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Amide group coordination to the Hg^{2+} ion. Potentiometric, ^1H NMR and structural study on Hg^{2+}–*N*-protected amino acid systems †

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The binary complexes of Hg^{2+} formed by *N*-carbonyl and *N*-sulfonyl amino acids, which are ligands containing peptide and sulfonamide groups respectively, are investigated in aqueous solution by ^1H NMR, UV spectroscopy and potentiometry. The corresponding ternary systems with 2,2′-bipyridine are studied in aqueous solution by potentiometry and in DMSO solutions by ^1H-NMR. All the amino acids behave as simple carboxylate ligands at acid pH, while, around neutrality, *N*-*p*-tolylsulfonylglycine ($tsglyH_2$), *N*-*p*-tolylsulfonyl-β-alanine (ts-β-$alaH_2$) and *N*-2-nitrophenylsulfonylglycine ($NO_2psglyH_2$) switch to dianionic N,O-bidentate chelating ligands due to the involvement of the deprotonated amide nitrogen as an additional donor site. The Hg^{2+} ion is ineffective in promoting peptide nitrogen deprotonation in *N*-benzoylglycine (bzglyH). The binary and ternary species formed in aqueous solution and their stability constants are determined and compared with those of the homologous complexes of Pd^{2+}, Cu^{2+}, Cd^{2+} and Pb^{2+}. The molecular structure of $[Hg(bpy)_2(NO_2\text{-}psgly\text{-}N,O)]\cdot 0.5H_2O$ is determined by X-ray crystallography. It represents a rare example of Hg^{2+} N,O coordination by an amino acid molecule. In the complex Hg^{2+} shows a distorted octahedral environment with a N_5O donor set. Four nitrogen atoms are derived from the two bpy ligands, while the oxygen and the fifth nitrogen are from the NO_2-psgly dianion. New information on the solution and solid state chemistry of Hg^{2+} with ligands of biological interest is provided which may be of great relevance in understanding the mechanism of metal toxicity.

Introduction

Mercury (Hg^{2+}) and methylmercury (CH_3Hg^+) cations are known to react extensively with living organisms [1] and the chain targets for this type of complexation are thiol ligands [2,3] such as the cysteine residues in proteins. Cysteine coordination to Hg^{2+}, in a mercury metalloregulatory protein (mercuric reductase), has been investigated.[4–7] Although Hg^{2+} coordination chemistry is a key factor in determining the biological effects of this metal, relatively few studies, concerning Hg^{2+} interactions with simple biomolecules, are available, while a great number of studies on the CH_3Hg^+ cation interacting with amino acids and peptides are reported either in solid [8] or in solution.[9]

Potentiometric data on Hg^{2+}–dipeptide systems [10] show the formation of 1 : 1 complexes of $[Hg(HL)]^+$ type and peptide nitrogen deprotonation is not observed. Crystal structures of Hg^{2+} complexes with ligands containing donor groups of biological relevance have never been throughly investigated and the few reported data often concern $HgCl_2$ adducts.[11]

N-Sulfonylamino acids are low molecular weight ligands that were found to reproduce the coordination behaviour of peptides and their selectivity towards metal ions. At pH values close to neutrality these amino acids bind dipositive metal ions as dianions, through one carboxylate oxygen (acting as primary binding site) and the deprotonated sulfonamide nitrogen with the formation of a five membered chelate ring. On increasing pH, the deprotonation of the sulfonamide nitrogen in *N*-*p*-tolylsulfonylglycine ($tsglyH_2$) takes place with a markedly lower pK_{NH} than that of the free ligand and is promoted to different extents according to the coordinated metal ($Pd^{2+} > Pb^{2+} > Cu^{2+} > Cd^{2+}$).[12–15]

N-Carbonylamino acids such as *N*-benzoylglycine (bzglyH) were found to undergo nitrogen deprotonation only in the presence of Pb^{2+} ion,[15] while Pd^{2+} was unable to form N,O-chelate complexes.[16]

In ternary 2,2′-bipyridine systems the additional heteroatomic ligand favours Cu^{2+} and Cd^{2+} substitution for the sulfonamide nitrogen-bound hydrogen and induces binding of these ligands as N,O-dianions also toward Zn^{2+}, Co^{2+} and Ni^{2+} ions.[17–19]

In this work we investigate the binding ability of *N*-carbonyl- and *N*-sulfonyl-amino acids toward Hg^{2+} in binary and 2,2′-bipyridine (bpy) containing ternary systems. The presence of a substituent in *ortho* position, such as a nitro group, on the aromatic moiety of the $ArSO_2N$ amino acids was found to enhance the coordination properties of such molecules both in the solid and in the solution state.[20–21] By means of potentiometric and ^1H NMR data we find that Hg^{2+} ion is effective in promoting sulfonamide nitrogen deprotonation in $ArSO_2$-N-protected amino acids in binary and in 2,2′-bipyridine containing ternary systems. In addition the crystal and molecular structure of the $[Hg(bpy)_2(NO_2psgly\text{-}N,O)]\cdot 0.5H_2O$ complex is reported (NO_2psgly = *N*-2-nitrophenylsulfonylglycinate dianion). It represents a rare example of Hg^{2+} N,O-coordinated by a amino acid molecule.

Experimental

Materials

N-*p*-Tolylsulfonylglycine ($tsglyH_2$), *N*-*p*-tolylsulfonyl-β-alanine (ts-β-$alaH_2$) and *N*-(2-nitrophenylsulfonyl)glycine ($NO_2psglyH_2$) were synthesized as in ref. 20, while *N*-benzoylglycine (bzglyH) was from Carlo Erba. Stock solutions of $HgCl_2$ were standardized by means of a SPECTROFLAME D ICP plasma spectrometer; samples contained 1% of HNO_3 (BDH-Aristar).

† Electronic supplementary information (ESI) available: IR bands, least-squares planes. See http://www.rsc.org/suppdata/dt/b1/b100310k/

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Preparation of the complexes

Hg(L-*N*,*O*). (In what follows O and N,O refer to ligands binding as carboxylate monoanions and as N,O-chelating dianions respectively; L = tsgly, ts-β-ala or NO_2psgly). A 10 ml portion of an $Hg(CH_3CO_2)_2$ aqueous solution (0.05 M) was added under continuous stirring to 20 ml of an aqueous ethanolic (2 : 1 v/v) solution of the appropriate amino acid solution (0.05 M) at pH 8. The pH was kept constant by adding small amounts of concentrated aqueous NaOH. On standing for a few days white powders separated. They were collected by vacuum filtration and washed with ethanol. Hg(tsgly-*N*,*O*): Calc. for $C_9H_9HgNO_4S$ C, 25.16; H, 2.11; N, 3.26; S, 7.46%; Found C, 25.12; H, 2.27; N, 3.23; S, 7.30%; yield = 50%. Hg(ts-β-ala-*N*,*O*): Calc. for $C_{10}H_{11}HgNO_4S$ C, 27.16; H, 2.51; N, 3.17; S, 7.26%; Found C, 27.32; H, 2.58; N, 3.21; S, 7.10%; yield 60%. Hg(NO_2psgly-*N*,*O*): Calc. for $C_8H_6HgN_2O_6S$ C, 20.93; H, 1.32; N, 6.11; S, 6.99%; Found C, 21.03; H, 1.50; N, 5.98; S, 7.10%; yield = 40%.

Hg(bzgly-*O*)$_2$. The compound was prepared as described above. Separation of solid compounds was achieved in the pH range 4.5–6. Calc. for $C_{18}H_{16}HgN_2O_6$: C, 38.80; H, 2.90; N, 5.03%. Found: C, 38.68; H, 3.02; N, 5.00%. Yield = 30%.

Hg(bpy)(L-*N*,*O*) (L = tsgly, ts-β-ala or NO_2psgly). A 10 ml portion of an aqueous solution of $Hg(CH_3CO_2)_2$ (0.05 M) was added under continuous stirring to an aqueous ethanolic (2 : 1 v/v) solution of the appropriate amino acid and bpy, both 0.05 M. After a few days of evaporation, white powders separated from the solutions. They were collected under vacuum filtration and washed with ethanol. [Hg(bpy)(tsgly-*N*,*O*)]: Calc. for $C_{19}H_{17}HgN_3O_4S$ C, 39.10; H, 2.93; N, 7.20; S, 5.49%; Found C, 40.58; H, 3.29; N, 6.69; S, 6.32%; yield = 40%. [Hg(bpy)(ts-β-ala-*N*,*O*)]: Calc. for $C_{20}H_{19}HgN_3O_4S$ C, 40.19; H, 3.20; N, 7.03; S, 5.36%; Found: C, 38.65; H, 3.36; N, 6.53; S, 5.10%; yield = 50%. [Hg(bpy)(NO_2psgly-*N*,*O*)]: Calc. for $C_{18}H_{14}HgN_4O_6S$: C, 35.17; H, 2.29; N, 9.11; S, 5.22%. Found: C, 34.92; H, 2.10; N, 8.90; S, 4.75%; yield = 40%.

[Hg(bpy)$_2$(NO_2psgly-*N*,*O*)]·0.5H$_2$O. By slow evaporation of mother liquor, after separation of the [Hg(bpy)(NO_2psgly-*N*,*O*)] complex, white crystals separated, useful for X-ray analysis. Calc. for $C_{28}H_{23}HgN_6O_{6.5}S$: C, 43.08; H, 2.97; N, 10.78; S, 4.11%. Found: C, 44.12; H, 2.80; N, 10.20; S, 3.98%. Yield = 10%.

[Hg(bpy)(bzgly-*O*)$_2$]. This was prepared as were the [Hg(bpy)-(L-*N*,*O*)] complexes. Calc. for $C_{28}H_{24}HgN_4O_6$: C, 47.17; H, 3.39; N, 7.86%. Found: C, 46.95; H, 3.50; N, 7.48%.

Spectroscopy

^{1}H NMR spectra were obtained on a Bruker Avance DPX-200 spectrometer at 200.13 MHz with a Broad Band 5 mm probe (inverse detection). The typical acquisition parameters are as follows: spectral bandwidth 2 KHz, pulse width 7.6 μs (90° pulse), pulse delay 1 s, number of scans 216–512. The ligands and binary complexes spectra were run on aqueous (D_2O) millimolar solutions. Small amounts of concentrated NaOH or HNO_3 were added in order to adjust the pD values. pD Values are reported throughout the paper. The ternary complexes, because of their low solubility in aqueous media, and binary complexes were run in deuteriated DMSO millimolar solutions. A combined electrode was standardized by titration of a known volume of pure dimethyl sulfoxide with a standard solution of salicylic acid in DMSO [$(pK_{HA})_{DMSO} = 6.8$; $(pK_{HA})_w = 3.0$].[22] An excellent adherence to the Nernst equation of E(mV) *vs.* pa_H was observed, with maximum deviations of ±1 mV over a range of 10^{-4} to 10^{-1} M acid. All the spectra were performed at 30 ± 0.1 °C and referenced to tetramethylsilane.

Spectrophotometric titration were performed using a Perkin-Elmer Lambda 19 spectrophotometer at 25 ± 0.1 °C in the 200–500 nm spectral range employing a 1 cm cell length. The solutions contained Hg^{2+}/L in 1 : 1 molar ratio, with $[Hg^{2+}] = 1 \times 10^{-4}$ M. The pH of the solutions was adjusted by adding small amounts of concentrated aqueous NaOH solutions.

Infrared spectra of solid compounds were recorded with a Perkin-Elmer FT-IR 1600 instrument as KBr pellets in the spectral range 4000–400 cm^{-1}; a table reporting the more relevant IR bands, with their tentative assignment, is available as ESI supplementary material.

Potentiometry

Potentiometric measurements were performed in aqueous solutions at 25 ± 0.1 °C using a fully automated ORION 960 Autochemistry system and following the general procedures previously reported.[23] All experiments were carried out in a nitrogen atmosphere at ionic strength 0.1 M (adjusted with solid $NaNO_3$); the equivalence point was determined by the first derivative technique with constant volume increments. The stability constants (β_{pqrs}), which are defined by eqns. (1) and (2),

$$pM + qA + rL + sH \rightleftharpoons M_pA_qL_rH_s \quad (1)$$

$$\beta_{pqrs} = [M_pA_qL_rH_s]/[M]^p[A]^q[L]^r[H]^s \quad (2)$$

where M is the metal, A 2,2′-bipyridine, L the amino acid in the deprotonated form and H is proton, were refined by least-squares calculation using computer program SUPERQUAD,[24] taking into account the presence of $[Hg(OH)]^+$ and $[Hg(OH)_2]$ species.[25]

The concentration of the starting solution of the amino acid together with the protonation constants was determined by at least four titrations of 5×10^{-3} M solutions.

In the binary system the starting solutions for each titration were prepared by addition of known volumes of $HgCl_2$ (0.1 M) and amino acid (0.01 M) solutions in 1 : 1, 1 : 2 and 1 : 4 metal-to-ligand molar ratios. Hg^{2+} concentration ranged from 5×10^{-3} to 5×10^{-4} M, for all systems. Aqueous NaOH (0.05 M) was used as titrant. In the ternary systems the starting solutions were prepared as in the binary with the addition of known volumes of aqueous bpy solution (0.01 M) to have 1 : 1 : 1, 1 : 1 : 2, 1 : 1 : 4 M : bpy : L molar ratios. Hg^{2+} was 1.25×10^{-4} M for all systems and the titrant was NaOH (5×10^{-3} M). Ten measurements at least were performed for each system with 40 data points in each titration in the pH range 3–10.

X-Ray crystallography

A single crystal of [Hg(bpy)$_2$(NO_2psgly-*N*,*O*)]·0.5H$_2$O was mounted on a glass fiber and data were collected on an Enraf Nonius CAD4 diffractometer. Crystallographic data are summarized in Table 1. All data were corrected for Lorentz-polarization effects, while an absorption correction was not applied due to the absence of appropriate reflections. The structure was solved by conventional Patterson and Fourier techniques. The structure was refined by full-matrix least-squares calculations with anisotropic thermal parameters for all non-hydrogen atoms. The phenyl ring of the amino acid molecule was refined as a rigid group. Hydrogen atoms were calculated and used as fixed contributors. All calculations were carried out on a personal computer with SHELX 76,[26] SHELXL 93[27] and ORTEP[28] programs.

CCDC reference number 156235.

See http://www.rsc.org/suppdata/dt/b1/b100310k/ for crystallographic data in CIF or other electronic format.

Table 1 Crystallographic data for the complex $[Hg(bpy)_2(NO_2psgly\text{-}N,O)]\cdot 0.5H_2O$

Formula	$C_{28}H_{23}HgN_6O_{6.5}S$
M	780.17
Crystal system	Monoclinic
Space group	$C2/c$
a/Å	20.459(3)
b/Å	18.653(3)
c/Å	17.972(3)
β/deg	109.97(2)
V/Å^3	6446(2)
Z	8
T/K	293
λ(Mo-Kα)/Å	0.71069
μ/cm^{-1}	48.89
Reflections collected	6633
Reflections used in the refinement ($I > 2\sigma I$)	2666
Unique reflections	5694
wR(all)	0.202
R [$I > 2\sigma(I)$]	0.086
wR(abs)	0.195

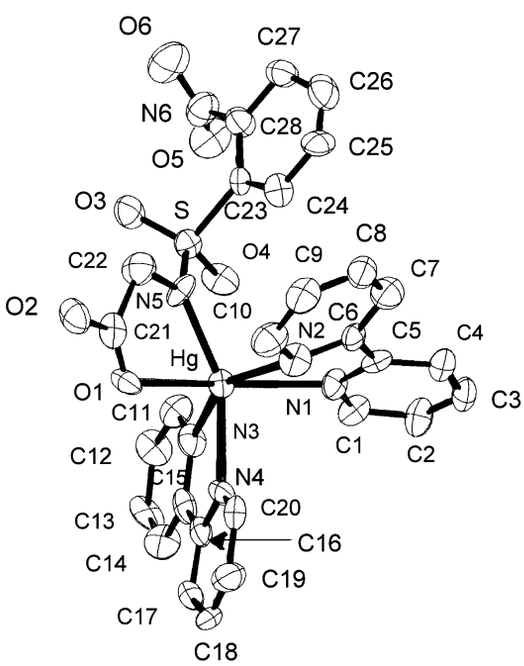

Fig. 1 An ORTEP view of the $[Hg(bpy)_2(NO_2psgly\text{-}N,O)]$ moiety showing atom numbering ellipsoids (40%) for non-hydrogen atoms.

Results and discussion

Description of the structure of $[Hg(bpy)_2(NO_2psgly\text{-}N,O)]\cdot 0.5H_2O$

Main bond distances and angles are reported in Table 2 with the atom numbering as in Fig. 1. In the complex molecule the Hg^{2+} environment can be described as distorted octahedral with a N_5O donor set. Four nitrogen atoms are derived from the two bpy ligands, while the oxygen and the fifth nitrogen are from the NO_2psgly dianion, so three five-membered chelate rings are formed. N(1), N(2), N(3) and O(1) act as equatorial ligands with deviation from the main plane of about 0.1 Å; N(4) and N(5), at shorter distances, are the apical ligands with a *trans* angle of 152.8(6)°. Six-coordination is rather common for Hg^{2+} ion although this structure represents a rare example of Hg^{2+} coordinated by an amino acid molecule acting as chelating agent *via* N,O donor atoms. The structure of bis[(*S*-methyl-L-cysteinato)mercury(II)][29] is known where the amino acid is N,O bonded with two almost collinear Hg–N bonds of 2.16(3) and 2.13(3) Å, and eight Hg · · · O distances in the range 2.54–3.75 Å. Hg^{2+} often prefers sulfur coordination as in the structure of methioninediperchloratomercury(II)[30] where Hg^{2+} ion is octahedrally coordinated by carboxylic oxygen atoms and sulfur atoms of methionine in a near collinear S–Hg–S bond. On the contrary CH_3Hg^+ has a strong tendency to form complexes with amino acids or small peptides, but it prefers linear or trigonal coordination geometry.[31]

Table 2 Selected bond distances (Å) and angles (deg) for $[Hg(bpy)_2(NO_2psgly\text{-}N,O)]\cdot 0.5H_2O$

Hg–N(5)	2.14(2)	Hg–N(4)	2.33(2)
Hg–N(3)	2.42(2)	Hg–N(1)	2.42(2)
Hg–O(1)	2.60(2)	Hg–N(2)	2.68(2)
N(1)–C(5)	1.33(2)	N(1)–C(1)	1.35(2)
N(2)–C(6)	1.36(2)	N(2)–C(10)	1.33(2)
N(3)–C(15)	1.31(2)	N(3)–C(11)	1.34(2)
N(4)–C(20)	1.31(2)	N(4)–C(16)	1.33(2)
O(1)–C(21)	1.18(3)	O(2)–C(21)	1.23(2)
C(21)–C(22)	1.63(4)	C(22)–N(5)	1.48(3)
N(5)–S	1.56(2)	S–O(3)	1.42(2)
S–O(4)	1.45(2)	S–C(23)	1.79(1)
C(28)–N(6)	1.49(3)	N(6)–O(5)	1.18(3)
N(6)–O(6)	1.22(3)		
N(5)–Hg–N(4)	152.8(6)	N(5)–Hg–N(3)	113.4(6)
N(4)–Hg–N(3)	71.4(6)	N(5)–Hg–N(1)	113.3(7)
N(4)–Hg–N(1)	92.0(6)	N(3)–Hg–N(1)	96.4(6)
N(5)–Hg–O(1)	69.7(6)	N(4)–Hg–O(1)	83.4(6)
N(3)–Hg–O(1)	110.2(6)	N(1)–Hg–O(1)	149.8(5)
N(5)–Hg–N(2)	89.6(6)	N(4)–Hg–N(2)	92.5(6)
N(3)–Hg–N(2)	155.3(6)	N(1)–Hg–N(2)	64.8(5)
O(1)–Hg–N(2)	85.5(5)	C(5)–N(1)–C(1)	124(2)
C(5)–N(1)–Hg	121.0(12)	C(1)–N(1)–Hg	115.4(12)
C(10)–N(2)–Hg	123.2(14)	C(10)–N(2)–C(6)	123(2)
C(11)–N(3)–Hg	124(2)	C(6)–N(2)–Hg	109.1(11)
C(20)–N(4)–Hg	119(2)	C(11)–N(3)–C(15)	121(2)
O(1)–C(21)–O(2)	127(3)	C(15)–N(3)–Hg	114.5(14)
C(22)–N(5)–S	117(2)	C(20)–N(4)–C(16)	122(2)
S–N(5)–Hg	119.7(9)	C(16)–N(4)–Hg	118(2)
O(3)–S–N(5)	112.6(9)	C(21)–O(1)–Hg	114(2)
O(3)–S–C(23)	103.8(9)	C(22)–N(5)–Hg	123(2)
N(5)–S–C(23)	107.5(9)	O(3)–S–O(4)	118.7(10)
O(5)–N(6)–C(28)	122(2)	O(4)–S–N(5)	106.5(9)
O(5)–N(6)–O(6)	126(3)	O(4)–S–C(23)	107.1(8)
O(6)–N(6)–C(28)	112(3)		

The N–Hg–N angles within the chelate rings range from 64.8(5) to 71.4(6)° and are similar to those found in other Hg^{2+}–bpy complexes.[32,33] The $Hg–N_{bpy}$ bond distances are in the range 2.33–2.68 Å, of the 2.22–2.56 Å interval of other Hg–bpy complexes.[32–34] The Hg–N(5) sulfonamide distance [2.14(2) Å] is the shortest, because of the great ligand strength of the deprotonated sulfonamide nitrogen, and is similar to those reported for complexes with amino acids.[29] The Hg–O(1) bond distance falls in the range [2.2–2.9 Å] found in carboxylate complexes.[35] In addition longer contacts with the S atom (3.22(2) Å) and sulfonic O4 atom (3.07(2) Å) are observed, within the sum of van der Waals radii. The glycine-like ring forms dihedral angles of 64.7(2) and 55.4(2)° with the chelate rings involving the bipyridine molecules; the angle between the chelate rings of bipyridine is 76.4(3)°. Bond distances and angles in the amino acid moiety are similar to those observed in the free ligand and in other NO_2psgly containing metal complexes.[20] The main difference is in the dihedral angle formed by the benzene ring and the NO_2 group, which is 59.9(2)° in this complex and 31.5(1)° in free $NO_2psglyH_2$. This may be attributed to the presence of intramolecular interactions between NO_2psgly and bpy(1) (range 3.7–3.9 Å), which causes also a distortion in bpy; in fact the internal rotation angle about the 2,2′ bond is 27.4° in bpy(1) and 2.4° in bpy(2). Ring stacking interactions involving bpy(1) and symmetry related bpy(2) (range 3.5–3.8 Å) are also present, while the water molecule is involved in a possible hydrogen bond with sulfonic oxygen O3.

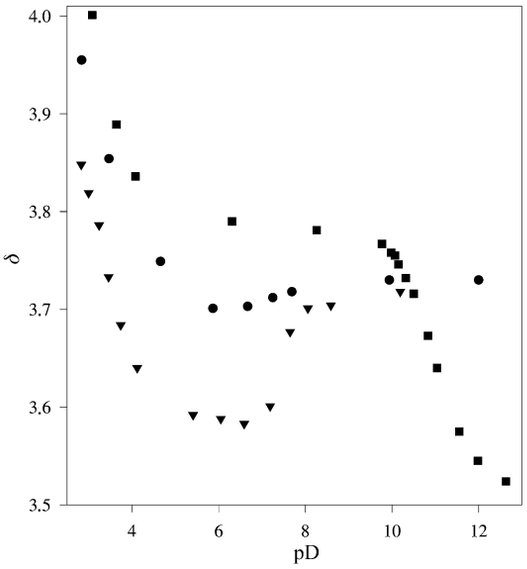

Fig. 2 pD Dependence of the chemical shift of the methylene resonances in the ^{1}H NMR spectra in D_2O: (■) NO_2psglyH$_2$ free ligand; (●) saturated solution of [Hg(NO_2psgly-*N*,*O*)]; (▼) saturated solution of [Hg(tsgly-*N*,*O*)].

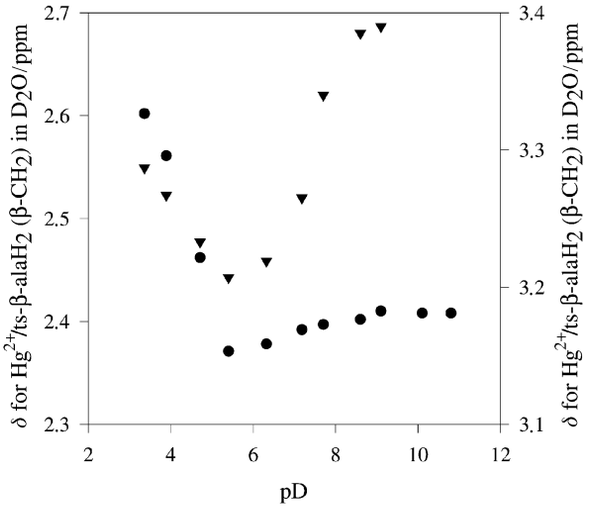

Fig. 3 pD dependence of the chemical shift of the methylene resonance in the ^{1}H NMR spectra of a saturated D_2O solution of [Hg(ts-β-ala)]: (●) α-CH_2; (▼) β-CH_2.

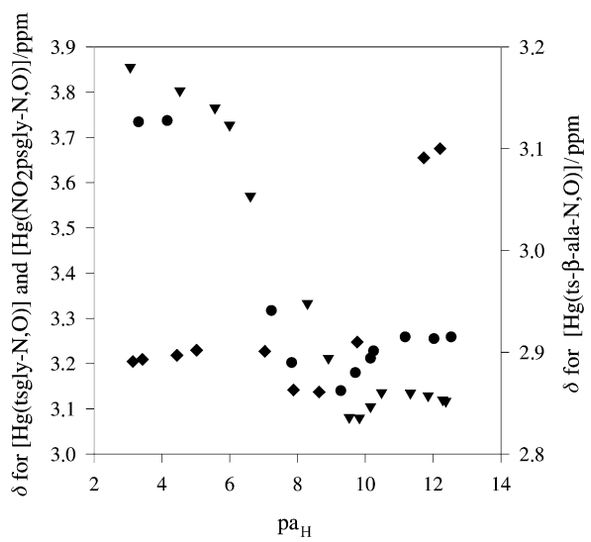

Fig. 4 pa_H dependence of the chemical shift of the methylene resonance in ^{1}H NMR spectra of saturated solutions of binary solid complexes in deuteriated DMSO: (●) [Hg(NO_2psgly-*N*,*O*)]; (▼) [Hg(tsgly-*N*,*O*)]; (◆) [Hg(ts-β-ala-*N*,*O*)] (β-CH_2).

Infrared spectra

A common feature for all the complexes is the position of ν_{asym}(OCO) (*ca*. 1600 cm^{-1}) as a consequence of the dissociation and coordination to the metal ion of the carboxylic group. In *N*-sulfonylamino acid containing complexes the disappearance of ν(N–H), which is present for bzgly containing complexes, is in line with the presence of a deprotonated and metal coordinated nitrogen atom.

As a general behaviour, the deprotonation and metal coordination of sulfonamide nitrogen increases the bond order of S–N and slightly decreases the S–O bond order.[36] This leads to ν(S–N) at higher frequency whereas asymmetric and symmetric $\nu(SO_2)$ are shifted to lower frequencies.

^{1}H NMR spectroscopy

D_2O solutions. The pD dependence of methylene peak(s)'s chemical shift are investigated for the free ligand NO_2psglyH$_2$ (tsglyH$_2$, bzglyH and ts-β-alaH$_2$ are reported in a previous work[15]) and for binary and ternary bpy containing Hg^{2+} systems.

The titration of NO_2psglyH$_2$ (Fig. 2) gives two pK_a values (correction applied pH = pD − 0.4;[37] all the calculated pK_a values have estimated error of ±0.2): 2.9 and 10.6 corresponding respectively to the equilibrium of carboxylic group dissociation and sulfonamide nitrogen deprotonation. These values are consistent with those found through potentiometric and spectrophotometric analysis.[21]

The titrations of binary systems (Figs. 2 and 3), performed on millimolar solutions, yield apparent pK_a values for the carboxylic group of 3.2, 3.3, 3.9 and 3.6 respectively for Hg^{2+}/NO_2psglyH$_2$, Hg^{2+}/tsglyH$_2$, Hg^{2+}/ts-β-alaH$_2$ and Hg^{2+}/bzglyH; these values are almost unchanged with respect to those of the free ligands.

In all binary systems (except bzglyH) at pD values greater than 6 the chemical shift (δ) of the methylene peak(s) increases to reach a maximum around pD 9. The Hg^{2+}/ts-β-alaH$_2$ spectrum shows a β-CH_2 peak broadening with loss of structure consistent with a quadrupolar interaction with a nitrogen atom. On increasing pD value the spectra reveal a triplet structure deformation of α-CH_2 due to the inequivalency of β-CH_2 protons in the chelate ring. Binary systems are stable and no precipitation of HgO is observed, even at high pD values.

In the Hg^{2+}/bzglyH binary system no hint of Hg^{2+} inducing amide nitrogen deprotonation is observed. The calculated pK_{NH} values are 6.5 for the Hg^{2+}/NO_2psglyH$_2$, 6.7 for Hg^{2+}/ts-β-alaH$_2$ and 7.1 for Hg^{2+}/tsglyH$_2$ systems.

DMSO solutions. The ^{1}H NMR study of the ligands in this solvent was prevented by the insolubility of their alkal-metal salts, while binary and ternary systems were investigated on varying pH. The titolative trend observed by adding NaOH and HNO_3 to a deuteriated DMSO solution of the solid binary complexes (Fig. 4) parallels the behaviour in D_2O. The pK_a values for the carboxylic group were 6.9, 7.2, 7.4 and 7.0 for Hg^{2+}/NO_2psglyH$_2$, Hg^{2+}/tsglyH, Hg^{2+}/ts-β-alaH$_2$ and Hg^{2+}/bzglyH respectively, while the pK_{NH} were 9.8 for Hg^{2+}/NO_2-psglyH$_2$ and 10.3 for Hg^{2+}/tsglyH$_2$ and Hg^{2+}/ts-β-alaH$_2$. In the binary Hg^{2+}/bzglyH system no further variation of the methylene group chemical shift is observed, after the carboxylic group dissociation.

The ^{1}H NMR spectra run on deuteriated DMSO solutions of the solid ternary complexes show only one equivalence point with pK_a of 6.8 for Hg^{2+}/NO_2psglyH$_2$ and Hg^{2+}/tsglyH$_2$, 8.6 for Hg^{2+}/ts-β-alaH$_2$ (Fig. 5). This behaviour suggests the simultaneous deprotonation of both carboxylic group and sulfonamide nitrogen, as was previously observed in the Pd^{2+}-

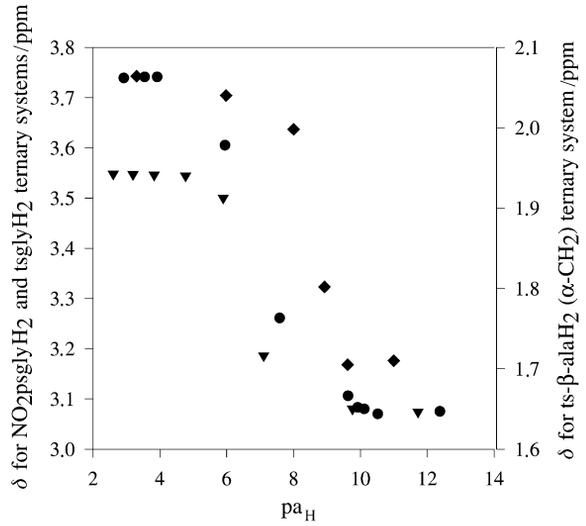

Fig. 5 pa_H dependence of the chemical shift of the methylene resonance in ^{1}H NMR spectra of saturated solutions of ternary solid complexes in deuteriated DMSO: (●) [Hg(bpy)(NO_2psgly-*N*,*O*)]; (▼) [Hg(bpy)(tsgly-*N*,*O*)]; (◆) Hg(bpy)(ts-β-ala-*N*,*O*)].

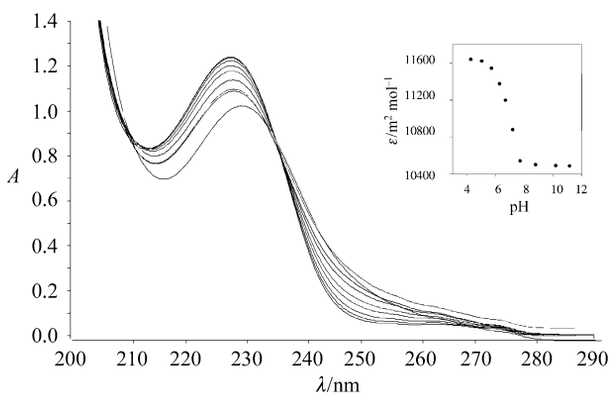

Fig. 6 Electronic spectra of the system Hg^{2+}/tsglyH_2 in the molar ratio 1 : 1, $[Hg^{2+}] = 10^{-4}$ M, at pH varying from 4.27, 5.04, 6.28, 6.69, 7.20, 7.70, 8.80, 10.20 to 11.16 in order of decreasing absorbance. The inset shows the overall pH dependence of the molar absorption coefficient at 228 nm.

containing systems with $ArSO_2N$-protected amino acids.[14,16] In the Hg^{2+}/bzglyH ternary system the pK_a value observed is quite similar to that found for the carboxylic group in the binary system; in addition a broad peak assigned to the sulfonamide nitrogen proton is observed in all spectra at different pa_H, confirming that the only complex species formed is of the carboxylate type.

UV Spectroscopy

The spectrophotometric titrations of Hg^{2+}/H_2L systems at 500–200 nm reveal the presence of an isosbestic point (Fig. 6); plotting absorbance *vs.* pH near 230 nm for tsglyH_2 and ts-β-alaH_2 and 270 nm for NO_2psglyH_2, a titration pattern is observed. The estimated values of apparent pK_{NH} observed are 6.9(2), 6.5(2) and 6.4(2) respectively, near to those found by ^{1}H NMR data. The trend is consistent with the order of basicity of the ligands confirming also for Hg^{2+} that the amount of metal induced decrease in pK_{NH} is independent of the nature of the amino acid, as was previously found for Cu^{2+}/and Cd^{2+}/*N*-sulfonylamino acid systems.[21] In the Hg^{2+}/bzglyH system no spectral changes are observed on increasing pH, therefore excluding any amide nitrogen deprotonation, also in the presence of Hg^{2+}, confirming ^{1}H NMR data.

Potentiometry

Binary systems. The pH-metric titration curves of Hg^{2+}/H_2L systems (L = tsgly, ts-β-ala or NO_2psgly in the dianionic form) in 1 : 1 and 1 : 2 molar ratio show two equivalence points in accordance to eqns. (3) and (4) where *m* stands for number of

$$m_{NaOH} = m_L + m_{Hg} \quad (3)$$

$$m_{NaOH} = m_L + 2m_{Hg} \quad (4)$$

moles and L is the amino acid. The first equivalence point (pH ≈ 5) is consistent with the formation of [HgL] or [Hg(HL)(OH)] species, the second (pH ≈ 8) with the species $[HgL(OH)]^-$ and $[HgL_2]^{2-}$. Analysis of the electronic and ^{1}H NMR spectra suggests that the deprotonated [HgL] species is formed beyond pH 6.5, so the prevailing species at pH ≈ 5.5 may reasonably be [Hg(HL)(OH)]; the presence of mixed-ligand hydroxo complexes has previously been found in Hg^{2+}-containing systems with carbohydrate α-amino acids,[38] while in Cu^{2+}/tsglyH_2[12] and Cd^{2+}/tsglyH_2[13] systems such species were suggested to be intermediates in the reaction mechanism of sulfonamide nitrogen deprotonation.

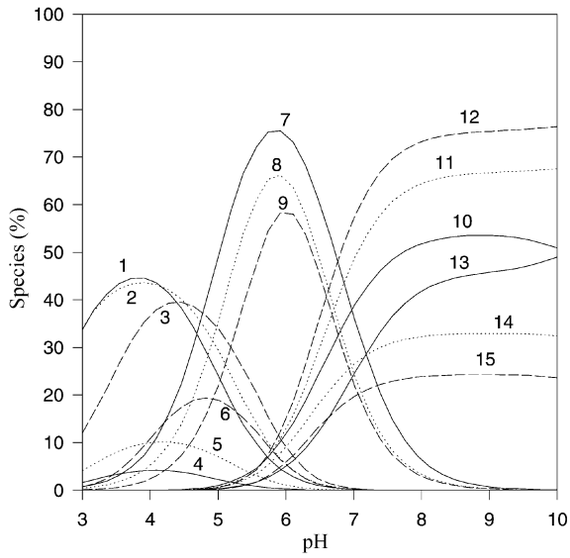

Fig. 7 Species distribution curves for the binary systems in $HgHg^{2+}$/ : H_2L 1 : 2 molar ratio, $[Hg^{2+}] = 10^{-3}$ M: 1, 2, 3 $[Hg(HL)]^+$; 4, 5, 6 $[Hg(HL)_2]$; 7, 8, 9 [Hg(HL)(OH)]; 10, 11, 12 $[HgL_2]^{2-}$; 13, 14, 15 $[HgL(OH)]^-$; ——, NO_2psglyH_2; ·····, tsglyH_2; – – – – ts-β-alaH_2.

The potentiometric titration of Hg^{2+}/bzglyH shows only one equivalence point corresponding to neutralisation of the carboxylic group of the ligand followed by precipitation of the metal hydroxide. The calculated formation constants of the complexes are reported in Table 3, while in Fig. 7 the species distribution curves are shown. In the Hg^{2+}/NO_2psglyH_2, Hg^{2+}/tsglyH_2 and Hg^{2+}/ts-β-alaH_2 systems the same prevailing species are observed. Owing to the electron-withdrawing effect of the sulfonyl group, log *β* values of $[Hg(HL)]^+$ and $[Hg(HL)_2]$ complexes are lower than those found for Hg^{2+} complexes with unsubstituted monocarboxylic acids (in the ranges 3.66–4.33 and 7.10–8.80 respectively),[40] while similar to those found for chloroacetate complexes (2.95 and 5.61 respectively).[40]

Regarding the deprotonated species $[HgL_2]^{2-}$ and the $[HgL(OH)]^{2-}$, the lower stability of the ts-β-ala complex with respect to the tsgly one is attributed to the effect of the greater strain of a six-membered chelate ring as compared to a five-membered one,[41] while the lower stability of NO_2psgly complexes is due to the steric hindrance of the NO_2 group which disfavours coordination of a second ligand molecule.

The log *β* values of $[HgL_2]^{2-}$ species are remarkably greater with respect to those of Hg^{2+} complexes with dipeptides (mean value 5.8 ± 0.8)[10] and amino acids such as 2-(benzylamino)-2-deoxy-D-glycero-D-guloheptonic acid (log *β* = 9.78)[42] owing to the greater basicity of the deprotonated sulfonamide nitrogen

Table 3 Logarithm of protonation constants of ligands and complex formation constants at $T = 298$ K, $I = 0.1$ M ($NaNO_3$)

Species		NO_2psgly	tsgly	ts-β-ala		bzgly
HL^-	$\log \beta_{0011}$	10.62(2)	11.35(3)	11.19(2)		
H_2L	$\log \beta_{0012}$	13.84(1)	14.56(2)	15.49(1)	HL^b	
	$\log K_{a1}{}^a$	3.22	3.21	4.30	$\log \beta_{0011}$	3.81(1)
$[Hg(HL)]^+$	$\log \beta_{1011}$	13.56(8)	14.32(5)	14.39(4)	$[HgL]^{+\,b}$	
	$\log K_1{}^c$	2.94	2.97	3.20	$\log \beta_{1010}$	3.01(6)
$[Hg(HL)_2]$	$\log \beta_{1022}$	26.08(5)	27.98(3)	28.38(4)	$[HgL_2]^b$	
	$\log K_2{}^d$	4.84	5.28	5.99	$\log \beta_{1020}$	5.73(4)
[Hg(HL)(OH)]	$\log \beta_{1010}$	8.87(2)	9.40(3)	9.20(3)		
$[HgL_2]^{2-}$	$\log \beta_{1020}$	15.72(3)	16.91(4)	16.42(4)		
$[HgL(OH)]^{2-}$	$\log \beta_{101\text{-}1}$	1.69(4)	2.69(3)	2.60(3)		
$[Hg(bpy)L]^e$	$\log \beta_{1110}$	18.58(2)	19.08(6)	18.88(6)		
$[Hg(bpy)L(OH)]^-$	$\log \beta_{111\text{-}1}$	10.95(5)	11.97(4)	11.76(5)		
	$\log \beta_{1120}$				$[Hg(bpy)L_2]^b$	17.59(2)
	$\log \beta_{112\text{-}1}$				$[Hg(bpy)L_2(OH)]^{-\,b}$	11.66(7)

[a] $\log K_{a1} = \log \beta_{0012} - \log \beta_{0011}$. [b] In the case of bzgly L stands for the carboxylate anion. [c] $\log K_1 = \log \beta_{1011} - \log \beta_{0011}$. [d] $\log K_2 = \log \beta_{1022} - 2\log \beta_{0011}$. [e] $\log \beta$ value for $[Hg(bpy)]^{2+}$ is 9.6.[39]

Table 4 Logarithm of stability constants of M^{2+} complexes, $T = 298$ K, $I = 0.1$ M ($NaNO_3$)

System	$[M(HL)]^+$	$[M(HL)_2]$	[ML]	$[ML_2]^{2-}$	$[ML(OH)]^-$	Ref.
$Hg/tsglyH_2$	14.32(5)	27.98(3)		16.91(4)	2.69(3)	
$Hg/ts\text{-}\beta\text{-}alaH_2$	14.39(4)	28.38(4)		16.42(4)	2.60(3)	
$Hg/NO_2psglyH_2$	13.56(8)	26.08(5)		15.72(3)	1.69(4)	
$Pb/tsglyH_2$	12.48(8)	24.83(7)	6.43(7)			15
$Pb/ts\text{-}\beta\text{-}alaH_2$	12.40(7)	24.52(7)	6.50(7)			15
$Pb/NO_2psglyH_2$	12.20(2)	25.73(5)	6.90(2)			21
$Cu/tsglyH_2$		28.0(1)	7.6(1)	11.3(1)		12
$Cu/ts\text{-}\beta\text{-}alaH_2$		27.9(1)				17
$Cu/NO_2psglyH_2$	12.36(6)		8.59(1)	14.22(3)	0.62(7)	21
$Cd/tsglyH_2$	15.00(1)[a]	30.10(1)[a]	4.90(1)	6.00(1)		13
$Cd/ts\text{-}\beta\text{-}alaH_2$		28.14(7)	5.34(4)	9.90(8)		43
$Cd/NO_2psglyH_2$	12.5(3)		6.24(3)	10.45(9)	−2.95(5)	21
$Pd/tsglyH_2$			17.8(1)	23.4(1)		14
$Pd/ts\text{-}\beta\text{-}alaH_2$			16.8(1)	20.5(1)		14

[a] Values in water–CH_3OH solutions.

compared to that of the terminal amine nitrogen in Hg^{2+}-coordinated amino acids or peptides.

Comparing the behaviour of these ligands with various metal(II) ions (Table 4) it is seen that Hg^{2+} forms more stable complexes with *N*-sulfonylamino acids, apart from Pd^{2+}. In the case of Pd^{2+} complexes the sulfonamide nitrogen deprotonation doesn't pass through the preliminary formation of carboxylate species, as is found for other investigated metal ions,[16,44] although other authors excluded the formation of carboxylate complexes of Cu^{2+} with *N*-*p*-amino-(or nitro)-phenylsulfonyl amino acid derivatives.[45,46] For Pb^{2+}, Cd^{2+}, Cu^{2+} and Pd^{2+} the effectiveness in substituting for the sulfonamide nitrogen-bound hydrogen and the stability of the complexes formed parallel well the behaviour toward oligopeptides;[47] even though the complex formation of Hg^{2+} with dipeptides has not been investigated frequently, it was found that Hg^{2+} was unable to undergo amide nitrogen deprotonation in dipeptides.[10] Therefore the behaviour of Hg^{2+} with *N*-sulfonylamino acids is rather surprising and may be attributed to its affinity for the sulfur atom, as evidenced by the bond interaction found in the crystal structure of the solid ternary complex, which may favour the sulfonamide nitrogen deprotonation reaction.

Ternary system. The complex species observed in the bpy containing systems are [Hg(bpy)L] and $[Hg(bpy)L(OH)]^-$ and their evaluated stability constants are reported in Table 3. Fig. 8 shows the species distribution curves for the ternary systems. The most interesting feature of these systems is that no carboxylate type complexes are detected, confirming the hypothesis suggested by ^1H NMR spectra that the sulfonamide nitrogen deprotonation takes place almost contemporarily with carboxylic oxygen coordination to the metal ion. The stabilising effect of 2,2′-bipyridine is reflected by a marked diminishing of pK_{NH}, whose value becomes independent of the nature of the amino acid ligand, being around 4.5 for all the three ligands as shown from the distribution curves. Regarding the stability of the complex species we may observe the same trend found in the corresponding binary systems; in fact $tsglyH_2$ is the ligand that forms more stable complexes. In particular the log *X* value[47] $\{\log X = 2\log\beta^{Hg}_{[Hg(bpy)L]} - [\log \beta^{Hg}_{[HgL_2]^{2-}} + \log \beta^{Hg}_{[Hg(bpy)_2]^{2+}}]\}$ is 5.74 for tsgly, 4.64 for ts-β-ala and 4.74 for NO_2psgly. These differences may be attributed to the different ability of bpy to give rise to π conjugation with the aromatic moiety of the amino acid molecule. In fact the formation of a non-planar six-membered chelate ring in the ts-β-ala complex and the presence of the NO_2 group in the NO_2psgly one handicap the π conjugation with the aromatic system of the bpy molecule, diminishing the value of log *X*.

The overall stability constants for these ternary complexes are similar to those found for Pb^{2+} complexes[15] and, in particular for the $tsglyH_2$ system, follow the order of stability $Pd^{2+} \gg Hg^{2+} > Pb^{2+} > Cu^{2+} > Ni^{2+} > Zn^{2+} > Co^{2+} > Cd^{2+}$; indicating that the metal affinity for N,O-donor ligands is the major factor determining the stability of the complexes.

In the bzglyH ternary system the complex species found are $[Hg(bpy)L_2]$ (where L, in this case, is the monoanionic form of the amino acid), isolated also in the solid state at pH ≈ 5, and [Hg(bpy)L(OH)], where the amino acid acts invariably as a carboxylate ligand; their stability constants are reported in Table 3.

In conclusion we have proved the ability of Hg^{2+} ion to coordinate N-protected amino acid molecules forming N,O-

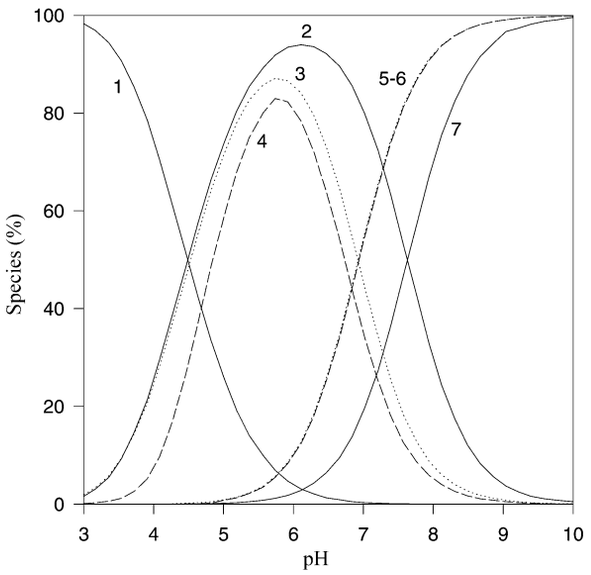

Fig. 8 Species distribution curves for ternary systems in Hg : bpy : H_2L 1 : 1 : 2 molar ratio, $[Hg^{2+}] = 1.25 \times 10^{-4}$ M: 1 $[Hg(bpy)]^{2+}$; 2, 3, 4 [Hg(bpy)L]; 5, 6, 7 $[Hg(bpy)L(OH)]^{-}$; ——, $NO_2psglyH_2$; ·····, $tsglyH_2$; – – – –, ts-β-alaH_2.

chelate complexes, either in the solid or solution state, in a wide range of pH. The stability of these complexes is greater than that found for Hg^{2+} complexes with methionine and L-cysteine[48] and is comparable to that of methylmercury(II)–thiol complexes.[49] The presence, in the ligand molecule, of a sulfur atom interacting with the metal ion, seems the driving force for the NH deprotonation and the formation of stable complexes.

These data confirm the great ability of Hg^{2+} to coordinate sulfur-containing biological ligands, when the sulfur atom is not considered a coordination site, a feature that can be the basis of its toxicity.

Acknowledgements

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