



A HIGHLY SELECTIVE CINAMMOYLTHIOAMIDE DERIVATIVE USED FOR SPECTROMETRIC DETERMINATION OF MERCURY CONTENT IN ENVIRONMENTAL SAMPLES

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abstract A spectroscopic study of Hg(II) complex of 3,4,5-trimethoxycinammoylthioamide ligand has been carried out. In aqueous solutions in a pH* range 1.00 – 9.00 the 3,4,5-trimethoxycinammoylthioamide forms with mercury (II) a 1:2 complex with the total stability constant, β equal to $1.56 \cdot 10^7$ at $20 \pm 2^\circ\text{C}$. The experimental data are correlated to some theoretical parameters obtained by semi-empirical AM1, PM3, ZINDO and ab initio 6-31G* methods. Based on the spectrometric study a selective and sensitive method has been developed for the determination of mercury (II) in various type of samples. The method was fully validated.

Introduction

Mining has long been an important source of environmental mercury levels. Weather events and anthropogenic processes can contribute frequently to the release of mercury through flood and soil erosion and increase its bioavailability: within the biogeochemical cycle mercury found at elemental state, Hg, can be oxidized to inorganic mercury, Hg (II), and then methylated during biotic and/or abiotic processes to form methyl mercury [1]. The chemical affinity of the mercury to the –SH groups, found in protein molecules, determines an inhibition of enzymes' activities which causes serious damage to basic metabolic reactions. Mercury and its compounds are considered health hazard [2]. The intense public concern about environmental mercury contamination generates a significant number of scientific papers focusing on the determination of mercury and its compounds. Excellent reviews on mercury speciation [3] and determination [4-6] have been published in recent times. Based on the chemical recognition between mercury and –SH groups numerous analytical methods employed for the determination of mercury by means of organic reagents containing –SH moiety have been described [7]. Of late, compounds like thioamides have been studied extensively and their abilities to form complexes with various cations have been reported [8,9]. Our research groups have focused on the chemistry of thioamides and N, N disubstituted thioamides and our results have been reported in previous paper [10,11]. This one presents a spectrometric study concerning the complexation of

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Hg(II) with 3,4,5 trimethoxycinnamoylthioamide (further noted by R) and a new selective and sensitive method for the determination of mercury (II) in various type of samples. The method proposed was fully validated according to the international rules [12÷13]. Formula of the reagent is presented in Fig. 1.

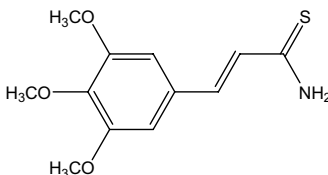


Fig. 1 Formula of the reagent R.

Experimental

Apparatus

The absorption spectra were recorded on a Jasco V 530 spectrometer (Japan) coupled with a personal computer running Jasco software. The spectrometer was validated [14]. The measurements have been made in quartz cells of 1 cm pathlength and the optimal condition for recording the spectra were: wavelength range: 200-400 nm; scan speed, 100 nm/min; slit width, 2 nm; wavelength interval 1nm; smooth, 5. The ORIGIN program (Micro Cal Inc, version 6.0.) was employed for the statistical analysis.

Reagents

All chemicals used were analytical reagent grade. All inorganic reagents were purchased from Merck. The synthesis of the reagent has been reported in a previous paper [15]. Solution of $0.001 \text{ mol}\cdot\text{L}^{-1}$ of organic reagent, R in ethanol 95%, was prepared from pure organic reagent. Solution of $0.001 \text{ mol}\cdot\text{L}^{-1}$ of Hg(II) was prepared using HgCl_2 . Stock aqueous solution of $0.001 \text{ mol}\cdot\text{L}^{-1}$ metallic cations: Na (I), K(I), Ca(II), Mg(II), Al(III), Cd(II), Co(II), Cr(VI), Fe(II), Fe(III), Mn(II), Pb(II), Ni(II), Zn(II), were prepared using corresponding salts commercial available. Working solutions ($5\cdot 10^{-4} \text{ mol}\cdot\text{L}^{-1}$) were prepared by dilution just before use. Double distilled water was used.

Procedure

The spectrometric study has been carried out using solution of $5\cdot 10^{-4} \text{ mol}\cdot\text{L}^{-1}$ of Hg(II) and $5\cdot 10^{-4} \text{ mol}\cdot\text{L}^{-1}$ of organic reagent, R. Exactly measured volumes of metallic cation and organic reagent solutions were added in 25 mL volumetric flasks and the double distilled water was used as solvent to fill them up to the mark. Absorbance of each solution was measured against a corresponding blank that was similarly prepared and does not contain the metallic cation solution.

Validation of the method

Linearity and range. For the calibration graph successive dilutions, starting from the working solution, were performed by means of 25 mL or 10 mL volumetric flasks. The

spectrum of each solution was recorded against a corresponding blank. The absorbances were measured at 290 nm.

Limit of detection and limit of quantification. According to the ICH recommendations [12,13], the approach based on the signal-to-noise ratio was used for determination of both the limit of determination, LOD, and the limit of quantitation, LOQ. Limits were established to be the concentration in the mercury (II) for a signal-to-noise ratio of three-to-one and ten-to-one for LOD and LOQ, respectively.

Selectivity. Interference studies have been done starting from working solution of metallic cations and organic reagent; for each metallic action investigated, samples were prepared in a 25 mL volumetric flask as follows: each flask contained 1 mL of aqueous solution of metallic ion, 5 mL of **R** and distilled water up to mark. All spectra were recorded against corresponding blank that does not contain metallic cation.

Precision and Accuracy. Precision and accuracy were estimated by 6 consecutive measurements, which were performed on solutions containing all the same amount of mercury. Sample were prepared in 25 mL volumetric flask by adding 1 mL solution of Hg(II) $5 \cdot 10^{-4} \text{ mol} \cdot \text{L}^{-1}$, 5 mL solution of **R** $5 \cdot 10^{-4} \text{ mol} \cdot \text{L}^{-1}$ and distilled water up to the mark. All spectra were recorded against corresponding blank that does not contain metallic cation. Intermediate precision has been determined under different operational conditions: different investigator on another day, using another batch of ethanol as solvent for the reagent **R**.

Stability of the solutions. The stability of stock aqueous solution of metallic ions and stock alcoholic solution of organic reagent has been evaluated. Working solutions were analyzed at well established period of time. These solutions were used to record, against a corresponding blank, the spectrum for a solution prepared in a 25 mL volumetric flask as follows: 1 mL of aqueous working solution of metallic ion, 5 mL of working solution of **R** and distilled water up to mark.

Analysis of real samples

The analysis of real samples of water were realised after removing the organic compounds, as described in Romanian standard [16] that is in agreement with the European one. Samples of soils were digested with a solution of concentrated nitric acid in a MILESTONE MLS – 1200 MEGA microwave oven. The working pressure was 110 bars. After digestion samples for spectrometric determination of Hg(II) were prepared as described in Romanian standard [17]. All the samples were taken around an important mining sector in the north of Romania.

Results and discussion

The interaction between Hg(II) and the organic reagent **R** occurs at the thioamide groups. The cinnamic moiety in the structure of the reagent determines an increase of the negative charge on both nitrogen and the sulfur atoms which improve their capability to form complex with metallic cations. A spectroscopic study was carried out in order to establish if the reagent **R** forms with mercury (II) a complex. Thus the UV spectrum of the solution that was prepared by adding 1 mL solution of Hg(II) $5 \cdot 10^{-4} \text{ mol} \cdot \text{L}^{-1}$ and 5 mL of **R** $5 \cdot 10^{-4} \text{ mol} \cdot \text{L}^{-1}$

in a 25 mL volumetric flask has been recorded against the corresponding blank, that contained only the reagent **R**. As it is shown in Fig. 2, the spectrum of the solution containing both mercury (II) and the reagent **R** has two maxima of the absorbance at 230 and 290 nm, wavelengths at which the reagent absorbs few, but enough to perform all further measurements versus a corresponding blank. In order to establish the stoichiometry of the complex, the influence of both organic reagent and Hg(II) concentrations on the absorbance was studied. All further measurements were made at 290 nm against a corresponding blank. The stability constant was determined using Job method [18], adapted according to the procedure described by J.M B. Sendra [19].

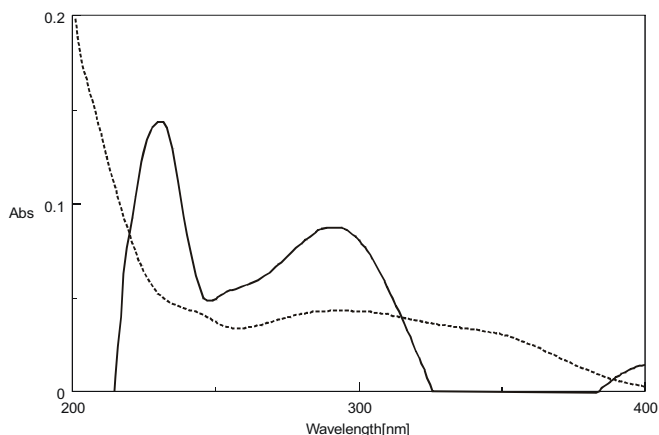


Fig. 2 Absorption spectra: — 4 ppm of Hg(II) + 25,3 ppm of **R** versus 25,3 ppm of **R**;
----- 25,3 ppm of **R** versus corresponding blank.

Stoichiometry of the complex

The stoichiometry of the complex was established by both spectrometric titration and continuous variation method, known as Job method for the isomolar solutions. The results that were obtained in the study of chemical interaction between Hg(II) and **R** indicate the formation of a complex having the stoichiometry $\text{Hg(II)} : \mathbf{R} = 1 : 2$. This stoichiometry has been confirmed by spectrometric titrations: of Hg(II) with **R**, of **R** with Hg(II) and by applying Job method for isomolar solutions. The results obtained are in agreement with those presented in literature as describing the ability of mercury, Hg(II), to forms 1 : 2 stoichiometric complexes with organic reagents having N, C, S, P, As and O atoms like donors [20].

Determination of the stability constant

The stability constant of the complex was determined from the experimental data obtained using the Job method. Thus, three series of solutions containing a fixed total number of moles of Hg(II) and organic reagent, but in which the ratio Hg(II)/organic reagent is systematically varied from large to small have been prepared and studied. These experimental conditions ensure the formation of a 1 : 2 complex between Hg(II) and **R**; this stoichiometry was confirmed by the inflexion point of the plot of absorbance versus the

molar fraction of the reagent. The average value of the stability constant is: $1.56 \cdot 10^7$ at $20 \pm 2^\circ\text{C}$. This value of the stability constant shows a very stable complex.

Validation of the method

Linearity and range. The fit of Beer's law is a measure of the linearity of any spectrometric method. The plot of the absorbance versus the concentration of mercury (II), in the range 0.10 – 20 ppm gives a calibration graph having the equation of linear regression: $A = 0,04428 + 0,03373C$, where A represents the absorbance of the sample measured at 290 nm and C the concentration of mercury (II) expressed in ppm; the correlation coefficient is 0.9995. The values obtained show a good linearity.

Limit of detection and limit of quantification. The detection limit was established by assessing the signal-to-noise ratio level in a proportion of 3:1 and it was found to be 0.014 mg.L⁻¹. The quantitation limit was 0.040 mg.L⁻¹ and corresponds to a signal-to-noise ratio in a proportion of 10:1.

Precision. Synthetic samples containing 1 ppm Hg(II) were studied. Precision has been estimated by: repeatability (or intra-day precision) using the results of six consecutive measurements that were performed on synthetic samples and by intermediate precision. Intermediate precision was determined under different conditions: different investigator, another batch of ethanol for the organic reagent and analysis carried out on another day. The results obtained for repeatability studies indicate that the precision expressed as relative standard deviation is 1.17% and 1.46% for intra-day and inter-day precision, respectively.

Accuracy. Accuracy was determined by calculating the recovery of mercury (II) from different synthetic samples. Method accuracy was determined in the interval 80 – 120% of the working concentration of the mercury (1 ppm) and was evaluated by the parameter "recovery". Values obtained were within the proposed limits ($100 \pm 2\%$), with results ranging from 98.7% to 101.3% and a RSD of 1.09%.

Stability of the solutions. The stock solutions are stable for three months if they are stored at room temperature and protected from light. The alcoholic solution of the organic reagent is stable for 5 months, if stored at $2 - 8^\circ\text{C}$.

Selectivity. The effect of potential interfering ions on the spectrometric determination of mercury (II) was investigated. The interference of Na (I), K(I), Ag(I), Ba(II), Ca(II), Mg(II), Al(III), Cd(II), Co(II), Cr(VI), Fe(II), Fe(III), Mn(II), Pb(II), Ni(II), Zn(II) has been studied. The ions: Na (I), K(I), Ba(II), Ca(II), Mg(II), Al(III) do not interfere even at a concentration greater than 100% of molar concentration of Hg(II). The ions Cd(II), Co(II), Cr(VI), Ni(II) and Zn(II) influence the determination of mercury up to 20% if they are present in the same molar concentration as mercury (II). The ions Cu(II), Ag(I), Fe(III), Mn(II), Pb(II) interfere if their concentrations are between 65 and 100% reported to the mercury concentration. The Fe(II) interferes only if its concentration exceeds 78 times the molar concentration of mercury. The interferences can be eliminated using a suitable masking agent. That is why the EDTA is one of the most used [21], but its efficiency depends on the stability constant of each complex that has been formed with the co-existing ions found in sample and on their number, too.

Table 1 Determination of Hg (II) in synthetic samples using proposed methods

No. Sample	Content in sample,(ppm)				Recovery of Hg(II),(%)		
	Hg(II)	Co-existing ions			proposed spectrometric method		reference method [21]
		¹ Group I	Fe(II)	Cu(II)	Without masking	Using EDTA	
1	0.500	0.025	0.150	0.150	98.14	100.3	99.71
2	0.500	0.500	0.300	0.250	83.79	98.54	98.92
3	1.000	0.075	0.150	0.150	99.48	98.95	100.8
4	1.000	1.000	0.300	0.250	79.15	101.2	99.35
5	1.000	2.500	0.300	0.250	63.15	98.64	99.67
6	1.000	3.500	0.350	0.250	56.14	100.7	101.2

Note: Recovery was calculated for 6 determinations;

¹Group I: Ag(I), Mn(II), Pb(II), Fe(III), Cd(II), Cr(VI), Co(II), Zn(II), Ni(II); each ion is present in the specified concentration; ² Cu(II) has been masked using EDTA;

Determination of mercury in synthetic samples. The results of the determination of mercury (II) in synthetic samples are presented in Table 1. The concentration of mercury (II) was determined by the proposed method as well as by AAS as reference method [22] for a better comparison. As shown in Table 2, mercury (II) can be determined in the presence of co-existing ions by proposed spectrometric method if the concentration of these ions does not exceed the limits established throughout this study. If the sample contains Cu(II) more than 100% by regard to the Hg(II) concentration, the determination of mercury(II) in the presence of this ion can be performed without any error by means of proposed spectrometric method. Yet, this happens only if Cu(II) is masked. For this purpose, the EDTA has been used. The Student's *t* distribution [21] has been calculated for each pair of methods: the spectrometric and AAS [21], respectively, in order to find out whether the results that have been obtained for mercury (II) determination estimate the same average. All calculated values *t*, of the Student's distribution, did not exceed the tabulated one for a probability of 95% and 10 degrees of freedom.

Table 2 Determination of Hg (II) in real samples

Sample	Content of Hg(II) determined in sample, (ppm)	
	proposed spectrometric method*	reference method [21]
River water upstream	0.995 ± 0.031	1.157 ± 0.028
River water downstream	3.129 ± 0.029	3.076 ± 0.015
Soil 1	0.517 ± 0.012	0.509 ± 0.031
Soil 2	1.469 ± 0.034	1.501 ± 0.006
Soil 3	0.782 ± 0.018	0.775 ± 0.009
Soil 4	3.282 ± 0.054*	3.364 ± 0.037

Note: Each result represents an average of 10 determinations ± standard deviation;

* content determined after masking co-existing ions using EDTA

Applications

The determination of mercury (II) at trace levels in real samples – of the kind of soil and water – is one of the main directives of environmental quality control. The methods developed and proposed in this paper have been tested and compared with the reference method and the results obtained are presented in Table 2. The proposed method ensures the determination of the mercury (II) found at trace levels in real samples.

Conclusion

A rapid, precise, accurate and sensitive spectrometric method has been developed for the quantitative determination of the mercury (II). The mercury (II) forms with 3,4,5-trimethoxycinammylthioamide a 1:2 complex with the stability constant equal to $1.56 \cdot 10^7$ at $20 \pm 2^\circ\text{C}$. The great selectivity of the spectrometric method recommend them for determination of Hg(II) in soil and water samples with small costs and without prior separation.

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