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Biophysical Chemistry 133 (2008) 19–27

Biophysical Chemistry

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Thermodynamic and spectroscopic study for the interaction of dimethyltin(IV) with L–cysteine in aqueous solution

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Received 24 October 2007; received in revised form 15 November 2007; accepted 15 November 2007
Available online 22 November 2007

Abstract

Thermodynamic and spectroscopic properties of the species formed by dimethyltin(IV) cation with L–cysteine (*cys*) were studied by potentiometric, calorimetric, UV and NMR investigations in aqueous solution. The resulting speciation model showed the formation of five complex species: $(CH_3)_2Sn(cys)H^+$, $(CH_3)_2Sn(cys)^0$, $(CH_3)_2Sn(cys)OH^-$, $(CH_3)_2Sn(cys)_2H^-$, $(CH_3)_2Sn(cys)_2^{2-}$. The stability and the formation percentages, for the mononuclear mixed species in particular, are very high, in a wide pH range. Thermodynamic parameters indicate that the enthalpy values are exothermic and the enthalpic contribution to the stability is higher than entropic one. Individual UV spectra of *cys* and dimethyltin(IV)–*cys* species were calculated. Spectroscopic results of UV and ^1H NMR investigations fully confirm the speciation model. The structures calculated from NMR investigations show that all the species have an eq–$(CH_3)_2$–tbp structure.

Keywords: Dimethyltin(IV) complexes; Thermodynamic parameters; Potentiometry; Calorimetry; Spectroscopy

1. Introduction

The study of the interactions between metal cations and amino acids is of fundamental relevance for the knowledge of natural occurring metalloprotein chelation as well as for human and environmental toxicology. The most relevant amino acids, able to bind metal cations, are histidine, cysteine, glutamic and aspartic acids [1]. *Cys* is the only naturally occurring sulfur-containing amino acid. It is a component of the antioxidant glutathione (L–γ–glutamyl–L cysteinyl–glycine). A living organism is able to synthesize *cys* from the essential amino acid methionine under normal physiologic conditions; furthermore, among the amino acids considered in blood plasma models it is present at the highest concentration, with a value ≥ 100 μmol L^{-1} [2]. *Cys* 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 enzymes cysteine proteases and in several peptides and proteins [3]. It is recognized that *cys* can coordinate metal cations through sulfur sites in several proteins and metalloenzymes [4–6].

In several investigations alkyltin(IV) cations, as metals, were used for studying their interaction with biological ligands. This choice is induced by the widespread use of organotin(IV) compounds in industrial, agricultural and biological field, which caused their release and accumulation into the environment and consequently in biological systems. It is well known that these alkyltin(IV) compounds are generally toxic and their toxicity depends on the nature of the alkyl groups bound to the tin(IV) atom and it is directly proportional to their number. In contrast, some others organotin compounds have been found active *in vitro* against human tumour cell lines [7]. For this reason the solution and structural studies on the interaction of the organotin moieties with several biologically relevant compounds such as carbohydrates, amino acids, peptides, nucleic acids and DNA has been carried out mostly in the last decade [8–21]. From some of these cited studies, the interaction of dimethyltin(IV) and trialkyltin(IV) (with R = methylic,

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doi:10.1016/j.bpc.2007.11.005

ethylic, and butylic groups) in aqueous solution, at physiological pH, with thiol groups of model molecules, as well as feline and rat haemoglobin, have been investigated by ^{119}Sn Mössbauer spectroscopy. The results showed that the above species form tetrahedral or trigonal bipyramidal tin sites, with covalent Sn–S bonds and further coordination by nitrogen donors, from *cys* and histidine side chain, respectively [14,15]. Since for understanding biological action of alkyltin chemicals, their speciation in biological systems must be known, it seems of interest to study quantitatively the interactions between alkyltin(IV) cation and *cys*. Thermodynamic data on metal–*cys* complexes are extremely poor. In particular, no calorimetric study has been performed. The only available data obtained by the van't Hoff equation, regard several metals, such as Pb^{2+}, Ni^{2+}, Hg^{2+} and others, but there are no enthalpic data on tin or alkyltin cations [21]. Spectroscopic studies on the characterization of several organotin(IV) derivatives of *cys* [17,18]; potentiometric [19,20] and Mössbauer [19] investigations on the coordination of *cys* and its derivatives towards trialkyltin(IV) cations can be found in literature.

This work represents a further step in our systematic investigations on the speciation of mono-, di- and tri-methyltin(IV) cations and their interactions with several carboxylic ligands in aqueous solution [22–30]. In this paper we give a complete picture of the thiolic group influence on the interaction of dimethyltin(IV) cation with *cys* by determining thermodynamic parameters by potentiometric and calorimetric measurements, describing the speciation in aqueous solution and confirming findings by spectroscopic (UV and ^{1}H NMR) investigations.

2. Experimental

2.1. Chemicals

Fresh dimethyltin(IV) solutions were prepared every day by weighing dichloride salt (Fluka product), twice re-crystallised before use. *Cys* (microselect >99.5%) (Fluka product) was used without further purification and its purity, which was always higher than 99.5%, was checked by potentiometric titrations with standard solutions of NaOH. Sodium hydroxide and hydrochloric acid solutions were prepared by diluting concentrated Fluka ampoules and were standardised against potassium biphthalate and sodium carbonate, respectively. All the solutions were prepared using analytical grade water (resistivity = 18 MΩ cm) and grade A glassware.

2.2. Potentiometric apparatus and procedure

Potentiometric titrations were carried out (at 25.0±0.1 °C) using an apparatus consisting of a Metrohm Model 713 potentiometer, equipped with a combined glass electrode (Ross type 8102, from Orion) and a Metrohm Model 765 motorized burette. Estimated accuracy was ±0.2 mV and ±0.003 mL for e.m.f. and titrant volume readings, respectively. The apparatus was connected to a PC, and automatic titrations were performed using a suitable computer program to control titrant delivery, data acquisition and to check for e.m.f. stability. All titrations were carried out under magnetic stirring and presaturated N_2 was bubbled through the purified solution in order to exclude O_2 and CO_2 inside. A volume of 25 mL of the solution containing *cys*, dimethyltin(IV) dichloride and NaCl was titrated with standard NaOH up to 80–90% neutralization. Details of experimental measurements are reported in Table 1. Separate titrations of HCl at the same ionic strength as the sample under study, were carried out to determine standard electrode potential E^0 and to obtain $pH = -\log [H^+]$ readings. The reliability of the calibration in the alkaline range was checked by calculating pK_w values.

2.3. Calorimetric apparatus and procedure

The measurements were carried out using a Tronac model 450 Isoperibolic Titration calorimeter, coupled with a Keithley 196 system Dmm digital multimeter. A volume of 50 mL of solution, containing dimethyltin(IV) dichloride at 25.000±0.001 °C was titrated with a solution of Na_3*cys*. Details of experimental measurements are reported in Table 1. The titrant was delivered by a 2.5 mL capacity Hamilton syringe, model 1002TLL. A computer program was used for the acquisition of calorimetric data. Accuracy was checked by titrating a THAM (tris–(hydroxymethyl)amino–methane) buffer with HCl. The heat of dilution was measured before each experiment. The accuracy of calorimetric apparatus was Q±0.008 J and v±0.001 cm^3.

2.4. UV spectra

The UV spectra were recorded using a Varian Cary 50 UV–VIS spectrophotometer with an optic fibre probe having a fixed 1 cm path length, from 220 to 280 nm. The spectrophotometer was connected to a PC for the acquisition of the spectra. The measurement cell and potentiometric apparatus are the same of those already described in the *potentiometric apparatus and procedure* paragraph. A volume of 25 mL of the solutions containing ligand or metal-ligand were prepared and analysed in a wide pH range values. Small increments of standard NaOH solution were added to each solution in order to obtain the maximum absorbance of each predominant species, according to the preliminarly determined speciation diagrams of the system. Details of experimental measurements are reported in Table 1.

2.5. NMR measurements

^{1}H NMR spectra were recorded on a Bruker AMX R-300 spectrometer. The proton chemical shifts were measured with respect to 1,4-dioxane as reference and converted relative to TMS, using $\delta_{dioxane} = 3.70$ ppm. Measurements were generally made in a 9:1 H_2O:D_2O solution. The ligand and metal concentrations were varied in the range 0.005–0.01 mol L^{-1}. In order to obtain the individual NMR parameters (δ, ^{2}J) of the various species, the protonic spectra were recorded at different pH ranging from 2 to 11. The individual chemical shifts as well as $^2J(^{119}Sn–^1H)$ coupling constants belonging to the dimethyltin(IV)–*cys* complexes were calculated assuming fast mutual exchange [12]. The heteronuclear couplings of the tin-

Table 1
Experimental conditions for potentiometric, calorimetric and spectrophotometric measurements at T=25 °C

Potentiometric measurements	C_{NaCl} [a]	$C_{(CH_3)_2SnCl_2}$ [a]	C_{cys} [a]	I [a b]	N_{tit} [c]	N_{pts} [d]
		0.002	0.003	0.008	2	125
		0.001	0.002	0.004	2	148
		0.002	0.006	0.009	2	136
		0.004	0.002	0.009	2	226
	0.14	0.002	0.003	0.142	2	146
	0.15	0.001	0.002	0.146	2	205
	0.14	0.002	0.006	0.141	2	163
	0.14	0.004	0.002	0.137	2	147
Calorimetric measurements	C_{NaCl} [a]	$C_{(CH_3)_2SnCl_2}$ [a]	C_{Na_2cys} [a]	I [a b]	N_{tit} [c]	N_{pts} [d]
	0.1	0.0025	0.2507	0.104	2	60
	0.1	0.005	0.2507	0.108	2	60
Spectrophotometric measurements	C_{HCl} [a]	$C_{(CH_3)_2SnCl_2}$ [a]	C_{cys} [a]	I [a b]	N_{tit} [c]	$N_{spectra}$ [e]
	0.012		0.0004	0.019	1	10
	0.020		0.0006	0.031	1	22
	0.101		0.002	0.129	1	15
	0.040		0.005	0.072	1	13
	0.004	0.002	0.0004	0.015	1	10
		0.0004	0.0008	0.004	1	10
	0.004	0.005	0.001	0.027	1	9
	0.003	0.0005	0.0005	0.009	1	32

[a] Concentrations in mol L^{-1}; [b] mean value of ionic strength; [c] number of titrations; [d] number of points; [e] number of spectra.

bound methyl groups $^2J(^{119}Sn-^1H)$ obtained in such a way were "converted" into C–Sn–C angles by using the published equation [31].

2.6. Calculations

All the parameters relative to alkalimetric purity determination were refined using the nonlinear least squares computer program ESAB2M. Formation constants were refined using the nonlinear least squares computer programs STACO and BSTAC. Speciation profiles were obtained using the computer program ES4EC. Details of calculation methods and programs have already been reported [32].

The interactions of dimethyltin(IV) with small amounts of Cl^- from the dimethyltin(IV) dichlorides and from NaCl used as ionic medium were taken into account in the calculations. Formation constants were extrapolated to zero ionic strength as already reported [33,34]. Both BSTAC and STACO computer programs can deal with potentiometric data obtained in variable ionic strength conditions. The dependence on ionic strength of formation constants was taken into account using the Debye–Hückel type equation:

$$\log\beta = \log^T\beta - z^*\sqrt{I}/\left(2+3\sqrt{I}\right) + CI \quad (1)$$

where

$C=(0.025\pm0.015)\ z^*$, at $I \le 0.15$
$z^* = \sum z^2_{reactants} - \sum z^2_{products}$
(β=stoichiometric formation constant; $^T\beta$=formation constant at infinite dilution; z is the component charge).

Calorimetric titration data were analysed by the computer program ES5CM [35]. UV spectra were analysed by the Hyperquad 2000 program [36], which allows to calculate stability constants and molar absorbance spectrum of each absorbing species, using experimental absorbances, analytical concentrations of reagents and the proposed chemical model as input. NMR calculations were performed using the general linear and nonlinear least squares computer program LIANA [32]. Concentrations and thermodynamic parameters are given in the molar scale. Errors are given as standard deviations. The overall formation equilibria are expressed as (charges omitted).

$$pM + qL + rH = M_pL_qH_r \quad (2)$$

where $r>0$ for protonated species and $r<0$ for hydrolytic species.

Thermodynamic parameters of protonation of *cys* used in the calculations, showed in Table 2 at I=0 mol L^{-1} and T=25 °C, have been published in the ref. [37]. Dimethyltin(IV) cation has a strong tendency to hydrolyze in aqueous solution, forming five hydrolytic species $[(CH_3)_2Sn(OH)^+$; $(CH_3)_2Sn(OH)^0_2$; $(CH_3)_2Sn(OH)^-_3$; $[(CH_3)_2Sn]_2(OH)^{2+}_2$; $[(CH_3)_2Sn]_2(OH)^+_3$, whose thermodynamic

Table 2
Thermodynamic parameters of protonation of *cys* at T=25 °C and I=0 mol L^{-1}

pqr	$\log\beta$ [a]	ΔH^{0} [a, b]
011	10.21	−42
012	18.39	−77
013	20.53	−81

[a] Ref. [37]; [b] expressed in kJ mol^{-1}.

Table 3
Thermodynamic parameters for hydrolysis and Cl^- complex formation of $(CH_3)_2Sn^{2+}$ at T=25 °C and I=0 mol L^{-1}

pqr	logβ [a, b]	ΔH^0 [c, d]
10-1	−2.86	33.1
10-2	−8.16	62.1
10-3	−19.35	97.7
20-2	−4.99	60.0
20-3	−9.06	85.0
11 0	0.78	–
11-1	−3.17	–

[a] Reaction: $pM^{2+}+qCl^-+rH_2O=M_pCl_q(OH)_r^{2p-(r+1)}+rH^+$; [b] Refs. [22,24]; [c] expressed in kJ mol^{-1}; [d] Ref. [39].

parameters are reported in Table 3 together with those regarding the complexes with Cl^-.

3. Results and discussion

3.1. Potentiometric and calorimetric measurements and speciation model

The stoichiometries and the stability constants of the species formed in the system dimethyltin(IV)–*cys* were obtained by performing on the potentiometric data several trials with different sets of complex species. The selected speciation model was that which had the best statistical fit, without giving systematic drifts in the residuals. This model very well fits experimental data, see for example Fig. s.1 in Supporting information. The results showed the formation of five complex species between $(CH_3)_2Sn^{2+}$ (M) and *cys* (L): three mononuclear, such as MLH, ML, MLOH and two binuclear, such as ML_2H and ML_2. The overall thermodynamic formation parameters values referring to reaction 2 are reported in Table 4. The thermodynamic formation parameter values obtained on the basis of the most probable reaction were collected in Table 5. The analysis of the thermodynamic results reported in Tables 4 and 5 indicates that all enthalpy values are exothermic and in some cases the enthalpic contribution is higher than the entropic one. The errors associated to the enthalpic values are acceptable, except for ML_2H species, which is affected by a high error, so it seems convenient to indicate approximately this value. Log$K$ values, referring mainly to the mononuclear species, show a very high stability, that allows to hypothesize high formation percentages in a wide pH range.

Table 4
Overall thermodynamic formation parameters of $(CH_3)_2Sn$–*cys* complexes at T=25 °C and I=0 mol L^{-1}

pqr [a]	logβ±3 s [b]	logβ [c]	$-\Delta G^0$ [d]	ΔH^0±s [b, d]	$T\Delta S^0$ [d]
1 1 1	18.81±0.04	18.03	107.4	−61.5±0.6	46
1 1 0	14.67±0.03	13.89	83.7	−44.1±0.3	40
1 1 -1	7.29±0.03	6.71	41.6	−10±1	32
1 2 1	27.77±0.04	26.60	158.5	∼−134	∼25
1 2 0	20.27±0.04	19.49	115.7	−48.7±0.7	67

[a] Reaction: $pM+qL+rH=M_pL_qH_r$; [b] standard deviation; [c] calculated at I=0.15 mol L^{-1} with Eq. (1); [d] expressed in kJ mol^{-1}.

Table 5
Thermodynamic formation parameters of $(CH_3)_2Sn$–*cys* complexes at T=25 °C and I=0 mol L^{-1}

Equilibrium	log$K$	$-\Delta G^0$ [a]	ΔH^0 [a]	$T\Delta S^0$ [a]
HL+M=MLH	8.6	49.1	−19.5	30
L+M=ML	14.67	83.7	−44.1	40
L+MOH=MLOH	10.1	57.7	−43	15
ML+HL=ML_2H	2.9	16.6	∼−48	−31
ML+L=ML_2	5.6	32.0	−4.6	27

[a] Expressed in kJ mol^{-1}.

The formation of MLH species in the acidic pH range ($2 \le pH \le 4$) is confirmed by the results obtained for the same system by Buzás et al., Silvestri et al. [18,19] and in the system containing trialkyltin(IV) studied by Hynes and O'Dowd [20]. Further deprotonation of *cys*, promoted by metal cation, occurs at higher pH values ($5 \le pH \le 7$), where the ML species is formed. At $6 \le pH \le 7$, when the L/M ratio increases, the species ML_2H becomes more significant. In the alkaline range ($8 \le pH \le 11$) the hydroxo-complex MLOH is predominant, while the ML_2 species, present only in our speciation model, prevails when M/L concentration ratio is at least equal to 0.75 or lower than this value.

By analysing the distribution of the species $(CH_3)_2Sn^{2+}$–*cys* vs. pH, it arises that the formation percentages of the mononuclear mixed species are very high in all the pH range investigated ($2 \le pH \le 11$), suppressing almost completely the hydrolysis of dimethyltin(IV) cation, as confirmed by speciation diagrams (Fig. 1a–c). When $C_{(CH_3)_2Sn}$=5 mmol L^{-1} and C_{cys}=10 mmol L^{-1} (Fig. 1a), in the acidic range $2.1 \le pH \le 4.1$ the predominant species MLH achieves a maximum percentage of 82.9 at pH=3.05, while in the same range the hydrolytic MOH species has a maximum percentage of 6.0. In the wide range $4.2 \le pH \le 7.0$ the predominant species is ML and at pH=7.0 its formation percentage is 92.9. In the alkaline range $8.0 \le pH \le 9.5$, where the ML_2 and MLOH species are present in high percentages: the former reaches 59.0 at pH=9.2 and this percentage lowers to 8.6 and 3.1 at $C_{(CH_3)_2Sn}=C_{cys}$=1 and 0.1 mmol L^{-1}, respectively (Fig. 1b–c). The decreasing of the concentration of the metal and the ligand induces a lowering of MLH as well as an increase of the hydrolytic species, such as MOH and $M(OH)_2$ formation percentages; on the contrary, the percentages referring to MLOH species remain very high throughout alkaline range. For example, at dimethyltin(IV)/*cys* concentration ratio=1 and $8 \le pH \le 11$, the MLOH species is absolutely predominant: at pH=9.5 its formation percentage is 87.3 at 1 mmol L^{-1} and 76.8 at 0.1 mmol L^{-1}.

In Table s.1 of Supporting information we report the percentages of the species in a pH range, which includes the physiological one (pH=7.4) and those of interest for many natural waters. The pH values reported are: 7; 7.5; 8.0 and the concentration ratios dimethyltin(IV)/*cys*: 0.5 and 1.

3.2. UV measurements

Before studying the UV properties of a complicated system containing metal complexes of an absorbing ligand it was

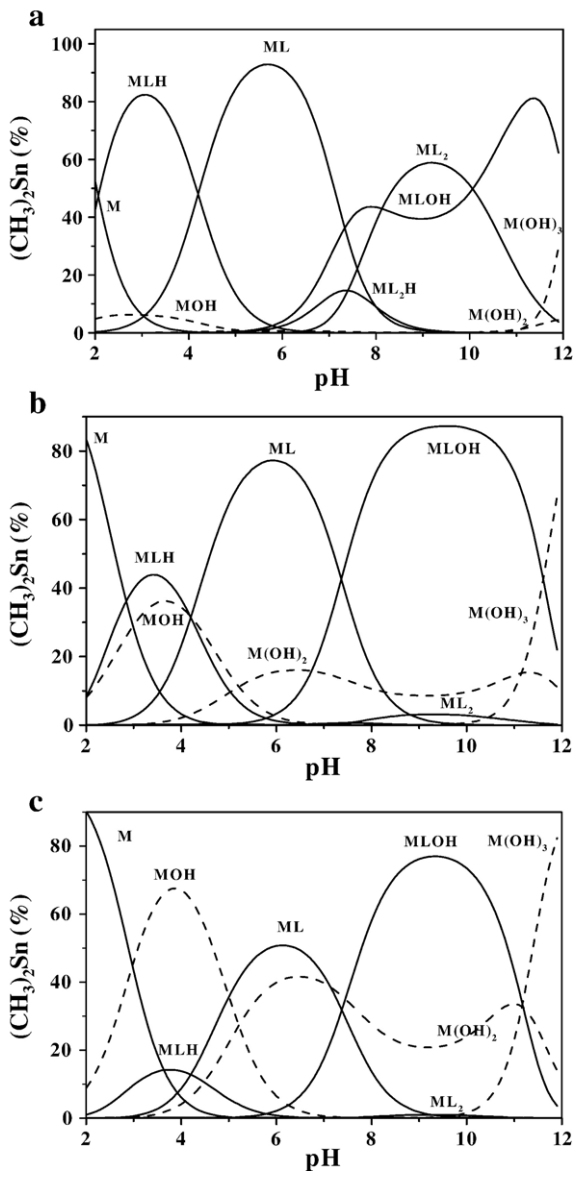

Fig. 1. a–c. Speciation diagrams of $(CH_3)_2Sn^{2+}$(M)–*cys* (L) *vs*. pH at I=0 mol L^{-1} and T=25 °C. Indexes refer to reaction (2)**.** Hydrolytic species are shown by dashed lines. a: C_M=5 mmol L^{-1}; C_L=10 mmol L^{-1}. b: C_M=C_L=1 mmol L^{-1}. c: C_M=C_L=0.1 mmol L^{-1}.

necessary to determine the molar absorbance spectrum of each protonated ligand form. The only paper [17] on the UV properties of complexes formed by *cys* with dimethyltin(IV) merely reports λ_{max} and ε_{max} values, obtained for solutions containing *cys* and dimethyltin(IV)–*cys*, at a specific pH value, without any hypothesys of speciation model and consequently without calculating the individual spectra of the species.

The molar absorbance spectra of protonated and unprotonated species of the ligand were calculated by using aqueous solutions of *cys* at different concentrations by means of spectrophotometric investigations in the 220–280 nm spectral range, by varying the pH through alkalimetric titration. While preliminary tests revealed that solutions containing dimethylti(IV) cation do not absorb in the spectral range investigated, the UV measurements on the *cys* solutions showed that the absor-

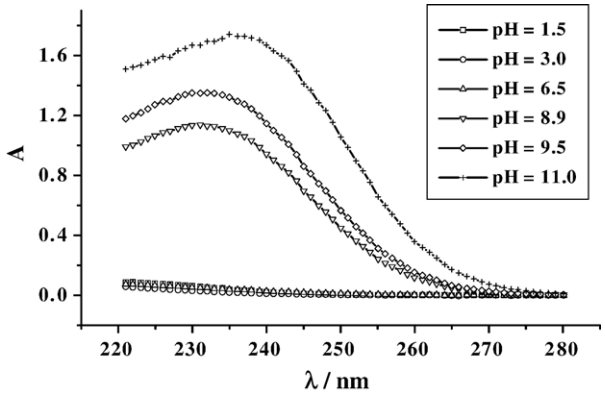

Fig. 2. Experimental UV spectra recorded on aqueous solutions of *cys* at C=0.4 mmol L^{-1} and T=25 °C.

bance intensity and the position of absorption maximum are clearly affected by the degree of the ligand protonation (Fig. 2). Experimental spectra referring to solutions containing *cys*, at C=0.4 mmol L^{-1}, show very low absorbance intensity from pH=1.5 (% LH_3=81.4 and % LH_2=18.6) to pH=6.5. On the contrary in the $8.9 \le pH \le 11.5$ range they show large spectral variations: 1. by increasing both pH and ligand deprotonation, absorbance values increase; 2. with the predominating of L species on the LH one, a batocromic shift from λ_{max}=230 nm to λ_{max}=235 nm occurs. As examples: at pH=8.9, where the formation percentages of the species are 18.9 and 75.8 for LH_2 and LH respectively, the absorbance intensity observed is A=1.14 at the λ_{max}=231 nm; at pH=9.5, where % LH_2=4.6, % LH=74.2 and % L=21.2, A=1.35, at the λ_{max}=232 nm; at pH=11.0, % LH=9.3 and % L=90.6, A=1.74 at the λ_{max}=235 nm. Individual UV spectra of the four species of *cys*, namely L^{2-}, HL^{-}, H_2L^0, H_3L^+ are obtained by deconvolution of experimental spectra into the component gaussian peaks, as shown in Fig. 3. Those relating to the L^{2-} and HL^{-} species display a higher molar absorbances with respect to LH_2 and LH_3 species, to attribute to the ionisation of the SH group, *i.e.* $n \rightarrow \sigma^*$ transition of the –S– chromophore, while the deprotonation of carboxylic and aminic group produces a little spectral variation in molar absorbance in the range investigated. Precisely, at λ_{max}=235 nm, L and LH species, reach ε_{max}=4279,

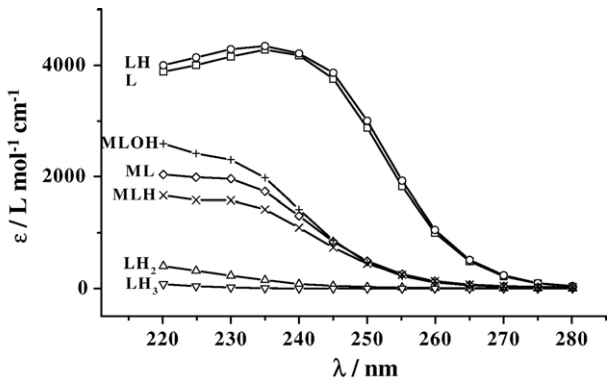

Fig. 3. Molar absorbances of protonated and unprotonated species of *cys* and of complexes with $(CH_3)_2Sn^{2+}$ cation at T=25 °C.

Table 6
Formation constants of dimethyltin(IV)–*cys* species obtained with two different techniques and calculation programs

Species	logβ±3s [a]	
	From potentiometry [b]	From UV spectroscopy [c]
MLH	18.81±0.04	18.5±0.3
ML	14.67±0.03	15.4±0.2
MLOH	7.29±0.03	7.7±0.3

[a] s = standard deviations; [b] at I=0 mol L^{-1}; [c] at I=0.013 mol L^{-1}.

4345 L mol^{-1} cm^{-1}, respectively; while, at λ_{max}=220 nm, LH_2 and LH_3 species, reach ε_{max}=402, 77 L mol^{-1} cm^{-1}, respectively. In literature [17] it was reported a value of ε_{max}=4300 L mol^{-1} cm^{-1}, at λ_{max}=236 nm and pH≈12, which is very close to our result for L species (ε_{max}=4279 L mol^{-1} cm^{-1}), at λ_{max}=235 nm.

With the aim to know the UV spectral properties of the complexes with alkyltin(IV) cations and to confirm the reliability of their formation constant values obtained by potentiometry, a wide number of spectra were performed by varying the pH, in the $1.5 \le pH \le 11.0$ range, and the stoichiometric ratio between the *cys* and the dimethyltin(IV). In Fig. s.2 of the Supporting Information some experimental spectra registered on solutions containing dimethyltin(IV) and *cys* at $C_M = C_L$=0.5 mmol L^{-1} are reported. They show slight absorptions up to pH=6.5 that increase to a great extent in the $8.7 \le pH \le 11.5$ range. Individual UV spectra of the MLH, ML and MLOH complex species are reported in Fig. 3 together with those of *cys*. The comparison of these individual spectra with those of *cys* species reveals: 1. a batocromic shift of molar absorbance maximum; 2. a decrease of molar absorbances; as an example, at λ_{max}=220 nm, ε_{max}=1666, 2038, 2591 L mol^{-1} cm^{-1} for MLH, ML and MLOH species, respectively. These results totally confirm the complex formation between the absorbing ligand *cys* and the dimethyltin(IV) cation.

The stability constants evaluation of these complexes can be performed by peak heights of UV spectra. By using the experimental spectra obtained on dimethyltin(IV)–*cys* solutions at several concentrations we refined the formation constants of the three main species (MLH, ML, MLOH) at I=0.013 mol L^{-1}. The results are shown in Table 6 together with those obtained from the potentiometric measurements. The comparison shows that the spectroscopic technique is less sensitive than the potentiometric one, consequently the latter allows to determinate the stabilities of all the species examined with lower standard deviations; nevertheless, it represents a further method of validation of the speciation model calculated on the basis of potentiometric evidences.

3.3. NMR measurements

The spectroscopic properties of the dimethyltin(IV)–*cys* complexes were studied by means of ^{1}H NMR investigations in H_2O/D_2O solutions. A wide number of spectra were performed by varying the pH in the range 3–11 and the stoichiometric ratio between the *cys* and dimethyltin(IV) as well. The evidence that the spectra show a single set of peaks despite the experimental conditions suggests that although several species may be present in solution, they are involved in fast mutual exchange; as a consequence, the resonances of the bound and the free ligands cannot be directly observed from the spectra. Nevertheless, some spectra of *cys* have been carried out in experimental conditions corresponding to the maxima of speciation curves, thus allowing the NMR characterization of LH_2 and *L* species, in particular conditions of concentration and pH.

As a common feature of all the spectra, on increasing the pH, upfield chemical shifts of all the signals occurred regardless of the dimethyltin(IV)/*cys* ratio employed. It is worth to mention that Silvestri et al. [18] studied the system dimethyltin(IV)–*cys* in aqueous solution by means of ^{1}H NMR, not considering the fast mutual exchange occurring in the experimental conditions. Furthermore some small signals clearly associated to the presence of low amount of cystine were wrongly ascribed to *cys*-containing species.

As far as the CH_3 signals are concerned, the ^{1}H NMR spectra of both dimethyltin(IV) and dimethyltin(IV)–*cys* solutions showed a single sharp signal featured by the satellite peaks of heteronuclear couplings, namely $^2J(^{117}Sn-^1H)$ and $^2J(^{119}Sn-^1H)$, with the two NMR active isotopes of tin. Fig. 4 shows the variations of δCH_3 vs pH for dimethyltin(IV) and dimethyltin(IV)–*cys* solutions in different stoichiometric ratio. The strong differences showed by the curves obtained with and without *cys* suggest that the presence of *cys* involves the formation of complex species in a wide pH range rather than

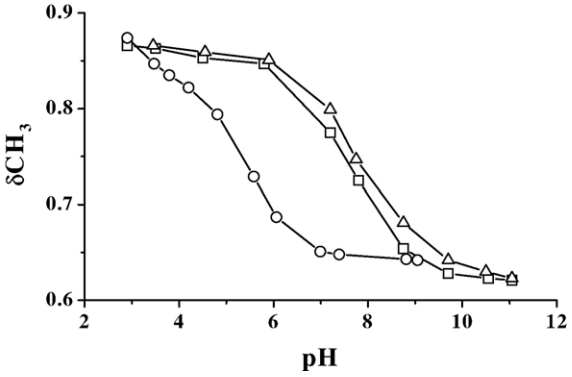

Fig. 4. Measured chemical shifts, at T=25 °C, of CH_3 vs. pH for: —○— solutions containing dimethyltin(IV) only; —□— dimethyltin(IV):*cys* 1:1 mixtures; –Δ– dimethyltin(IV): *cys* 1.5:2 mixtures.

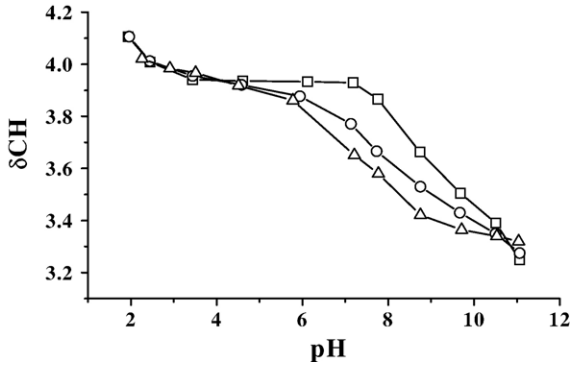

Fig. 5. Measured chemical shifts , at T=25 °C, of CH vs. pH for: –□– solutions containing *cys* only; –○– dimethyltin(IV): *cys* 1.5:2 mixtures; –Δ– dimethyltin(IV): *cys* 1:1 mixtures.

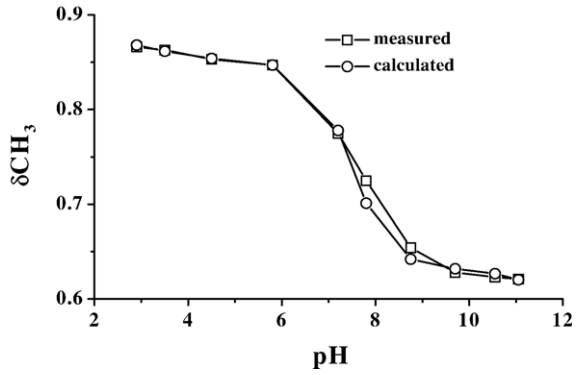

Fig. 6. Chemical shifts of CH_3 vs. pH in dimethyltin(IV)–*cys* solutions at $C_{(CH3)2Sn}=0.00869$ mol L^{-1}; $C_{cys}=0.01114$ mol L^{-1} and $T=25$ °C.

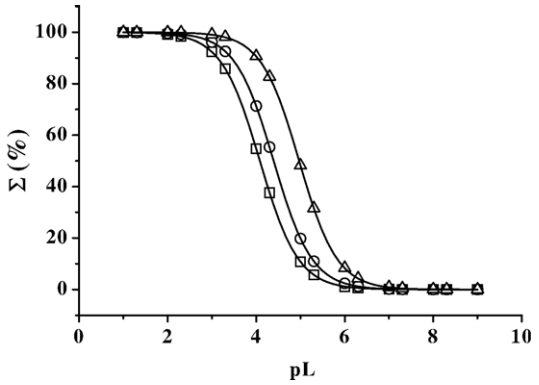

Fig. 7. Sequestration diagram of $(CH_3)_2Sn^{2+}$ cation by *cys* at $T=25$ °C and $C_{(CH3)2Sn}=10^{-3}$ μmol L^{-1}. □: pH=5;○: pH=6; Δ: pH=8.

simple hydrolysis. Furthermore, the curves corresponding to dimethyltin(IV)–*cys* solutions show a different behavior depending on the stoichiometric ratio employed, starting at pH~6. These conclusions fully support the potentiometric data interpretation that suggests the formation of complexes such as ML_2 or ML_2H whenever the measurements are performed in excess of *cys*.

As can be inferred from Fig. 5, the δCH of the *cys* alone shows a first variation at low pH supporting the deprotonation of COOH group in this range; furthermore, on increasing the pH, the CH resonance keeps almost constant in a wide region (namely 3.4–7.2) while after neutral pH it decreases steadily. The dimethyltin(IV) containing mixtures are featured by a CH signal very close to the one showed by *cys* solutions up to 4.5, whilst at higher pH the signals behavior is different; this evidence may be ascribed to the formation of dimethyltin(IV)–*cys* containing species [8]. Furthermore, while the ^1H NMR spectra of *cys* registered in the presence of dimethyltin(IV) at pH <4.5 show that the resonance associated to CH is comparable with the one obtained for the free ligand in the same pH region, some differences can be detected in the above range in the CH_2 signals suggesting an interaction between *cys* and dimethyltin(IV) at low pH by means of a distant group from the CH, i.e. the thiol. The absence of interactions between the two species present in solution must be discarded on the basis of potentiometric analysis.

Even though the direct measurements of NMR parameters cannot be carried out owing to fast mutual exchange among the formed species, it is possible to obtain both δ and ^2J for each supposed complex formed starting from speciation study. In fact, since the concentration of each species over the whole pH range is known from potentiometric data, individual chemical shifts and coupling constants can be easily calculated [31]. The aim of obtaining these calculated values is twofold: on one hand these individual "indirect" δ and ^2J were used to recalculate the mean corresponding to experimental pHs (simulating a fast mutual exchange) in order to validate the model used for speciation (by comparing the average recalculated and experimental values); on the other hand individual ^2J calculated for all the complexes allows some structural considerations to be discussed.

For example, Fig. 6 shows the good agreement between calculated and observed chemical shifts of the tin-bound methylic groups in the mixture with $C_{(CH_3)_2Sn}=0.00869$ mol L^{-1} and $C_{cys}=0.01114$ mol L^{-1}. As a result the NMR data are consistent with the model proposed for the interpretation of potentiometric investigations. Furthermore, the $^2J(Sn-^1H)$ values can be conveniently converted into C–Sn–C angles by means of the empiric relation proposed by Lockhart and Manders [31]. The $^2J(Sn-^1H)$ relative to the single species were calculated (Table 7) and the corresponding angles obtained show that all the complexes are characterized by an angle ranging from 123° to 130° suggesting a pentacoordinated trigonal bipyramidal arrangement around the tin with equatorial CH_3 groups for all the species formed in solution, confirming literature evidences [38].

3.4. Sequestering power of L–cysteinate towards dimethyltin (IV) cation

The sequestering capacity of a ligand towards a metal can be expressed by the function $\bar{\Sigma}(\%)$ vs. pL, where $\bar{\Sigma}(\%)$ is the total percentage of alkyltin(IV) cation complexed and pL = −log

Table 7
Calculated individual ^1H NMR parameters

Species	$\delta(CH_3)$ (ppm)	$^2J(Sn-H)$ (Hz)	∠C–Sn–C (°)
MLH	0.871±0.005	79.5±0.2	130
ML	0.853±0.001	75.5±0.1	125
MLOH	0.617±0.005	75.2±0.2	125
ML_2H	1.19±0.04	74±1	123
ML_2	0.67±0.01	74.9±0.2	124

Table 8
Literature comparisons at $T=25$ °C

Species	logβ		
	$M=(CH_3)_2Sn^{2+}$ [a]	$M=(C_2H_5)_2Sn^{2+}$ [b]	$M=(C_2H_5)_3Sn^{+}$ [b]
	This work	Ref. [19]	Ref. [20]
M(*cys*)H	19.43	19.43	15.21
M(*cys*)	14.86	14.77	–
M(*cys*)OH	7.29	6.98	–
$M(cys)_2H$	28.88	28.35	–
$M(cys)_2$	20.83	–	–

[a] At I=0.1 mol L^{-1} in NaCl; [b] at I=0.1 mol L^{-1} in $NaClO_4$.

Table 9
Literature comparisons at T=25 °C

Species	logβ		
	$M=(CH_3)_2Sn^{2+}$ L=*cys*[a]	$M=(CH_3)_2Sn^{2+}$ L=alanine[b]	$M=(C_2H_5)_2Sn^{2+}$ L=tiolactic acid[c]
	This work	Ref. [10]	Ref. [40]
MLH	18.81	11.20	–
ML	14.67	8.25	14.13
MLOH	7.29	3.23	7.48
$ML(OH)_2$		−5.95	–
ML_2H	27.77	–	–
ML_2	20.27	–	–

[a]At I=0 mol L^{-1}; [b] at I=0.1 mol L^{-1} in KNO_3; [c] at I=0.3 mol L^{-1} in $NaClO_4$.

$[L]_{tot}$. Since this function is a typically sigmoidal curve (or a dose response curve), increasing rapidly over a relatively small change in concentration, we can use the Boltzmann type equation (with asymptotes of 100 for pL→−∞ and 0 for pL→+∞):

$$\Sigma(\%) = 100 \times \left[\frac{1}{1 + e^{(pL - pL_{50})/S}} - 1\right] \quad (3)$$

where pL_{50} and S are empirical parameters, which define the ligand concentration necessary to sequester 50% of metal ion, while S is a measure of the slope in flex of the function (Σ%) vs pL. The pL_{50} parameter is very useful because gives a representation of the binding ability of a ligand (L) toward a specific cation in the investigated conditions.

Fig. 7 shows sequestration diagram of *cys* towards dimethyltin(IV) cation, at $C_{RSn}=10^{-3}$ μmol L^{-1} and T=25 °C and at several pH values. pL_{50} values are 4.082, 4.394, 4.970 at pH=5, 6, 8, respectively; while S=0.434, 0.433, 0.428. With increasing pH value, increases the binding ability of the ligand, too. These results are interesting for the toxicological implications, because they indicate that the sequestering ability of *cys* is very high; for example at pH=8 when $C_{(CH_3)_2Sn}=10^{-3}$ μmol L^{-1}, the concentration of the ligand necessary to sequester 50% of the dimethyltin(IV) is equal to 10 μmol L^{-1}, i.e. 10 times lower than *cys* concentration in blood plasma models (≥100 μmol L^{-1}) [2].

4. Literature comparisons

In Table 8 the speciation model and the stability constants of the species formed between *cys* and dimethyltin(IV) at I=0.1 mol L^{-1} in NaCl are compared with those referring to the system containing the same ligand and diethyltin(IV) at I=0.1 mol L^{-1} in $NaClO_4$. The two speciation models, proposed by us and by Buzás et al. [19], respectively, are different only for the ML_2 species, present in our model only. The stability constants referring to MLH, ML, MLOH, and ML_2H species are similar, precisely the species of the dimethyltin(IV) with *cys* are slightly higher than those of diethyltin(IV) (the highest difference is 0.53 and refers to the ML_2H species), as expected for higher steric crowding of the ethyl groups with respect to methyl ones in the interaction with the ligand. The results obtained by Hynes and O'Dowd on the triethtyltin(IV)–*cys* system at I=0.3 mol L^{-1} in $NaClO_4$ are reported in Table 8, too [20]. The only species proposed is the MLH and the corresponding stability is much lower than that referring to dimethyltin(IV)–*cys* system.

The comparisons of the complex stabilities of dimethyltin (IV)–*cys* system with those formed by dimethyltin(IV) with a simple amino acid, such as alanine, are reported in Table 9. The high extra stability obtained for the dimethyltin(IV)–*cys* system, with respect to dimethyltin(IV)-alanine (from 4.1 for MLOH to 7.6 for MLH species), is to attribute unequivocally to the –SH group of the *cys*. From the same Table, the little differences between the stabilities of dimethyltin(IV)–*cys* and diethyltin(IV)–tiolactate species confirm what was already discussed.

5. Conclusions and biological implications

The results obtained by different equilibrium (pH-metric, calorimetric and spectrophotometric) and structural (^{1}H NMR) methods for the study of the interaction of dimethyltin(IV) cation with *cys*, can be summarized as follows.

- The formation of five complex species, with very high stabilities and formation percentages (mainly for mononuclear species), in a wide pH range, was found.
- Exothermic enthalpy values and in some cases with an enthalpic contribution higher than entropic one, are observed.
- The speciation model proposed on the basis of potentiometric results was also validated by means of spectroscopic technique.
- Pentacoordinated trigonal arrangement around the tin, with equatorial CH_3, for all the species, was found by NMR data.
- Very high sequestering ability of *cys* toward dimethyltin(IV), was observed.
- The high extra stability of dimethyltin(IV)–*cys* species with respect to those of dimethyltin(IV) with simple amino acids, such as alanine, confirms that −S− interaction leads to more stable coordination than COO^- or NH_2.

As pointed up by Pellerito and Nagy in a recent review [9] on the interaction of organotin(IV) cations with biologically active ligands, this field is still open. Equilibrium data on the different systems are largely missing. Therefore the study of the interaction of dimethyltin(IV) with a biological ligand such as cysteine, is useful to understand the biological action of dimethyltin(IV) complexes. The information obtained on these complexes has biological implications, because *cys* is a sulfur containing amino acid, present in the blood, which plays an important role in the metabolism of living cells (it participates in many biochemical processes, e.g. in the synthesis of tripeptides such as glutathione).

Acknowledgements

The authors thank University of Messina for financial support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bpc.2007.11.005.

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