

ARTICLES

Amperometric Detection of Unithiol Complexes of Heavy Metals in High-Performance Liquid Chromatography

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Abstract—It was demonstrated that Pb(II), Cd(II), Hg(II), Ni(II), Co(II), and Cu(II) can be indirectly determined as their unithiol complexes by amperometric detection under static and HPLC conditions. Factors affecting the chromatographic separation and amperometric detection of metal complexes of unithiol were studied. Two designs of flow electrochemical cells (thin-layer and wall-jet cells) and three electrode materials (platinum, graphite, and glassy carbon) were compared. The best sensitivity was attained for an amperometric detector with wall-jet flow cell and a graphite indicator electrode. The detection limits for Hg(II), Pb(II), and Cd(II) were 0.9, 0.3, and 0.1 $\mu\text{g/mL}$, respectively. The chromatographic determination of heavy metals in a sample of waste water was carried out using the amperometric detector.

High-performance liquid chromatography (HPLC) finds increasing use for monitoring the concentration of organic and inorganic toxicants, including heavy metals, in various naturally occurring samples. Metal ions are usually separated as chelate complexes using reversed-phase and ion-pair HPLC. Unithiol, which meets all requirements imposed on chelating agents used in HPLC, is one of the promising ligands. It reacts with many metals to form stable and labile complexes. Heavy and transition metals can be separated and determined by chromatography using unithiol [1].

As a rule, metal complexes are detected in HPLC with spectrophotometric detectors. Up to now, unfairly little attention has been given to the capabilities of electrochemical detectors, including the simplest electrochemical detectors, amperometric. The evident advantages of these detectors are wide linearity ranges of the calibration graphs, small volumes of cells, rapid responses to a change in the concentration of the ionophore, low background signals, and the simplicity of their design [2]. It was shown previously [3] that penicillamine complexes of heavy metals can be successfully determined with an amperometric detector after their separation on a microcolumn chromatograph.

The use of readily oxidizable unithiol can also extend the range of electrochemical detectors for determining metal ions after their chromatographic separation. The goal of this work was to study the indirect amperometric detection of unithiol complexes of Pb(II), Cd(II), Hg(II), Ni(II), Co(II), and Cu(II) in HPLC. Special attention was given to the choice of the most suitable material for the indicator electrode and to the design of a cell.

EXPERIMENTAL

A PO-5122 (Model 03) polarograph with a two-electrode cell supplied with a silver-silver chloride (saturated KCl) reference electrode was used in this work. A setup for the amperometric detection under static conditions consisted of an M45M potential controller, an M-95 microammeter, a two-electrode cell with a silver-silver chloride reference electrode, and a magnetic stirrer [3]. Current strength was measured in the potential range from 0.5 to 1.0 V. An indicator electrode was polarized by switching the cell into the circuit only during the measurements. A paraffin-impregnated graphite electrode (GE) with an apparent surface area (S) of 28.3 mm^2 , a glassy-carbon electrode (GCE) with $S = 9.6 \text{ mm}^2$, and a platinum electrode (Pt-E) with $S = 85 \text{ mm}^2$ from Radelkis served as indicator electrodes.

The chromatographic determination of complexes was carried out on a chromatographic installation including a Marathon 11 HPLC pump and an injector valve with a loop volume of 50 μL provided by Rheodyne. A steel chromatographic column (150 mm \times 3 mm i. d.) packed with a Separon C_{18} sorbent with the grain diameter 5.0 μm was used. Two amperometric detectors obtained from Biotronik were used in this work. The first detector with a thin-layer three-electrode flow cell with a glassy-carbon disk ($S = 7.1 \text{ mm}^2$) or a Pt disk ($S = 8.0 \text{ mm}^2$) working electrode and a silver-silver chloride reference electrode; the steel case of the cell served as an auxiliary electrode. The second cell consisted of an AD-01 potential controller and a two-electrode wall-jet flow electrochemical cell [4] with a GE ($S = 1.8 \text{ mm}^2$) as an indicator electrode and the stainless-steel case as an auxiliary electrode. A thin-layer flow cell with a Pt electrode for the detector from Biotronik was made in our laboratory from Teflon F-4.

Standard stock solutions of Ni(II) (10 mg/mL), Cu(II) (10 mg/mL), and Pb(II) (2 mg/mL) were prepared by dissolving accurately weighed samples of metals of high-purity grade in HNO₃ (1 : 1) under heating followed by the triple evaporation with 5 mL of HCl (1 : 1) to wet salts to remove nitrogen oxides. After cooling, the residue was dissolved in 100 mL of 0.1 M HCl. Standard stock solutions of Co(II) (10 mg/mL) and Cd(II) (2 mg/mL) were prepared by dissolving accurately weighed samples of metals of high-purity grade in 10 mL of HCl (1 : 1) followed by the dilution to 100 mL with 0.1 M HCl. The standard stock solution of Hg(II) (2 mg/mL) was prepared by dissolving an accurately weighed sample of Hg(NO₃)₂ · 9H₂O in HCl (1 : 1) under heating followed by the triple evaporation with 5 mL of HCl to wet salts and dilution to 100 mL with 0.1 M HCl. The working solutions (100 µg/mL) were prepared by diluting the standard stock solutions. The solutions of supporting electrolytes were prepared from chemically pure CH₃COONa, H₂SO₄ and CH₃COOH and KCl of high-purity grade. A 5% unithiol solution from injection ampoules, tetrabutyl ammonium bromide (TBAB) of reagent grade provided by Diagnostikum, and acetonitrile of analytical grade were used without additional purification.

RESULTS AND DISCUSSION

Electrooxidation of unithiol at solid electrodes. It is known [5] that unithiol can be oxidized at solid electrodes to give irreversible diffusion waves. Based on this fact, procedures of the amperometric titration of a number of metals with unithiol were developed [5] and it was supposed that metal ions can be indirectly detected as their complexes with unithiol under HPLC conditions with an amperometric detector. First, it was important to study the electrochemical behavior of unithiol at electrodes made of different materials (platinum, graphite, and glassy carbon) in different supporting electrolytes in order to select an optimal electrode material and the conditions for detecting the unithiol oxidation current. Therefore, the behavior of unithiol at the above electrodes was studied by cyclic voltammetry in 0.1–2.0 M H₂SO₄, 0.1–1.0 M KCl, 0.1–1.0 M CH₃COONa solutions, and in a 0.1 M acetate buffer solution. These supporting electrolytes were successfully used previously in the amperometric titration of heavy metal ions with unithiol [5]; a CH₃COONa solution and an acetate buffer solution are often included into mobile phases in HPLC, especially into those used for the separation of metal complexes. Cyclic voltammograms were recorded in the potential range from 0 to 1.2 V at a potential sweep rate of 0.5 V/s using the anodic–cathodic sweep.

It was found that unithiol was irreversibly oxidized at all electrodes and in all supporting electrolytes listed above, because in cyclic voltammograms, only anodic peaks were detected at 0.5–0.7 V; the heights of these

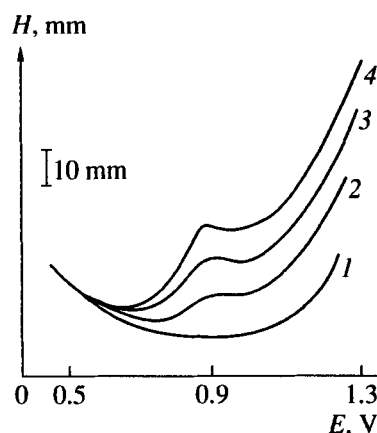


Fig. 1. Anodic branches of cyclic voltammograms obtained for (1) 0, (2) 1.3×10^{-3} , (3) 2.5×10^{-3} , and (4) 3.6×10^{-3} M unithiol solution at a Pt electrode in a 0.2 M KCl solution (potential sweep rate, 0.5 V/s).

peaks increased in the order GCE < GE < Pt-E. The peaks of unithiol oxidation at GCE were very unclear.

We managed to observe the peaks of unithiol oxidation in H₂SO₄ solutions only at the Pt electrode when concentrations of the supporting electrolyte were higher than 0.5 M. As the concentration of the H₂SO₄ solution increased from 0.8 to 2 M, peak heights increased, while their potentials remained virtually unchanged (0.62–0.64 V). The height of unithiol oxidation peak linearly depended on its concentration in the range from 1×10^{-3} to 1×10^{-2} M, which supports the diffusion character of its electrooxidation.

We observed well-defined peaks of unithiol oxidation at both the Pt and the graphite electrodes in CH₃COONa and KCl solutions and also in an acetate buffer solution. As an example, anodic branches of cyclic voltammograms of unithiol obtained at the Pt electrode in a 0.2 M KCl solution are shown in Fig. 1. It follows from the plots of the anodic peak current versus the potential sweep rate that the rate of unithiol oxidation under these conditions was diffusion-controlled and complicated with adsorption (the slope of the $\log(I_p)$ – $\log V$ plot was 0.67 ± 0.04). The heights of peaks of unithiol oxidation at the Pt and graphite electrodes linearly depended on the concentrations of both KCl in the range from 0.2 to 0.9 M and unithiol in the range from 7×10^{-4} to 8×10^{-3} M. The longest linear portion in the plots of unithiol oxidation peak height versus the unithiol concentrations was observed in a 0.5 M KCl solution. Similar dependences for Pt and graphite electrodes in a 0.1 M acetate buffer solution were linear in the range of unithiol concentrations from 1×10^{-4} to 1×10^{-3} M. It is interesting to note that, on the addition of 0.01 to 0.25 M KCl to a 0.1 M acetate buffer solution, the height of the unithiol oxidation peak increased almost three times.

Table 1. Conditions for the amperometric detection of metal ions at electrodes made of different materials under static conditions and the equations of straight lines ($I = A + Bx$ (I is the unithiol oxidation current, μA , $x = c_{\text{Me(II)}} \times 10^5 \text{ M}$) (0.1 M acetate buffer solution (65 vol %) + acetonitrile (35 vol %) + 5×10^{-4} M unithiol solution + 2×10^{-2} M TBAB solution served as the supporting electrolyte)

Metal ion	Electrode material	Potential, V	Straight line equation	Linearity range, M
Ni(II)	Graphite	0.7	$I = -1.4x + 62.2$	$3 \times 10^{-5} - 1 \times 10^{-4}$
	Platinum	0.7	$I = -1.8x + 75.1$	$1 \times 10^{-5} - 1 \times 10^{-4}$
Co(II)	Graphite	0.8	$I = -0.2x + 7.8$	$3 \times 10^{-5} - 1 \times 10^{-4}$
	Platinum	0.7	$I = -5.6x + 154.6$	$5 \times 10^{-5} - 1 \times 10^{-4}$
Cu(II)	Platinum	0.5	$I = -1.4x + 75.4$	$1 \times 10^{-5} - 5 \times 10^{-5}$
Pb(II)	Graphite	0.7	$I = -0.7x + 15.6$	$1 \times 10^{-5} - 1 \times 10^{-4}$
Cd(II)	Graphite	1.0	$I = -3.7x + 194.4$	$1 \times 10^{-5} - 1 \times 10^{-4}$
	Platinum	0.8	$I = -2.1x + 131.4$	$1 \times 10^{-5} - 1 \times 10^{-4}$
Hg(II)	Graphite	0.9	$I = -1.6x + 216.4$	$2 \times 10^{-5} - 1.5 \times 10^{-4}$
	Platinum	1.0	$I = -6.4x + 132.5$	$1 \times 10^{-5} - 1.5 \times 10^{-4}$

Amperometric detection of unithiol complexes of metals under static conditions. The current of unithiol oxidation at the Pt electrode in a 0.1 M CH_3COONa solution in the potential range from 0.5 to 1.0 V increased as its concentration increased from 5×10^{-5} to 1×10^{-4} M; the slope of the calibration graph increased on going to more positive potentials (from 0.5 to 0.7 V). As anodic potential continued to grow, the reproducibility of the current peaks became somewhat worse. In a 0.1 M acetate buffer solution, the unithiol oxidation current can be measured in the wider range of potentials (from 0.5 to 0.9 V) than in a 0.1 M CH_3COONa solution. The linear dependence of the unithiol oxidation current on the unithiol concentration was observed at 0.9 V in the range from 3×10^{-5} to 1×10^{-4} M (the straight line equation is $I = 1.63 \times 10^3 x - 133$, where I is the oxidation current of unithiol, μA and x is the unithiol concentration, M). The addition of KCl to a 0.1 M acetate buffer solution extended the linearity range of the concentration dependence of the unithiol oxidation current. The equation of a straight line obtained for 1×10^{-5} to 1.1×10^{-4} M unithiol solutions in a 0.1 M acetate buffer solution + 0.1 M KCl solution at 0.9 V was as follows: $I = 1.67 \times 10^3 x + 220$, where I is the oxidation current of unithiol, μA and x is the unithiol concentration, M. The slope of the calibration graph increased as the concentration of potassium chloride was increased to 0.25 M.

The possibility of the amperometric detection of unithiol complexes of heavy and transition metals (Pb(II), Cd(II), Hg(II), Co(II), Ni(II), and Cu(II)) was examined at electrodes made of different materials. The results are presented in Table 1. It is important that, as the concentration of each of the above-listed metals in a solution containing unithiol increased, the unithiol oxidation current decreased. This points to the possibility of the indirect amperometric detection of the studied metals. It follows from the data given in Table 1 that the

detection potentials obtained at the graphite electrode for the majority of metals under study were more positive than those obtained at the Pt electrode.

It was found in preliminary experiments that the unithiol complexes of heavy metals can be efficiently separated using a mobile phase consisting of a 0.1 M acetate buffer solution with pH 6.0 (65 vol %), acetonitrile (35 vol %), and 2×10^{-2} M tetrabutyl ammonium bromide (TBAB) as an ion-pair reagent. The addition of up to 40 vol % acetonitrile and up to 2×10^{-2} M TBAB solution to the supporting electrolyte solution only slightly affected the unithiol oxidation current. We had to rule out the addition of potassium chloride to the mobile phase, because it deteriorated the resolution of chromatographic peaks.

It follows from the results obtained that the indirect amperometric detection of Pb(II), Cd(II), and Hg(II) ions was possible in the potential range from 0.5–1.0 V, where the linear dependence of a drop in the unithiol oxidation current on the concentration of a metal ion is observed. For Ni(II), Co(II), and Cu(II) ions, a drop in the unithiol oxidation current was observed in a narrower range of potentials (0.5–0.8 V) and in a narrower range of metal concentrations. Therefore, the possibility of the amperometric detection of unithiol–metal complexes after their HPLC separation was examined in greater detail using unithiol complexes of Pb(II), Cd(II), and Hg(II) as examples.

Amperometric detection of metal–unithiol complexes under HPLC conditions. It is known [6] that unithiol forms negatively charged complexes with the majority of metals; therefore, these complexes should be separated by ion-pair reversed-phase HPLC. A hydrophobic silica gel, Separon C_{18} , was used as a stationary phase. Mixtures of acetonitrile and acetate buffer solution were used as the mobile phase. We chose acetonitrile as an organic modifier of the mobile phase because of its high elution ability, low viscosity,

and complete miscibility with water [7]. Acetonitrile dissolves both the selected organic reagent and the metal complexes under study.

The composition of the aqueous component of the mobile phase has a significant effect on the chromatographic behavior of chelates, because the degree of dissociation of the reagents and their complexes, as well as the composition of the complexes are changed with a change in pH, ionic strength, and anionic composition of the mobile phase. Unithiol forms stable complexes with metals in the pH range from 5 to 8 [6]; because of this, we used an acetate buffer solution with pH 6.0.

The chromatographic behavior of unithiol complexes strongly depended on the procedure of their preparation. A comparison of the chromatographic behavior of unithiol complexes obtained beforehand and *in situ* on a column by the addition of unithiol to the mobile phase showed that the efficiency and selectivity of the chromatographic system was higher if the complexes was obtained *in situ* on the column. The concentration of unithiol in the mobile phase was 5×10^{-4} M.

It is important to properly choose an ion-pair reagent. Because unithiol chelates of metals are negatively charged, for their separation by ion-pair reversed-phase HPLC, one should use ion-pair reagents with hydrophobic cations, for example, quaternary ammonium salts. It was found previously that the maximum selectivity of metal separation was achieved with tetrabutyl ammonium bromide as an ion-pair reagent. Thus, the optimal composition of the mobile phase for the chromatographic separation of unithiol complexes of metals was the following: acetonitrile (35 vol %), an acetate buffer solution with pH ~ 6.0 (65 vol %), a 2×10^{-2} M TBAB solution, and a 5×10^{-4} M unithiol solution.

The potential of a working electrode is the most important parameter of the amperometric detection. Because the electrochemical behavior of substances under the dynamic conditions may differ from that under the static conditions, we constructed a hydrodynamic voltammogram to select the potential range for the amperometric detection of unithiol complexes of heavy metals under HPLC conditions using the flow system of a Milikhrom chromatograph and an electrochemical detector with the wall-jet cell and a graphite indicator electrode. A 5×10^{-4} M unithiol solution was injected into the flow of the mobile phase (acetonitrile (35 vol %), acetate buffer solution of pH ~ 6.0 (65 vol %), and 2×10^{-2} M TBAB) in 10- μ L portions. The flow was sent to the detector cell. The potential of the graphite electrode was varied from 0.5 to 1.2 V, and heights of the unithiol oxidation peaks were recorded at a given potential and measured (Fig. 2). It was found that the heights of the peaks obtained at the indicator electrode in the potential range from 1.0 to 1.1 V differed little and were reproduced better than the peak heights obtained at more positive potentials. The injection of Pb(II), Cd(II), and Hg(II) into the flow of the mobile

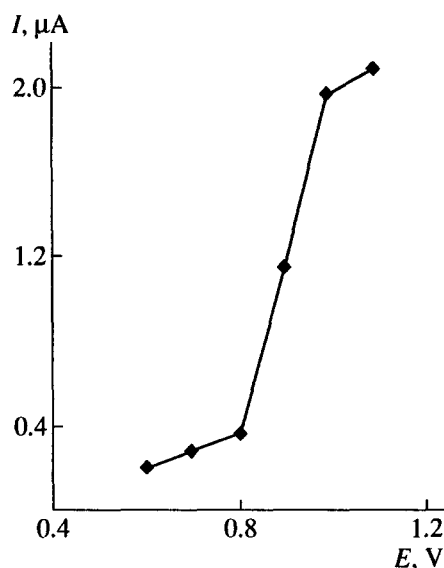


Fig. 2. Hydrodynamic voltammogram obtained after the injection of 10 μ L of a 5×10^{-4} M unithiol solution into the flow of the mobile phase (a wall-jet cell; working electrode, GE; mobile phase, acetonitrile (35 vol %) + acetate buffer solution with pH 6.0 (65 vol %) + 5×10^{-4} M unithiol + 2×10^{-2} M TBAB).

phase (acetonitrile (35 vol %), acetate buffer solution of pH ~ 6.0 (65 vol %), and 2×10^{-2} M TBAB) containing 5×10^{-4} M unithiol resulted in the formation of reverse peaks caused by a decrease in the background signal due to the current of unithiol oxidation at the indicator electrode. The heights of reverse peaks depended on the potential of the indicator electrode in the range from 0.6 to 1.2 V. The maximum signal was observed at 1.1 V. This potential was used in the further work. Similar studies demonstrated that the best reproducibility of the results was achieved with thin-layer flow cells at the potential of the indicator electrode equal to 0.8 V (versus a silver-silver chloride electrode).

Table 2. Detection limits and determination limits for Pb(II), Cd(II), and Hg(II) ions attained with amperometric detectors including cells of various design with different electrodes (mobile phase, acetonitrile (35 vol %) + an acetate buffer solution with pH 6.0 (65 vol %) + 5×10^{-4} M unithiol + 2×10^{-2} M TBAB; eluant flow rate, 400 μ L/min; electrode potential, 1.1 V; stationary phase, Separon C₁₈)

Cell design	Electrode	Element	Lower boundary of determinable concentrations, μ g/mL	Detection limit, μ g/mL
Wall-jet	GE	Pb	0.7	0.3
	GE	Cd	0.3	0.1
	GE	Hg	2.7	0.9
Thin-layer	Pt-E	Pb	0.6	0.2
	GCE	Pb	0.6	0.2

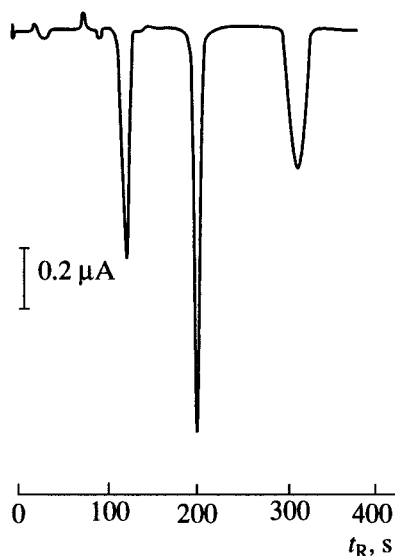


Fig. 3. Chromatogram of a mixture of unithiol complexes of Pd(II), Cd(II), and Hg(II) (mobile phase, acetonitrile (35 vol %) + acetate buffer solution with pH 6.0 (65 vol %) + 5×10^{-4} M unithiol + 2×10^{-2} M TBAB; eluant flow rate, 400 $\mu\text{L}/\text{min}$; electrode potential, 1.1 V; stationary phase, Separon C₁₈; $c_{\text{Me}} = 10 \mu\text{g}/\text{mL}$).

The eluant flow rate may have a significant effect on the sensitivity of amperometric detection [2]. It was found that a 1.5-fold increase in the flow rate of the mobile phase resulted only in a 10% increase in the unithiol oxidation current, i.e., no considerable gain in sensitivity was achieved. The flow rate of the mobile phase used in our work was 400 $\mu\text{L}/\text{min}$.

We compared the potentialities of the amperometric detection of unithiol complexes of Pb(II), Cd(II), and Hg(II) after their chromatographic separation for a wall-jet flow cell with the graphite electrode, a flat thin-layer flow cell with the glassy-carbon electrode, and a flat thin-layer flow cell with the Pt electrode. The maximum sensitivity to Cd(II) and Hg(II) was attained with the detector including the wall-jet-type cell with the graphite electrode. As might be expected, the carbon-glassy electrode exhibited the minimum sensitivity

Table 3. Parameters of chromatographic separation of a mixture of unithiol complexes of metals (mobile phase, acetonitrile (35 vol %) + an acetate buffer solution with pH 6.0 (65 vol %) + 5×10^{-4} M unithiol + 2×10^{-2} M TBAB; eluant flow rate, 400 $\mu\text{L}/\text{min}$; electrode potential, 1.1 V; stationary phase, Separon C₁₈; $c_{\text{Me}} = 10 \mu\text{g}/\text{mL}$)

Components to be separated	t_R	k'	N	α	R_S
Pb	135	0.69	121	2.21	2.75
Cd	202	1.53	272	2.54	4.13
Hg	390	3.88	961	—	—

to these metal ions: we failed to obtain a signal for Hg(II) with this detector. Linear calibration curves were obtained for Pb(II) with both types of cells and with all electrodes tested. The linearity ranges in the plots of the detector signal against the Pb(II) concentration extended in the series GCE < Pt-E for the thin-layer cell and were even wider for the wall-jet cell with the graphite electrode. The determination limits and the detection limits for Pb(II), Cd(II), and Hg(II) calculated from 3s test are presented in Table 2. The background signal only slightly changed with time for all three detectors, but the values of the background signal were different. The maximum background signal was observed for the detector with the Pt electrode; the sensitivity of determining metal ions with this detector was lower than that with the detector with a graphite electrode. Note that it was not necessary to regenerate the surface of the graphite electrode in the wall-jet cell as distinct from the electrodes in the thin-layer flow cell. This specific feature of the wall-jet type detecting systems were noted previously along with the fact that these detectors provide the lowest residual currents and wide linearity ranges of calibration graphs [8].

A chromatogram of a mixture of unithiol complexes of three metal ions (Pb(II), Cd(II), and Hg(II)) recorded with an amperometric detector with the graphite electrode is shown in Fig. 3. The parameters of chromatographic separation of these complexes are listed in Table 3.

A sample of industrial waste water from an electroplating plant was first purified by filtration through a coarse calico filter filled with sand and then analyzed under the selected conditions.

Determination procedure. A sample is filtered through an Acrodise CR PTFE 0.2- μm filter (Dionex) and diluted with distilled water in a ratio of 1 : 16. A 50- μL portion of the solution is injected into the chromatographic column, and the chromatographic analysis is repeated at least three times at a working electrode potential of 1.1 V. The concentration of Pb(II), Cd(II), and Hg(II) is found from the calibration graphs (Table 4) or by the standard addition method.

Table 4. Equations of straight lines $H = A + BX$ (H is the peak height in the chromatogram of a mixture of unithiol complexes of Pb(II), Cd(II), and Hg(II), mm; $X = c_{\text{Me}}$, $\mu\text{g}/\text{mL}$) (mobile phase, acetonitrile (35 vol %) + an acetate buffer solution with pH 6.0 (65 vol %) + 5×10^{-4} M unithiol + 2×10^{-2} M TBAB; eluant flow rate, 400 $\mu\text{L}/\text{min}$; electrode potential, 1.1 V; stationary phase, Separon C₁₈)

Metal ion	Straight line equation	Correlation coefficient	Linearity range, $\mu\text{g}/\text{mL}$	RSD, %
Pb	$H = 5.8x + 10.8$	0.98	2.5–20	3
Cd	$H = 11.6x + 7.6$	0.99	2.5–20	5
Hg	$H = 1.7x + 0.1$	0.99	2.5–20	2

Table 5. Results of an analysis of a waste water sample ($P = 0.95$, $n = 3$) (mobile phase: acetonitrile (35 vol %) + acetate buffer solution with pH 6.0 (65 vol %) + 5×10^{-4} M unithiol + 2×10^{-2} M TBAB; eluant flow rate, 400 $\mu\text{L}/\text{min}$; electrode potential, 1.1 V; stationary phase, Separon C₁₈)

Analyte	Found, $\mu\text{g}/\text{mL}$ $\bar{x} \pm \delta$	RSD, %	Concentration of an element, $\mu\text{g}/\text{mL}$
Pb	85 ± 4	2	$78 \pm 6^*$, $85 \pm 5^{**}$
Hg	198 ± 10	2	$198 \pm 7^*$
Cd	74 ± 4	2	$74 \pm 5^*$, $69 \pm 3^{**}$

* Found by HPLC with spectrophotometric detection at $\lambda_{\text{max}} = 264$ nm.

** Found by atomic absorption spectroscopy.

The results of analysis are presented in Table 5. The developed procedure was verified by comparing the obtained results with the data of atomic absorption determination and with the results obtained by HPLC with the spectrophotometric detection. The proposed procedure is rapid (the time of analysis varies from 5 to 15 min, depending on the sample) and simple; it does not require preliminary isolation of metal complexes by solvent extraction.

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