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SYNTHESIS AND CHARACTERIZATION OF A THIOSEMICARBAZONE FLUORESCENT CHEMOSENSOR FOR MERCURY(II) AND ORGANOMERCURY(II) IONS

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Fluorescence-based sensors show great promise for heavy metal ion detection since they can provide high sensitivity, selectivity, and the possibility for real-time monitoring of these high-priority pollutants. Herein, we report the synthesis and characterisation of a fluorescent thiosemicarbazone ligand containing a phenothiazine moiety. The complexation behavior of the ligand towards several heavy metal ions was assessed through both UV-VIS and fluorescence spectroscopy. Upon complexation with mercury (II) and organomercury (II) ions, the fluorescence emmision was effectively quenched, suggesting that the ligand can be used as a turn-off fluorescence chemosensor for these analytes with LOQ of 5×10^{-6} M for mercury (II) ions, and 1×10^{-5} M for organomercury (II) ions respectively.

Keywords: phenotiazine, fluorescence chemosensors, mercury ions

1. Introduction

Heavy metal ions are a critical concern for public health, since they bioaccumulate readily, inducing severe health issues in both humans and animals. For this reason, metals such as mercury, cadmium, lead *etc.* are classified as high priority pollutants by the World Health Organization, their presence in drinking water being limited to values as low as 1 pbb for Hg^{2+} and 5 ppb for Cd^{2+} and Pb^{2+} [1,2]. In particular, organomercury compounds from both natural and

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anthropogenic sources are considered to be potent neurotoxins. Hence, the existence of these pollutants in both aquatic and terrestrial ecosystems emphasize the need for developing innovative, effective, and reliable detection methods.

According to IUPAC, *"a chemical sensor is a device that transforms chemical information, ranging from the concentration of a specific sample component to total composition analysis, into an analytically useful signal. The chemical information, mentioned above, may originate from a chemical reaction of the analyte or from a physical property of the system investigated"* [3]. Based on their working principle – molecular recognition and signal transduction – chemosensors can be defined as chemical systems consisting of two main components: a receptor (which selectively binds with the analyte) and an active unit with properties that change upon complexation with the analyte. Additionally, a spacer may be inserted between the receptor and the active unit to modify the system's geometry and enhance the electronic interaction between these components [4,5].

Electrochemical and optical sensing techniques are powerful analytical methods due to their remarkable sensitivity, rapid response time, and capacity for real-time monitoring of heavy metal ions [6-9]. In particular, fluorescence-based sensors have been extensively developed, due to their increased accuracy and selectivity. Among the numerous fluorophores reported to date, phenothiazines represent a promising class of materials for sensor development, since the fluorescence exhibited in their free state is diminished via a static quenching mechanism following complexation with metal ions [10-12]. In the same time, thiosemicarbazone-based ligands exhibit a high affinity towards heavy metal ions, forming stable complexes that can induce distinct photophysical changes in the system [13-15]. Thus, by combining the optical properties of phenothiazine derivatives with the heavy metal ions complexing ability of thiosemicarbazone ligands, novel optical sensors for heavy metal ions screening can be developed.

In this work we report on the synthesis and characterization of a fluorescent thiosemicarbazone ligand containing a phenothiazine moiety. The complexation behavior of the ligand towards several heavy metal ions was assessed through UV-VIS and fluorescence spectroscopy. We show that the fluorescence emission of the ligand is effectively quenched in the presence of both Hg^{2+} and $PhHg^{+}$, suggesting that it can function as a *turn-off* fluorescence chemosensor for these analytes.

2. Experimental

2.1 Equipment

ATR FT-IR spectra were recorded on a Bruker Vertex 70 spectrometer at a resolution of 4 cm^{-1}, in the range: 4000 - 600 cm^{-1}. A Bruker Avance III 500 spectrometer was used for recording of the NMR spectra (^{1}H: 500 MHz, ^{13}C: 125 MHz). A Jasco V-660 double beam spectrometer, using 1 cm standard quartz cuvettes, was employed to record UV-VIS spectra in the 200 - 800 nm range. Fluorescence emmision spectra were recorded in the 460 - 800 nm range on a Cary Eclipse Fluorescence Spectrometer, with an excitation wavelength of 450 nm and both emission and excitation slits set to 5 nm.

A Metrohm Autolab 128N potentiostat/galvanostat fitted with a SCAN250 analog waveform generator module and an ADC10M ultrafast analog-to-digital converter was employed for the electrochemical mesurements. The experimental apparatus consists of a conventional three-electrode cell, with a bare glassy carbon (GC) disk working electrode (3 mm diameter, ALS Co. Ltd.), a platinum rod counter electrode using Ag/Ag$^+$ (0.1 M $AgNO_3$ and 0.1 M $TBABF_4$ in MeCN) as reference electrodes for nonaqueous solutions.

2.2 Reagents and Syntheses

6-Aminohexanoic acid, carbon disulfide, ethyl chloroformate, trimethychlorosilane, triethylamine, methanolic HCl solution (1.25 M), hydrazine hydrate, phenothiazine, potassium *tert*-butoxide, methyl iodide, phosphoryl chloride, chloroform, petroleum ether (b.p. = 40-60°C), anhydrous toluene, anhydrous hexane, absolute ethanol (EtOH), methanol (MeOH), ethyl acetate (AcOEt), diethyl ether (Et_2O), anhydrous tertahydrofuran (THF), anhydrous *N*,*N*-dimethylformamide (DMF), anhydrous dichloroethane (DCE), acetonitrile (MeCN, HPLC grade), tetrabutylammonium perchlorate ($TBAClO_4$, electrochemical grade), hydrochloric acid, anhydrous magnesium sulfate, sodium acetate and sodium hydroxide were purchased from Merck or Sigma-Aldrich and used without further purification. The following analytical grade salts were purchased from Merck, Sigma-Aldrich or Alfa Aesar and used as received: $Hg(CF_3SO_3)_2$, $PhHg(OCOCH_3)$, $Cd(ClO_4)_2{\cdot}6H_2O$, $Pb(ClO_4)_2{\cdot}3H_2O$, $Ni(ClO_4)_2{\cdot}6H_2O$, $Zn(ClO_4)_2{\cdot}6H_2O$, $Cu(ClO_4)_2{\cdot}6H_2O$.

6-(N-dithiocarbonylethoxycarbonyl)hexanoic acid (**1**) [16]: To a stirred solution of 9 g (200 mmol) sodium hydroxide in 60 mL water, 13.12 g (100 mmol) of 6-aminohexanoic acid were added. The mixture was cooled to 10°C under an argon atmosphere and 6.1 mL (7.68 g, 101 mmol) carbon disulfide were added dropwise, stirred for two hours and then keep to achieve room temperature. After 6 hours, 40 mL water were added, and the reaction mixture was cooled to 0°C. Next, 9.6 mL (10.89 g, 100 mmol) ethyl chloroformate were added dropwise and the reaction mixture was stirred for 15 min and then a solution of 8.5 mL HCl 37% in 10 mL was added dropwise. The resulting precipitate was dissolved in 50 mL chloroform and the solution was dried over anhydrous $MgSO_4$ and filtred. Next, 200 mL petroleum ether (b.p. = 40-60°C) were added and then the mixture was cooled to

-20°C and allowed to stand for 20 hours. The resulting precipitate was filtred, tritured with petroleum ether and dried at room temperature under reduced pressure to yield 12.2 g of 6-(*N*-dithiocarbonylethoxycarbonyl)hexanoic acid (43%). **^{1}H-NMR** (500 MHz, $CDCl_3$, ppm) δ: 1.35 (t, J = 7.1 Hz, 3H); 1.46 (dt, J = 15.4 Hz, 7.8 Hz, 2H); 1.69 (dd, J = 15.3 Hz, 7.7 Hz, 2H); 1.75 (dd, J = 15.0 Hz, 7.5 Hz, 2H); 2.39 (t, J = 7.4 Hz, 2H); 3.77 (dd, J = 12.5 Hz, 7.0 Hz, 2H); 4.35 (q, J = 7.1 Hz, 2H); 10.35 (s, 1H). **^{13}C-NMR** (125 MHz, $CDCl_3$, ppm) δ: 14.04; 24.10; 26.21; 27.52; 33.66; 45.58; 64.78; 170.94; 179.41; 186.72. **IR** (ATR, cm^{-1}) $\bar{\nu}$: 3246 (ν_{NH}); 3194 (ν_{NH}); 1702 ($\nu_{C=O}$); 1194 ($\nu_{C=S}$)

6-isothiocyanatohexanoic acid trimethylsilyl ester (**2**) [17]: A solution of 12 g (43 mmol) 6-(*N*-dithiocarbonylethoxycarbonyl)hexanoic acid in 120 mL anhydrous toluene was treated with 11 mL (9.41 g, 86.6 mmol) trimethylchlorosilane under an argon atmosphere. Next, 13 mL (9.43 g, 93.2 mmol) triethylamine were added dropwise, and the reaction mixture was refluxed for 2 hours. After allowing it to cool to room temperature, the triethylamine hydrochloride precipitate was filtred thorugh celite and the filtrate was evaporated under reduced pressure to yield 4.93 g (46%) pure 6-isothiocyanatohexanoic acid trimethylsilyl ester. **^{1}H-NMR** (500 MHz, $CDCl_3$, ppm) δ: 0.27 (s, 9H); 1.44 (dt, J = 15.3 Hz, 7.6 Hz, 2H); 1.62 (dt, J = 15.1 Hz, 7.4 Hz, 2H); 1.71 (dt, J = 14.0 Hz, 7.5 Hz, 2H); 2.32 (t, J = 7.3 Hz, 2H); 3.51 (t, J = 6.6 Hz, 2H). **^{13}C-NMR** (125 MHz, $CDCl_3$, ppm) δ: -0.31; 24.06; 25.98; 29.65; 35.47; 44.79; 129.90; 173.85. **IR** (ATR, cm^{-1}) $\bar{\nu}$: 2185 ($\nu_{N=C=S}$); 2093 ($\nu_{N=C=S}$); 1712 ($\nu_{C=O}$); 1252 ($\nu_{Si\text{-}C}$).

6-isothiocyanatohexanoic acid (**3**) [17]: To 25 mL anhydrous hexane, 4.9 g (20 mmol) 6-isothiocyanatohexanoic acid trimethylsilyl ester were added under an argon atmosphere. The resulting mixture was cooled to 0°C and 2.3 mL (1.81 g, 39.4 mmol) absolute ethanol were added, followed by a drop of methanolic HCl solution, and then stirred for 1 hour. The resulting oil was decanted, washed with cold anhydrous hexane and dried under reduced pressure to yield 1.66 g (48%) 6-isothiocyanatohexanoic acid.

^{1}H-NMR (500 MHz, $CDCl_3$, ppm) δ: 1.46 (dt, J = 15.5 Hz, 7.5 Hz, 2H); 1.65 (dd, J = 15.3 Hz, 7.6 Hz, 2H); 1.72 (dd, J = 14.6 Hz, 7.3 Hz, 2H); 2.38 (t, J = 7.4 Hz, 2H); 3.52 (t, J = 6.6 Hz, 2H); 10.89 (s, 1H). **^{13}C-NMR** (125 MHz, $CDCl_3$, ppm) δ: 23.75; 25.92; 29.55; 33.68; 44.76; 129.91; 179.65. **IR** (ATR, cm^{-1}) $\bar{\nu}$: 3036 (ν_{OH}); 2184 ($\nu_{N=C=S}$); 2092 ($\nu_{N=C=S}$); 1703 ($\nu_{C=O}$).

4-(5-carboxypentyl)-3-thiosemicarbazide (**4**), adapted from [18,19]: A solution of 0.5 mL (0.51 g, 98% purity, 10 mmol) hydrazine hydrate in 10 mL methanol was cooled to 0°C under an argon atmosphere for 15 min. and then treated with a solution of 1.66 g (9.6 mmol) 6-isothiocyanatohexanoic acid in 5 mL methanol. The reaction mixture was kept to achieve room temperature and then stirred for 20 hours. Then, the solvent was evaporated under reduced pressure and the residue was washed with 10 mL ethyl acetate and 10 mL diethyl ether, decanted and dried under reduced pressure to yield 1.85 (94%) 4-(5-carboxypentyl)-3-thiosemicarbazide.

^{1}**H-NMR** (500 MHz, DMSO-d_6, ppm) δ: 1.24 (dt, *J* = 15.5 Hz, 7.5 Hz, 2H); 1.45-1.51 (m, 4H); 2.14 (t, *J* = 7.3 Hz, 2H); 3.41 (dd, *J* = 13.0 Hz, 6.5 Hz, 2H); 7.80 (s, 1H); 8.53 (s, 1H). ^{13}**C-NMR** (125 MHz, DMSO-d_6, ppm) δ: 24.59; 26.01; 28.90; 34.37; 42.77; **175.04**; 181.16. **IR** (ATR, cm^{-1}) $\bar{\nu}$: 3280 ($\nu_{OH/NH}$); 1701 ($\nu_{C=O}$); 2184 ($\nu_{N=C=S}$); 1541 ($\nu_{C=N}$); 1240 ($\nu_{C=S}$).

10-methylphenothiazine (**5**), adapted from [20]: To a solution of 3.7 g (33 mmol) potassium *tert*-butoxide in 50 mL anhydrous THF cooled at 0°C, a solution of 4.4 (22 mmol) phenothiazine in 15 mL anhydrous THF was added dropwise under an argon atmoshpere. After stirring for 5 min., 2.1 mL (4.78 g, 33 mmol) methyl iodide were added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for 20 h. Next, 4 mL water were added and the mixture was evaporated to a volume of ~10 mL, under reduced pressure. The residue was added to 200 mL water and resulted precipitate was filtered, washed with 100 mL water and dried at room temperature, under reduced pressure. The crude product was purified by recrystalization from absolute ethanol to yield 2.14 g (45%) 10-methylphenothiazine.

IR (ATR, cm^{-1}) $\bar{\nu}$: 2972, 2891 ($\nu_{C_{sp^3}-H}$)

10-methyphenothiazine-3-carbaldehyde (**6**) [21]: A solution of 1 mL (0.944 g, 12.9 mmol) anhydrous *N*,*N*-dimethylformamide in 5 mL anhydrous dichloroethane was cooled to 0°C under an argon atmosphere, treated with 1.2 mL (1.98 g, 12.9 mmol) phosphoryl chloride, and stirred for 1 hour at 5°C. Next, a solution of 2.133 (10 mmol) 10-methylphenothiazine in 10 mL anhydrous dichloroethane was added dropwise and the mixture was refluxed for 6 hours. The reaction mixture was allowed to cool to 0°C and then treated with 15 mL of a saturated sodium acetate aqueous solution. The mixture was transferred to a separatory funnel and the organic layer was twice washed with 25 mL water, dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by dry column vacuum chromatography (silica gel, toluene) to yield 1.1 g (45%) pure 10-methyphenothiazine-3-carbaldehyde.

^{1}**H-NMR** (500 MHz, $CDCl_3$, ppm) δ: 3.43 (s, 3H); 6.84 (d, *J* = 4.7 Hz, 1H); 6.86 (d, *J* = 5.0 Hz, 1H); 6.99 (t, *J* = 7.5 Hz, 1H); 7.13 (d, *J* = 7.6 Hz, 1H); 7.19 (t, *J* = 8.1 Hz, 1H); 7.60 (d, *J* = 1.5 Hz, 1H); 7.65 (dd, *J* = 8.4 Hz, 1.5 Hz, 1H); 9.80 (s, 1H). ^{13}**C-NMR** (125 MHz, $CDCl_3$, ppm) δ: 35.82; 113.70; 114.76; 122.55; 123.63;

123.99; 127.29; 127.76; 127.94; 130.45; 131.21; 144.09; 151.07; 189.95. **IR** (ATR, cm^{-1}) $\bar{\nu}$: 2887, 2825 ($\nu_{C_{sp}{}^3\text{ - H}}$); 2770, 2734 ($\nu_{C(O)\text{-}H}$);1675 ($\nu_{C=O}$).

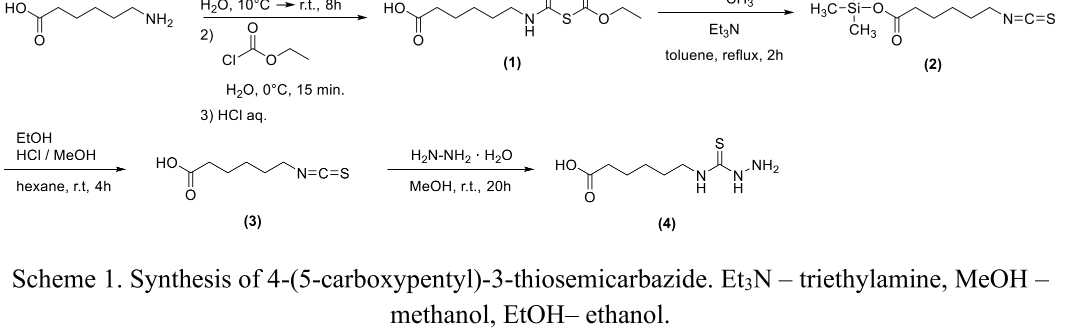
Phenothiazine-based thiosemicarbazone ligand (**Me_PHTSC**): A mixture of 0.2 g (1 mmol) 4-(5-carboxypentyl)-3-thiosemicarbazide, 250 mg (1 mmol) 10-methyphenothiazine-3-carbaldehyde and 5 mL methanol was refluxed for 20 hours under an argon atmoshpere. The mixture was evaporated under reduced pressure to approximately 2 mL, and the residue was cooled to -20°C for 24 hours. The resulting precipitate was filtred, washed with cold methanol and dried under reduced pressure at room temperature. The crude product was purified by recrystallization from acetonitrile to yield 290 mg (65%) pure **Me_PHTSC**.

^{1}H-NMR (500 MHz, DMSO-d$_6$, ppm) δ: 1.29 (dd, J = 15.0 Hz, 7.7 Hz, 2H); 1.45-1.65 (m, 4H); 2.21 (t, J = 7.3 Hz, 2H); 3.35 (s, 3H); 3.52 (dd, J = 13.9 Hz, 6.6 Hz, 2H); 6.93 (d, J = 8.5 Hz, 1H); 6.96 (d, J = 3.0 Hz, 1H); 6.97 (d, J = 4.1 Hz, 1H); 7.15 (d, J = 7.8 Hz, 1H); 7.21 (t, J = 7.2 Hz, 1H); 7.47 (dd, J = 8.4 Hz, 1.4 Hz, 1H); 7.71 (d, J = 1.4 Hz, 1H); 7.92 (s, 1H); 8.50 (t, J = 5.8 Hz, 1H); 11.30 (s, 1H). **^{13}C-NMR** (125 MHz, DMSO-d$_6$, ppm) δ: 24.76; 26.35; 29.15; 34.07; 35.81; 43.77; 114.83; 115.36; 121.98; 123.16; 123.32; 124.79; 127.29; 128.36; 128.42; 129.14; 141.37; 145.05; 146.89; 174.91; 177.10. **IR** (ATR, cm^{-1}) $\bar{\nu}$: 3358 (ν_{NH}); 3141 (ν_{NH}); 1699 (ν_{CO}); 1538 ($\nu_{C=N}$); 1255 ($\nu_{C=S}$).

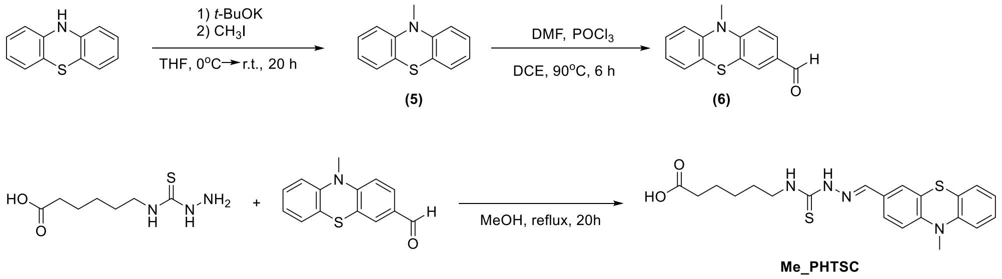
3. Results and discussion

3.1 Synthesis and characterization of Me_PHTSC ligand

The phenothiazine-based thiosemicarbazone ligand (**Me_PHTSC**) was synthesized through the reaction sequence shown in Scheme 2 and Scheme 2. Specifically, the key intermediate 4-(5-carboxypentyl)-3-thiosemicarbazide (**4**), obtained starting from 4-aminohexanoic acid, was condensed with 10-methylphenotiazine (**5**) to give **Me_PHTSC**.



Scheme 1. Synthesis of 4-(5-carboxypentyl)-3-thiosemicarbazide. Et$_3$N – triethylamine, MeOH – methanol, EtOH– ethanol.



Scheme 2. Synthesis of **Me_PHTSC** ligand. DMF – *N*,*N*-dimethylformamide, THF – tetrahydrofuran, DCE – dichloroethane.

3.2 Electrochemical behavior of Me_PHTSC ligand

The electrochemical properties of **Me_PHTSC** in solution were examined using cyclic voltammetry at glassy carbon (GC) electrodes. As anticipated, **Me_PHTSC** displays two different redox processes (Fig. 1), a reversible one at ~0.5V and an irreversible one at ~1V.

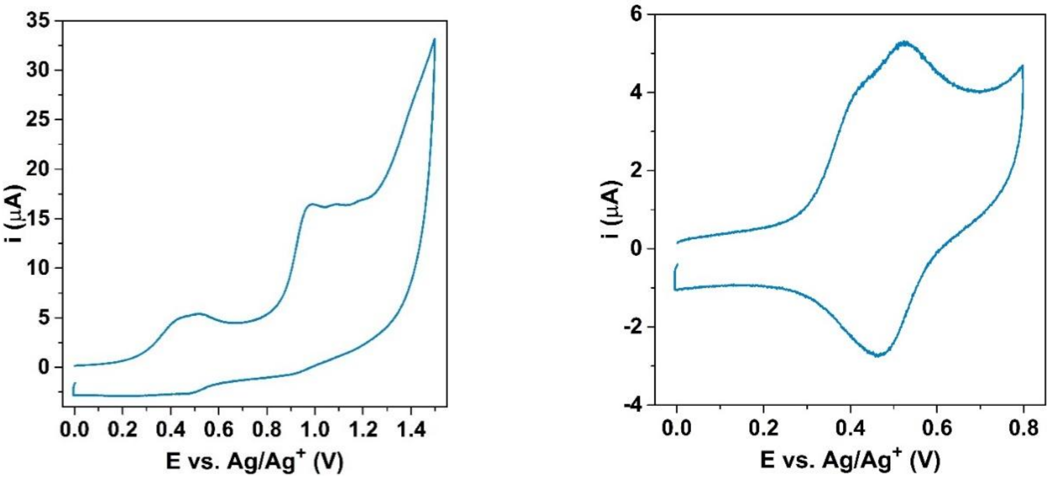
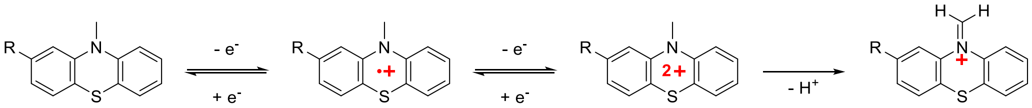


Fig. 1. Cyclic voltammograms (0.1 V·s^{-1}) recorded at a GC eletrode in a solution of **Me_PHTSC** (0.1 mM in MeCN with 0.1 M TBACl$_4$), in the potential range 0 - 1.5 V (left) and 0.0 - 0.8 V (right).

The two different processes can be tentatively explained through the mechanism shown in Scheme 3. In the first step, the phenotiazine moiety is oxidized to a radical-cation, which is further oxidized to a dication in the second step. However, the latter species is not stable and tends to deprotonate spontaneously to form an imino-cation species which cannot be subsequently reduced, thus making the entire process irreversible [22].



Scheme 3. Proposed mechanism for electrochemical oxidation of 10-methylphenotiazine derivatives.

Additionally, a closer examination of the voltammogram obtained for the first redox process (Fig. 1) indicates the existence of two overlapping voltammetric waves during the anodic scan. This is an indication that electrochemical oxidation occurs not only in the solution but also on the electrode surface, suggesting that the ligand adsorbs spontaneously onto the electrode surface, due to π-π stacking and hydrophobic interactions between the phenothiazine ring and the GC basal plane. This behavior is common for phenothiazine derivatives, such as methylene blue, promazine, chlorpromazine *etc.* [23-25]

The electrochemical behavior of **Me_PHTSC** in solutions containing heavy metal ions was also investigated through CV. As shown in Fig. 2, no significant changes in the voltammograms can be observed for any heavy metal ions tested. Only the first voltammetric wave (ascribed to the adsorbed ligand) disappears partially in the presence of Cu^{2+} and completely in the presence of Hg^{2+}. Presumably, the voltammetric wave associated with the complex completely overlaps with the wave corresponding to the free ligand in solution, so the two voltammetric waves cannot be distinguished.

Furthermore, the disappearance of the first voltammetric wave following complexation with Hg^{2+} ions may be attributed to the desorption of the complex from the surface of the GC electrode. Therefore, **Me_PHTSC** is unsuitable for obtaining modified electrodes for heavy metal ion sensing, unlike other thiosemicarbazone-based ligands previously developed by our group. [8,9]

3.3 Heavy metal ions detection using Me_PHTSC ligand through UV-VIS and fluorescence spectroscopy

The UV-VIS spectra of **Me_PHTSC** recorded in acetonitrile displays several absorption bands: the short wavelength bands (λ_{max} = 243 nm and λ_{max} = 262 nm) are attributed to π-π* transitions, while the longer wavelength bands, λ_{max} = 310 nm and λ_{max} = 373 nm, are attributed to n-π* transitions, with the former originating from the thiosemicarbazone moiety and the latter from the phenothiazine ring [26, 27].

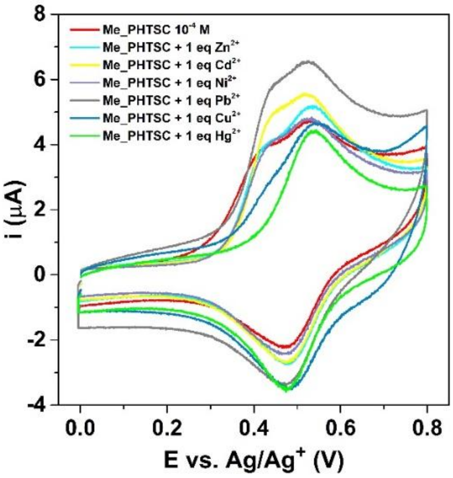


Fig. 2. Cyclic voltammograms (0.1 $V \cdot s^{-1}$) recorded at a GC eletrode in a solution of **Me_PHTSC** (0.1 mM in MeCN with 0.1 M $TBACl_4$) upon addition of 1 eq. of heavy metal ions (Zn^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+}, Cu^{2+}, Hg^{2+}).

The complexation behavior of **Me_PHTSC** ligand was assessed through UV-VIS spectroscopy, by recording the spectra of **Me_PHTSC** in the presence of 1 eq. of several heavy metal ions (Zn^{2+}, Cd^{2+}, Ni^{2+}, $Pb^{2+,}$ Cu^{2+}, Hg^{2+}), as well as an organomercury compound, i.e. phenyl mercuric acetate ($PhHg^{+}OAc^{-}$) (Fig. 3). The spectra recorded in the presence of Zn^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+} ions do not show any significant differences with respect to the spectrum of the free ligand, suggesting that no complexation process occurs. The spectrum recorded in the presence of 1 eq. Cu^{2+} displays hypochromic shifts of the 310 nm and 373 nm bands and a hyperchromic shift of the 262 nm band. The presence of Hg^{2+} ions induces a bathochromic shift of the 373 nm band, a hyperchromic shift of the 243 nm band and a hypochromic shift of the 310 nm one. Moreover, in the presence of $PhHg^{+}$, the spectrum displays small batochromic shifts of the 310 nm and 373 nm bands, as well as an important hypochromic shift of the 310 nm band. The hypochromic shifts of the 310 nm band suggest that Cu^{2+}, Hg^{2+} and $PhHg^{+}$ form complexes with **Me_PHTSC**, since the thiosemicarbazone lone pairs participating in the coordination of the metal cation are less susceptible to excitation. The selecitvity of the ligand towards the Hg^{2+} ions can be assesed based on the bathochromic shift of the 373 nm absorption band in contrast with the other heavy metal ion species investigated. This outcome is supported by *Hard and Soft Acid and Base Theory* (HSAB), whereby the Hg^{2+} ion is classified as a soft acid and the thiosemicarbazone unit functions as a soft base and is consistent with our group's prior findings [8,9].

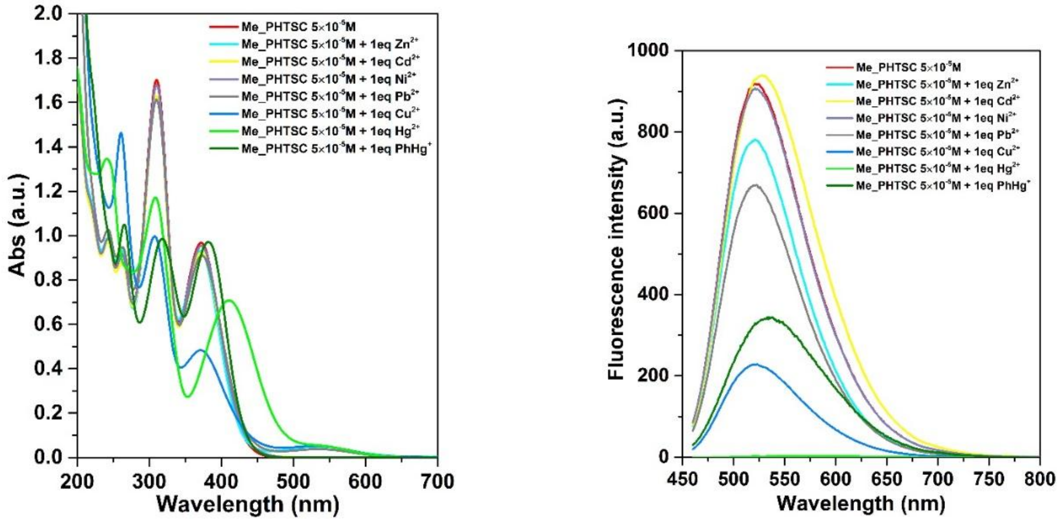


Fig. 3. UV-VIS (left) and fluorescence (right) spectra of **Me_PHTSC** (5×10^{-5} M in MeCN) recorded in the presence of 1 eq. of heavy metal ions (Zn^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+}, Cu^{2+}, Hg^{2+}, $PhHg^{+}$).

Furthermore, in order to determine the binding stoichiometry of **Me_PHTSC** with Hg^{2+} and $PhHg^{+}$, a Job's analysis was performed (Fig. 4).

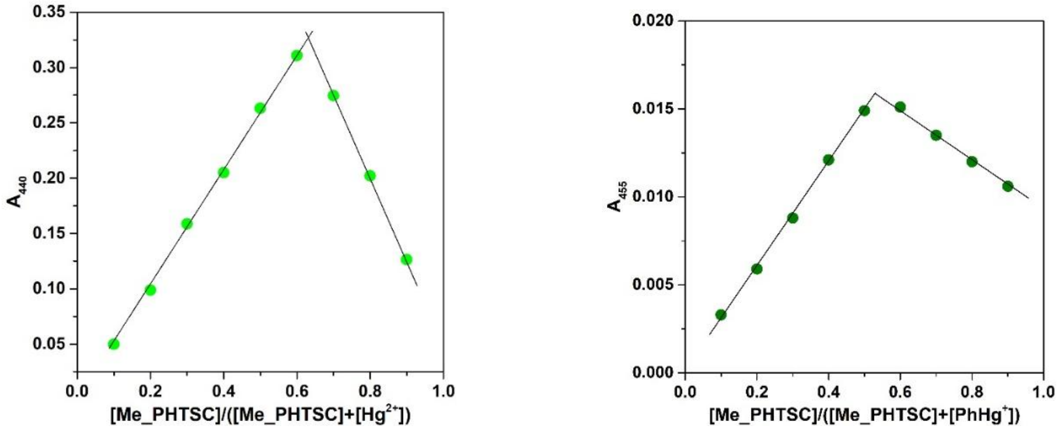


Fig. 4. Job plots corresponding to the spectrophotometric titration of **Me_PHTSC** with Hg^{2+} (left) and $PhHg^{+}$ (right).

The corresponding titration results showed that a complex with 2:1 metal to ligand stoichiometry forms between Hg^{2+} and **Me_PHTSC**, while $PhHg^{+}$ forms a 1:1 complex.

Finally, the complexation behavior of **Me_PHTSC** was also investigated through fluorescence spectroscopy. The emission spectrum of the free ligand recorded in acetonitrile with an excitation wavelength of 450 nm, displays an emission band with a maximum centered at 521 nm. No significant changes in the emission profile could be identified in the presence of 1 eq. Zn^{2+}, Cd^{2+}, Ni^{2+} and Pb^{2+} ions (Fig. 3). On the other hand, Cu^{2+}, Hg^{2+} and $PhHg^{+}$ induced fluorescence quenching, presumably through a *chelation enhanced quenching effect* (CHEQ) [28]. Moreover, as shown in Fig. 5Fig. 3, the fluorescence is effectively quenched in the presence of 1 eq. of Hg^{2+}, consitent with previous reports from the literature [29,30]. Unfortunately, the detection sensitivity is rather low, with a limit of quantitation (LOQ) of 5×10^{-6} M for Hg^{2+}, and 1×10^{-5} M for $PhHg^{+}$ as compared to other Hg^{2+} fluorescence-based sensors reported in the literature (

Table *1*).

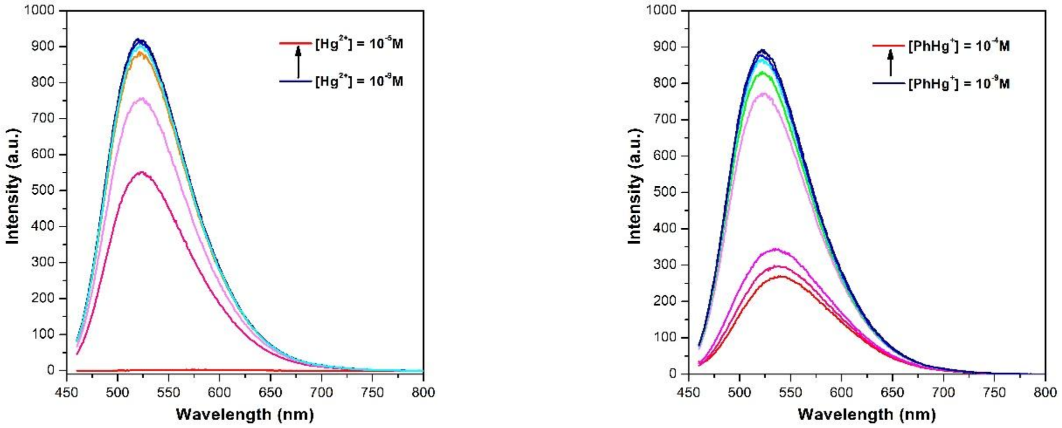


Fig. 5. Fluorescence spectra of **Me_PHTSC** (5×10^{-5} M in MeCN) recorded in the presence of increasing concentrations of Hg^{2+} (left) and $PhHg^{+}$ (right).

Table 1.

Literature reported fluorescence-based Hg^{2+} sensors and their correspondig LOD values.

Fluorescence-based sensor	**LOD for Hg^{2+}**	**Reference**
1,8-naphtalimide derivative	1.8×10^{-6} M	[31]
His-NMI-Bu	0.52×10^{-6} M	[32]
PY-2TH	22×10^{-9} M	[33]
Cou-S	2.3×10^{-9} M	[34]
aza-BODIPY 2b	1.0×10^{-7} M	[35]
Imidazole derivative	5.3×10^{-9} M	[36]

4. Conclusions

In this work, we report the synthesis and characterization of a fluorescent thiosemicarbazone ligand containing a phenothiazine moiety. The **Me_PHTSC** ligand was synthesized through a condensation reaction between 4-(5-carboxypentyl)-3-thiosemicarbazide and 10-methyphenothiazine-3-carbaldehyde. The complexation behavior of **Me_PHTSC** towards several heavy metal ions was studied through both UV-VIS and fluorescence spectroscopy. The photophysical properties of **Me_PHTSC** remained unchanged in the presence of Zn^{2+}, Cd^{2+}, Ni^{2+} and Pb^{2+}, suggesting that no complexation process occurs. In contrast, the fluorescence emission was effectively quenched in solutions containing Cu^{2+}, Hg^{2+} and $PhHg^{+}$ ions. These results suggest that **Me_PHTSC** ligand can be used as a *turn-off* fluorescence chemosensor for Hg^{2+} and $PhHg^{+}$ ions.

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