empirical parameter, which defines the ligand concentration necessary to sequester 0.5 of the cation fraction.[26,27] The pL_{50} parameter is very useful because it gives a representation of the binding ability of a ligand (L) toward a specific cation in the investigated conditions. Table 8 reports pL_{50} values regarding the Hg^{2+}-TLA, -MPA, and -TMA systems, at different pH values (5.0, 7.4, and 8.1) and different

Table 8. pL_{50} Values of eq 5 for Mercaptocarboxylate Ligands at Different Ionic Strengths (in $mol \cdot L^{-1}$) in $NaNO_3$, at pH = 5.0, 7.4, and 8.1, T = (298.15 and 310.15) K

		pL_{50}				
ligand	I	pH = 5.0; T = 298.15 K	pH = 7.4; T = 298.15 K	pH = 7.4; T = 310.15 K	pH = 8.1; T = 298.15 K	pH = 8.1; T = 310.15 K
TLA	0.10	24.45	22.78	22.08	22.38	21.37
	0.25		22.61		22.07	
	0.50		22.54		21.91	
	1.00		22.17		21.46	
MPA	0.10	23.24	20.93	19.90	20.23	19.20
TMA	0.10	26.01	23.76	22.65	23.03	21.95
Suc[a]	0.10	5.58			1.56	
btc[a]	0.10	6.76			3.06	2.71
mlt[a]	0.10	11.00			9.68	
en[a]	0.10	5.94			6.16	
dien[a]	0.10	7.78			6.68	5.32
spd[a]	0.10	3.60			4.23	
trien[a]	0.10	10.33			11.69	
Gly[a]	0.10	5.76			3.88	
His[a]	0.10	7.00			4.23	
Asp[a]	0.10	6.58			5.14	

[a] Ref 11. Abbreviations: Suc, succinic acid; btc, butanetetracarboxylate; mlt, benzenehexacarboxylate; en, ethylenediamine; dien, diethylenetriamine; spd, spermidine; trien, triethylenetetramine; Gly, glycine; His, histidine; Asp, aspartic acid.

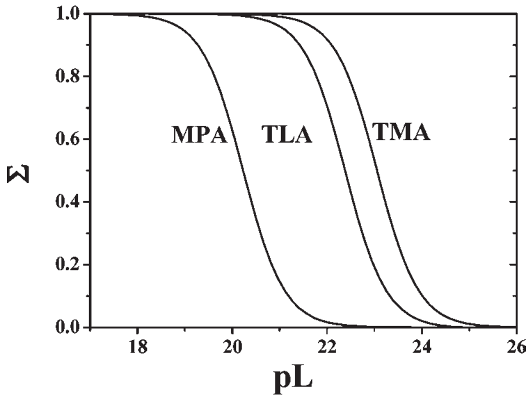


Figure 4. Calculated sum of fractions of Hg^{2+}-mercaptocarboxylate species vs pL in $NaNO_3$ at I = 0.1 $mol \cdot L^{-1}$, pH = 8.1, and T = 298.15 K.

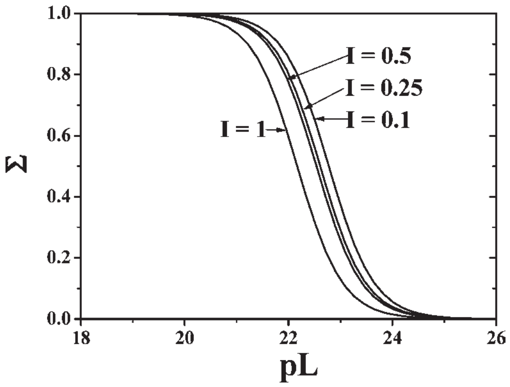


Figure 5. Calculated sum of fractions of Hg^{2+}-TLA species vs pL in $NaNO_3$ at $0.1 \leq I$ ($mol \cdot L^{-1}$) ≤ 1, pH = 7.4, and T = 298.15 K.

temperatures [(298.15 and 310.15) K]. Figure 4 shows that, at I = 0.1 $mol \cdot L^{-1}$ in $NaNO_3$, pH = 8.1, and T = 298.15 K, TMA has a sequestering ability slightly higher than that of TLA and much higher than that of MPA. The dependence on ionic strength of the sequestering power of TLA toward Hg^{2+}, shown by Figure 5 at $0.1 \leq I$ ($mol \cdot L^{-1}$) ≤ 1, pH = 7.4 and T = 298.15 K, indicates a smooth decreasing trend of the sequestering ability with increasing ionic strength. For TLA, by considering the data of Table 8, the dependence is fairly well described by the linear equations:

$$\text{at pH} = 7.4 \qquad pL_{50} = 23.1 - 0.9I^{1/2} \tag{6}$$

$$\text{at pH} = 8.1 \qquad pL_{50} = 22.2 - 1.3I^{1/2} \tag{7}$$

The increase of the temperature from (298.15 to 310.15) K, at pH = 8.1, and I = 0.1 $mol \cdot L^{-1}$ is expected to induce a considerable lowering of the sequestering ability for all three mercaptocarboxylate ligands investigated, as shown by Figure 6.

It is possible to compare the sequestering ability of a mercaptocarboxylate, such as TLA, toward Hg^{2+} with that of several ligands previously studied,[11] in the same conditions of temperature, pH, and ionic strength (Table 8). For example, at T = 298.15 K, pH = 8.1, and I = 0.1 $mol \cdot L^{-1}$, if we compare TLA with: a dicarboxylic acid, such as succinic acid (Suc), $pL_{50TLA} - pL_{50Suc} = 20.82$; a simple amino acid, such as glycine (Gly), $pL_{50TLA} - pL_{50Gly} = 18.50$; a diamine, such as ethylenediamine (en), $pL_{50TLA} - pL_{50en} = 16.22$. This indicates that, in these conditions of pH, temperature, and ionic strength, the substitution of a thiolic group with a carboxylic group induces the lowering of the sequestering ability by over 20 orders of magnitude and the substitution with an amino group of by over 18 orders of magnitude.

Literature Comparisons. Only a few studies can be found in the literature on the role of organic sulfides or thiols [RSH; e.g., cysteine, glutathione, penicillamine, dimercaptosuccinic acid (DMSA)] in mercury speciation.[5,6,16,28−34] Some examples are reported in Table 9. As concerns the ligands considered here,

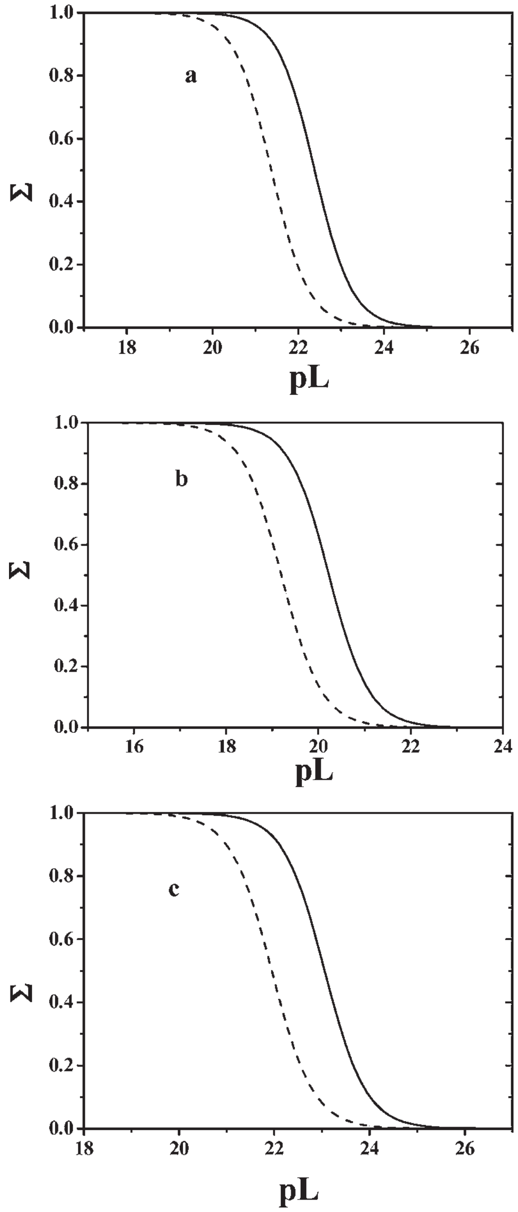


Figure 6. Calculated sum of fractions of Hg^{2+}-mercaptocarboxylate species vs pL in $NaNO_3$ at $I = 0.1$ mol·L^{-1}, pH = 8.1, and $T = 298.15$ K (solid line), $T = 310.15$ K (dashed line). (a) TLA; (b) MPA; (c) TMA.

Table 9. Formation Constants[a] for Hg^{2+}-S-Containing Ligand Species

ligand	pqr	log β	I/mol·L^{-1}	T/K
penicillamine[b]	110	18.86	0.1	298.15
	120	24.95		
meso-DMSA[c]	111	32.4	0.1	298.15
	110	27.5		
	120	34.2		
glutathione[d]	111	32.5	0.1	298.15
	110	26.0		
	120	33.4		
TMA[e]	110	9.94	0.1	298.15
	120	18.07		
TMA[f]	112	28.08	0.15	310.15
	111	16.24		
	110	11.78		
	120	23.16		
	11−1	2.79		
	12−1	12.73		
	124	38.82		
	123	36.04		
	122	32.55		
TLA[g]	111	36.33	0.1	298.15
	110	34.17		
	120	42.68		
MPA[g]	111	35.33	0.1	298.15
	110	32.10		
	120	39.54		
TMA[g]	111	39.00	0.1	298.15
	110	35.10		
	120	42.92		

[a] Referred to the reaction 2. [b] Ref 34 in KNO_3. [c] Ref 33 in KNO_3. [d] Ref 16. [e] Ref 6 in KNO_3. [f] Ref 30 in NaCl. [g] This work, in $NaNO_3$.

no data are reported for the Hg^{2+}-TLA or -MPA systems, while formation constants of the Hg^{2+}-TMA species were determined at $T = 298.15$ K and $I = 0.1$ mol·L^{-1} in KNO_3,[6] at $T = 310.15$ K and $I = 0.15$ mol·L^{-1} in NaCl,[30] by potentiometric measurements only. In both papers, formation constant values are much lower than those obtained in this investigation. For example, the difference, for the ML species, between the formation constant of ref 6 and that obtained by us is over 20 orders of magnitude. This enormous difference confirms that, in the case of very strong affinity between a metal and a ligand, due to the complete displacement of H^+, the determination of the complex formation constants by potentiometric (ISE-H^+, proton displacement) method cannot be performed unless in the presence of a ligand that competes strongly for the metal. In contrast with our potentiometric measurements, those reported in both refs 6 and 30 were performed in the absence of a strong competitive ligand, such as iodide.

To our knowledge, there are no literature enthalpy data on species formed by Hg^{2+} and mercaptocarboxylate ligands used in this investigation. If we compare enthalpy values, for the ML species, of the complex formed by Hg^{2+} with a mercaptocarboxylate containing two carboxylic groups (TMA), with that of the species formed by Hg^{2+} and a polycarboxylate, such as butanetetracarboxylate (btc), reported in ref 11, the difference $\delta(\Delta H) = \Delta H_{(\text{Hg-TMA})} - \Delta H_{(\text{Hg-btc})} = -128 - (-30) = -98$ kJ·mol^{-1} is attributable to the thiolic group. This enthalpy contribution, being much more exothermic, confirms the character of the interaction.

■ FINAL REMARKS

Formation constant values of Hg^{2+}-TLA, -MPA, and -TMA species show a very high stability. Because of the lack of literature

Table 10. Thermodynamic Contributions[a] Calculated for Different Hg^{2+}-Ligand Species at T = 298.15 K

group	$-\Delta G$	ΔH	$T\Delta S$
carboxylic	22	−9	13
amino	57	−43	14
thiolic	164	−119	45

[a] In $kJ \cdot mol^{-1}$.

data, it was not possible to compare the results of this investigation with data regarding similar systems. So, for the system Hg^{2+}-TLA, two independent techniques (potentiometry and ^1H NMR spectroscopy) were employed to study the equilibria. Both techniques fully support the chemical model proposed, providing fairly similar formation constant values. All enthalpy values are strongly exothermic. The sequestering ability toward Hg^{2+} is very high, even at physiological pH, and is markedly higher than that of di- and tetra-carboxylic ligands, di- and triamines, and aminoacids. The sequestering ability of these three mercaptocarboxylate ligands were analyzed and compared at different ionic strengths, pH, and temperatures.

On the basis of the results obtained in this and the previous work,[11] the contributions of Gibbs energy, enthalpy, and entropy, related to the ML species, for the different donor groups (amino, carboxylic, and thiol), have been roughly calculated. These values are given in Table 10. By considering the ML species for TLA, MPA, and TMA altogether, the resulting mean value of Gibbs energy is (-186 ± 9) $kJ \cdot mol^{-1}$. By subtracting the carboxylate contribution approximately equal to -22 $kJ \cdot mol^{-1}$,[11] the contribution of the thiolate group is -164 $kJ \cdot mol^{-1}$. Similarly, by subtracting the contribution of carboxylate group (-9 $kJ \cdot mol^{-1}$),[11] from the mean enthalpy value $[(-128 \pm 9)$ $kJ \cdot mol^{-1}]$ of the ML species formed by mercaptocarboxylate ligands, it is possible to obtain the thiolate contribution that is -119 $kJ \cdot mol^{-1}$. The contributions to the entropy of carboxylic and thiolic groups are (13 and 45) $kJ \cdot mol^{-1}$, respectively. By considering that, for the aminoacid glycine $\Delta G = -79.2$ $kJ \cdot mol^{-1}$, $\Delta H = -52$ $kJ \cdot mol^{-1}$,[11] the contributions of amino group to the Gibbs energy and enthalpy are (-57 and -43) $kJ \cdot mol^{-1}$, respectively. So this rough calculation confirms that increasingly covalent interactions between "soft entities" will result in more exothermic enthalpies (see Table 10).[24] Nevertheless a more positive entropy of formation depends on a increased release of coordinated solvent molecules.[24]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Formation constants for the Hg^{2+}-mercaptocarboxylate systems in $NaNO_3$ at I = 0.1 $mol \cdot L^{-1}$ and T = 310.15 K. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: ogiuffre@unime.it; fax: 0039090392827.

Funding Sources

The authors thank the University of Messina for financial support.

■ REFERENCES

(1) Al-Kandary, J. A.; Mandani, F. M. A.; Al-Jimaz, A. S.; El-Sherif, A. A.; Shoukry, M. M. Sinthesys and thermodynamic investigation of 4-amino-6-hydroxy-2-mercapto pyrimidine (AHMP) complexes with some selected divalent metal(II) ions. *J. Solution Chem.* **2007**, *36*, 247–257.

(2) Sirois, J.; Atchinson, W. D. Effect of mercurials on ligand- and voltage-gated ion channels: a review. *Neurotoxicology* **1996**, *17*, 63–84.

(3) Zalups, R. K. Molecular interactions with mercury in the kidney. *Pharmacol. Rev.* **2000**, *52*, 113–143.

(4) Andersen, O. Principles and Recent Developments in Chelation Treatment of Metal Intoxication. *Chem. Rev.* **1999**, *99*, 2683–2710.

(5) Ngu-Schwemlein, M.; Merle, J. K.; Healy, P.; Schwemlein, S.; Rhodes, S. Thermodynamics of the complexation of Hg(II) by cysteinyl peptide ligands using isothermal titration calorimetry. *Thermochim. Acta* **2009**, *496*, 129–135.

(6) Lenz, G. R.; Martell, A. E. Metal chelates of mercaptosuccinic and α,α′-dimercaptosuccinic acids. *Inorg. Chem.* **1965**, *4*, 378–384.

(7) Boyd, C. E. *Water Quality: An introduction*; Kluwer Academic Publishers: Boston, 2000.

(8) Billinge, S. J. L.; McKimmy, E. J.; Shatnawi, M.; Kim, H. J.; Petkov, V.; Wermeille, D.; Pinnavaia, T. J. Mercury binding sites in thiol-functionalized mesostructured silica. *J. Am. Chem. Soc.* **2005**, *127*, 8492–8498.

(9) *Capsule report: aqueous mercury treatment*; Office of Research and Development, EPA: Washington, DC, 1997.

(10) Zhang, J.; Wang, F.; House, J. D.; Page, B. Thiols in wetland interstitial waters and their role in mercury and methylmercury speciation. *Limnol. Oceanogr.* **2004**, *49*, 2276–2286.

(11) Foti, C.; Giuffrè, O.; Lando, G.; Sammartano, S. Interaction of inorganic mercury(II) with polyamines, polycarboxylates and amino acids. *J. Chem. Eng. Data* **2009**, *54*, 893–903.

(12) 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.

(13) De Robertis, A.; De Stefano, C.; Rigano, C. Computer Analysis of Equilibrium Data in Solution. ES5CM Fortran and Basic Programs for Computing Formation Enthalpies from Calorimetric Measurements. *Thermochim. Acta* **1986**, *138*, 141–146.

(14) Frassineti, C.; Ghelli, S.; Gans, P.; Sabatini, A.; Moruzzi, M. S.; Vacca, A. Nuclear Magnetic Resonance as a Tool for Determining Protonation Constants of Natural Polyprotic Bases in Solution. *Anal. Biochem.* **1995**, *231*, 374–382.

(15) Powell, K. J.; Brown, P. L.; Byrne, R. H.; Gajda, T.; Hefter, G.; Sjoberg, S.; Wanner, H. P. Chemical speciation of environmentally significant heavy metal with inorganic ligands. Part 1: the Hg^{2+}-Cl^-, OH^-, CO_3^{2-}, SO_4^{2-}, and PO_4^{3-} aqueous systems. *Pure Appl. Chem.* **2005**, *77*, 739–800.

(16) Martell, A. E.; Smith, R. M.; Motekaitis, R. J. *Critically Selected Stability Constants of Metal Complexes*; National Institute of Standard and Technology, NIST: Gaithersburg, MD, 2004.

(17) Daniele, P. G.; De Robertis, A.; De Stefano, C.; Sammartano, S.; Rigano, C. On the Possibility of Determining the Thermodynamic Parameters for the Formation of Weak Complexes Using a Simple Model for the Dependence on Ionic Strength of Activity Coefficients. Na^+, K^+ and Ca^{2+} Complexes of Low Molecular Weight Ligands in Aqueous Solution. *J. Chem. Soc., Dalton Trans.* **1985**, 2353–2361.

(18) Bretti, C.; De Stefano, C.; Foti, C.; Giuffrè, O.; Sammartano, S. Thermodynamic protonation parameters of some sulphur-containing anions in NaClaq and $(CH_3)_4NCl_{aq}$ at t = 25 °C. *J. Solution Chem.* **2009**, *38*, 1225–1245.

(19) Jawaid, M.; Ingman, F. Potentiometric studies on the complex formation between methylmercury(II) and some keto- and amino-carboxylic acids. *Talanta* **1981**, *28*, 137–143.

(20) Alderighi, L.; Gans, P.; Midollini, S.; Vacca, A. Co-ordination chemistry of the methylmercury(II) ion in aqueous solution: a thermodynamic investigation. *Inorg. Chim. Acta* **2003**, *356*, 8–18.

(21) Cardiano, P.; Giuffrè, O.; Pellerito, L.; Pettignano, A.; Sammartano, S.; Scopelliti, M. Thermodynamic and spectroscopic study of the binding of dimethyltin(IV) by citrate at 25 °C. *Appl. Organomet. Chem.* **2006**, *20*, 425–435.

(22) Cardiano, P.; De Stefano, C.; Giuffrè, O.; Sammartano, S. Thermodynamic and spectroscopic study for the interaction of dimethyltin(IV) with L-cysteine in aqueous solution. *Biophys. Chem.* **2008**, *133*, 19–27.

(23) Cardiano, P.; Giuffrè, O.; Napoli, A.; Sammartano, S. Potentiometric, ^{1}H-NMR, ESI-MS investigation on dimethyltin(IV) cation-mercaptocarboxylate interaction in aqueous solution. *New J. Chem.* **2009**, *33*, 2286–2295.

(24) Nancollas, G. H. The thermodynamics of metal-complex and ion-pair formation. *Coord. Chem. Rev.* **1970**, *5*, 379–415.

(25) Foti, C.; Sammartano, S. Ionic Strength Dependence of Protonation Constants of Carboxylate Ions in $NaCl_{aq}$ $(0 \leq I \leq 5.6 \text{ mol} \cdot \text{kg}^{-1})$ and KCl_{aq} $(0 \leq I \leq 4.5 \text{ mol} \cdot \text{kg}^{-1})$: Specific Ion Interaction Theory and Pitzer Parameters and the Correlation between Them. *J. Chem. Eng. Data* **2010**, *55*, 904–911.

(26) Crea, P.; De Robertis, A.; De Stefano, C.; Sammartano, S. Speciation of phytate ion in aqueous solution. Sequestration of magnesium and calcium by phytate at different temperatures and ionic strengths, in $NaCl_{aq}$. *Biophys. Chem.* **2006**, *124*, 18–26.

(27) De Stefano, C.; Gianguzza, A.; Giuffrè, O.; Pettignano, A.; Sammartano, S. Interaction of methyltin(IV) compounds with carboxylate ligands. Part 2: Formation thermodynamic parameters, predictive relationships and sequestering ability. *Appl. Organomet. Chem.* **2008**, *22*, 30–38.

(28) Fuhr, B. J.; Rabenstein, D. L. Nuclear Magnetic resonance studies of the solution chemistry of metal complexes. IX. The binding of Cadmium, Zinc, Lead and Mercury by glutathione. *J. Am. Chem. Soc.* **1973**, *17*, 6944–6950.

(29) Oram, P. D.; Fang, X.; Fernando, Q.; Letkeman, P.; Letkeman, D. Formation constants of Mercury(II)-glutathione complexes. *Chem. Res. Toxicol.* **1996**, *9*, 709–712.

(30) Maeda, M.; Okada, K.; Wakabayashi, K.; Honda, K.; Kaname, I. Complexation of thiomalic acids with mercury(II) and lead(II) under physiological conditions. *J. Inorg. Biochem.* **1991**, *42*, 37–45.

(31) Shoukry, M. M. Complex formation equilibria between mercury(II) complexes of penicillamine and glutathione and transition metal ions. *Transition Met. Chem.* **1990**, *15*, 1–4.

(32) Patil, P. R.; Krishnan, V. Thiomalates of divalent zinc, cadmium, mercury and lead. *J. Inorg. Nucl. Chem.* **1978**, *40*, 1255–1257.

(33) Fang, X.; Hua, F.; Fernando, Q. Comparison of rac- and meso-2,3-dimercaptosuccinic acids for chelation of mercury and cadmium using chemical speciation models. *Chem. Res. Toxicol.* **1996**, *9*, 284–290.

(34) Strand, R.; Lund, W.; Aaseth, J. Complex formation of zinc, cadmium, and mercury with penicillamine. *J. Inorg. Biochem.* **1983**, *19*, 301–309.