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Ion-Pair Chromatography of Metal Complexes of Unithiol in the Presence of Quaternary Phosphonium Salts

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Abstract—The chromatographic behavior of heavy-metal (Pb, Cd, Cu, Hg, and Ni) complexes of unithiol was studied by ion-pair reversed-phase chromatography using new ion-pair reagents: tetrabutylphosphonium and tributylhexadecylphosphonium bromides. The dependence of the retention of metal unithiolates on the nature and concentration of ion-pair reagents, concentrations of an organic solvent and inorganic salts in the mobile phase, and the pH of the mobile phase was studied. It was found that the retention of complexes increases upon increasing the hydrophobicity and concentration of the ion-pair reagent and upon decreasing the concentrations of acetonitrile and inorganic salts ($NaClO_4$ and $NaNO_3$). Optimal conditions of the separation of Pb, Cd, Cu, Hg, and Ni unithiolates were selected, and the possibility of their separate determination in a mixture was demonstrated. Concentrations of metals in process water were determined.

The determination of heavy-metal ions in the monitoring of natural and waste waters, vegetable materials, and soils requires highly sensitive and selective methods of analysis. The use of high-performance liquid chromatography (HPLC) for solving this problem attracts increasing attention from researchers. The most popular is the determination of heavy metals by adsorption HPLC as alkyldithiocarbamates [1–5]; however, the preliminary preparation of metal chelates is necessary in this case. The determination is more rapid when metals are separated as complexes that are formed directly in a chromatographic column on the addition of a chelating agent to the mobile phase. It was found [6] that this technique can be implemented with the use of sodium 2,3-dimercaptopropanesulfonate (unithiol), which is a well-known analytical group reagent and a widespread antidote for the treatment of poisoning with heavy metals [7]. Previously, we demonstrated that mercury can be efficiently determined by ion-pair HPLC in different materials using unithiol [6].

The aim of this work was to reveal main regularities in the retention of Cu(II), Cd(II), Ni(II), Pb(II), Fe(III), and Hg(II) unithiolates by ion-pair chromatography using quaternary phosphonium salts and to develop a procedure for the determination of heavy metals in process and waste waters.

EXPERIMENTAL

Stock solutions of Cu(II), Ni(II), and Pb(II) (0.50 mg/mL) were prepared by the dissolution of weighed portions of the metals of high-purity grade in HNO_3 (1 : 1) on heating and subsequent threefold evaporation with HCl (5 mL each time) to wet salts for removing nitrogen oxides. After cooling, the residue was dissolved in 100 mL of 0.1 M HCl. Stock solutions of Cd(II) and Fe(III) (0.50 mg/mL) were prepared by the dissolution of weighed portions of the metals of high-purity grade in 10 mL of HCl (1 : 1) and subsequent dilution to 100 mL with 0.1 M HCl. A stock solution of Hg(II) (1.0 mg/mL) was prepared by the dissolution of a weighed portion of chemically pure HgO in 0.1 M HCl. Working solutions of metals (100 μg/mL) were prepared by the dilution of corresponding stock solutions.

In this work, we used $NaClO_4$ and $NaNO_3$ of chemically pure grade, unithiol from Sigma, tetrabutylphosphonium bromide (TBPB) and tributylhexadecylphosphonium bromide (TBHDPB) from Merck, and acetonitrile of chemically pure grade without additional purification. Acetate buffer solutions were prepared according to recommendations from [8].

The chromatography of complexes was performed on a Milikhrom-2 chromatograph (Nauchpribor, Orel, Russia) equipped with a spectrophotometric detector (264 nm). We used a steel column (120 × 2 mm) filled with the Silasorb C_{18} sorbent with a particle size of 10 μm (Elsiko, Russia). The volume of a sample was 10 μL. In this work, we also used a Kontron liquid chromatograph (Italy) with a steel column (150 × 3 mm) filled with the Silasorb C_{18} sorbent with the particle size 10 μm (Elsiko, Russia). The volume of a sample was 100 μL.

RESULTS AND DISCUSSION

Sodium 2,3-dimercaptopropanesulfonate (unithiol) and heavy and transition metal ions form stable anionic complexes of composition 1 : 1 in weakly acidic solutions and 1 : 2 in solutions with pH > 6 [9–12]. These

Table 1. Capacity and selectivity factors of metal unithiolate complexes with the use of TBAB and CTMAB as ion-pair reagents
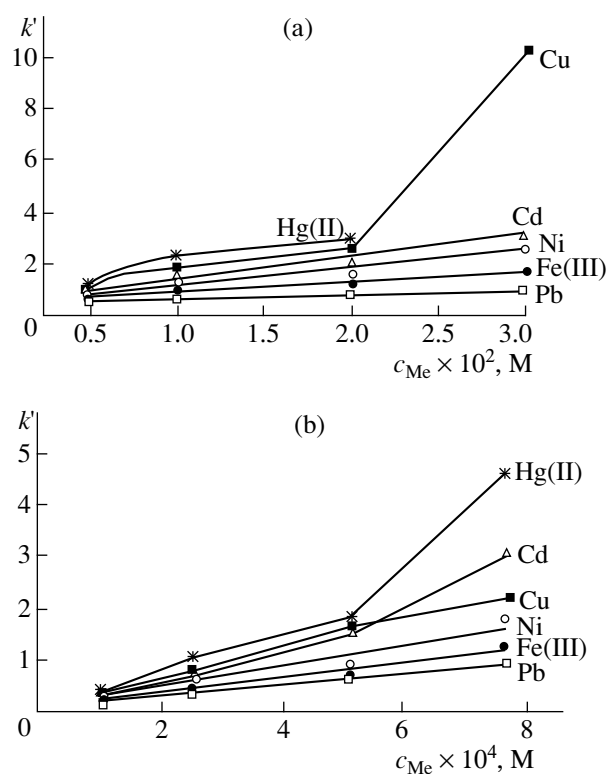
Metal	(40 : 60) Acetonitrile–acetate buffer solution (pH 6.3), 2×10^{-2} M TBAB		(35 : 65) Acetonitrile–acetate buffer solution (pH 6.3), 5×10^{-3} M CTMAB	
	capacity factor	selectivity factor	capacity factor	selectivity factor
Pb	1.00	–	–	–
Fe	1.17	1.17	1.25	–
Ni	1.67	1.42	–	–
Cd	3.50	1.06	2.70	1.47
Cu	3.30	1.16	1.83	1.46
Hg	2.83	1.69	1.83	1.00

complexes can be successfully separated by ion-pair chromatography.

It is known that retention and selectivity in ion-pair chromatography are particularly strongly affected by the nature and concentration of the ion-pair reagent, the concentration of an organic modifier, the addition of neutral salts, an the pH of the eluent. The effect of these factors was studied for revealing the main regularities in the retention of metal complexes of unithiol and for optimizing the conditions of their separation.

Effect of the nature and concentration of the ion-pair reagent. Quaternary ammonium bases are most frequently used as ion-pair reagents in ion-pair chromatography. Previously we demonstrated that mercury unithiolate can be separated from Fe(III), Pb(II), and Ni(II) complexes by their elution with a mobile phase containing tetrabutylammonium bromide (TBAB) [6]. However, the selectivity of this system was rather low, which can be related to the rather weak retention of metal complexes (k' was no higher than 3 (Table 1)). The use of more hydrophobic cetyltrimethylammonium bromide (CTMAB) leads to an increase in the retention of sorbates; however, upon increasing the hydrophobicity of the ion-pair reagent, the efficiency of a chromatographic column is decreased, which precludes an improvement in the resolution of the chromatographic peaks of metal complexes (Table 1). For changing the selectivity of the chromatographic separation of metal unithiolates, it is of interest to change not only the hydrophobicity of the ion-pair reagent, but also the nature of the heteroatom. Quaternary phosphonium salts are analogs of quaternary ammonium salts. It is known that anion exchangers based on quaternary phosphonium salts exhibit higher selectivity than their ammonium analogs [13]. In addition, quaternary phosphonium salts are more active interphase transfer catalysts in nucleophilic substitution reactions [14]. These data suggest that the use of quaternary phosphonium salts as ion-pair reagents will improve both the selectivity and efficiency of the separation of metal complexes of unithiol. We studied the chromatographic behavior of metal chelates when TBPB and TBHDPB were used as ion-pair reagents.

Figure 1 presents the dependences of the k' of metal unithiolates on the concentrations of TBPB and TBHDPB in the mobile phase. It is seen that the retention of all complex increases with the concentration of TBPB; however, the shapes of the dependences are different. The retention of the Pb(II) complex only insignificantly changes upon increasing the concentration of the ion-pair reagent; its capacity factor increases less than two times. On the contrary, the retention of mercury and copper unithiolates increases by a factor of 5–10, and the Hg(II) complex is not eluted from the column at the concentration of TBPB higher than 2×10^{-2} M.

Fig. 1. Effect of the concentration of (a) TBPB and (b) TBHDPB on the retention of metal unithiolates; mobile phase: (a) (60 : 40) and (b) (70 : 30) acetonitrile–acetate buffer solution (pH 6.0), 5×10^{-3} M unithiol.
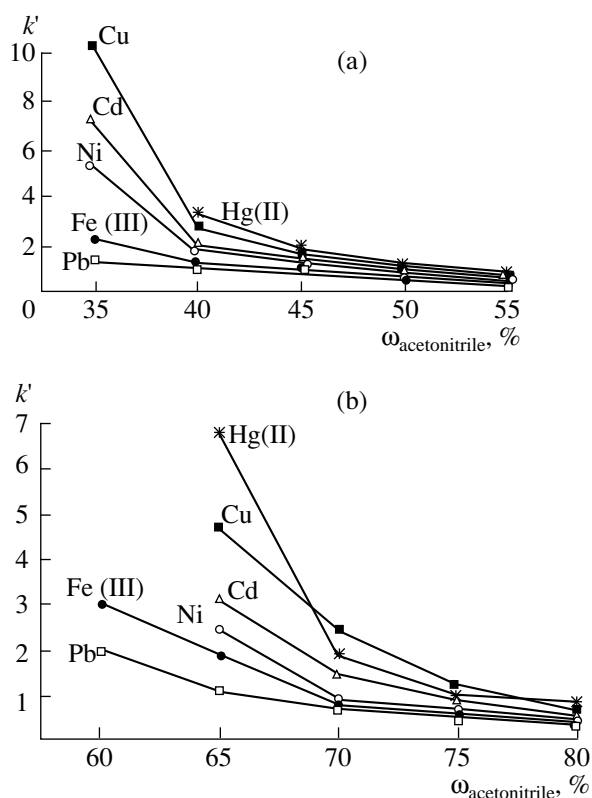
Fig. 2. Effect of the concentration of acetonitrile on the retention of metal unithiolates in the presence of (a) TBPB and (b) TBHDPB.

The shapes of the dependences obtained for TBH-DPB are nearly identical to those for TBPB. However, the comparison of the values of the k' of unithiolate complexes demonstrates that the retention of sorbates is significantly higher in the presence of TBHDPB. This is evidenced by the fact that, at the concentrations of TBHDPB higher than 7.5×10^{-4} M, unithiolates of all studied metals except Pb are virtually not eluted from the column, whereas in the presence of TBPB this effect was observed only for the most strongly retained mercury unithiolate at a significantly higher (2×10^{-2} M) concentration of the ion-pair reagent. This difference in the effect on the retention of complexes is evidently explained by the different hydrophobicity of tetrabutylphosphonium and tributylhexadecylphosphonium cations and is in agreement with the regularities of reversed-phase ion-pair chromatography. As known, the retention of sorbates increases with the hydrophobicity of the ion-pair reagent, in our case, on switching from TBPB to TBHDPB.

Note that for metals that can enter into redox reactions (copper and iron) at TBPB and TBHDPB concentrations higher than 2×10^{-2} and 7.5×10^{-4} M, respectively, the chromatograms exhibit two peaks with different retention times, which is evidently due to the fact

that complexes differing in composition are formed in the column.

By the values of the capacity factors of unithiolates in the systems under study, metals can be arranged in the order Pb < Fe < Ni < Cd < Cu < Hg. This is due to both the charge of the complex ion and the stability of the complexes. Retention increases with the charge and stability of complexes. When TBHDPB is used as the ion-pair reagent, the elution order of complexes is changed upon increasing the concentration of TBHDPB in the mobile phase; for the concentration 7.5×10^{-4} M, the order is as follows: Pb < Fe < Ni < Cu < Cd < Hg. Possibly, this is due to the larger increase in the retention of doubly charged cadmium complexes compared to singly charged copper complexes upon increasing the concentration of the ion-pair reagent.

The largest differences in the retention factors were obtained when TBPB was used as the ion-pair reagent. Probably, this is due to the fact that tetrabutylphosphonium cations are smaller in size than tributylhexadecylphosphonium cations and, therefore, do not mask differences in the structure and size of the chelates of different metals. It is not inconceivable that different mechanisms of the retention of complexes predominate in systems with TBPB and TBHDPB. TBHDPB is stronger sorbed at the surface of nonpolar silica gel, and in this case retention by the dynamic ion exchange mechanism can be predominant, whereas TBPB more readily forms ion pairs, which is favorable for retention by the mechanism including the formation of the ion pair in the mobile phase. The maximum selectivity of separation can be attained at the following concentration of the ion-pair reagents: TBPB, 1×10^{-2} M; TBHDPB, 7.5×10^{-4} M.

The comparison of the k' of metal unithiolates for the systems with TBAB and TBPB as well as with CTMAB and TBHDPB demonstrates that the effect TBPB on retention is somewhat larger and the effect of TBHDPB is significantly larger than the effect of bromides of quaternary ammonium bases, which is due to the higher hydrophobicity of the phosphonium analogues and the formation of more stable ion pairs. Our results also indicate that the selectivity of the separation of unithiolates is increased when quaternary phosphonium salts are used as ion-pair reagents.

Effect of the concentration of an organic solvent. As the mobile phase, we used a mixture of acetonitrile with acetate buffer solutions, which is most frequently used in the ion-pair chromatography of chelates. Acetonitrile was selected as the organic solvent because of several reasons: high elution ability, low viscosity, and weak absorption in the UV region. In addition, water–acetonitrile mixtures readily dissolve complexes under study and the reagent as well as the resulting ion pairs.

We studied the effect of the concentration of acetonitrile in the mobile phase on the retention of metal unithiolates at constant concentrations of ion-pair reagents: TBPB, 1×10^{-2} M and TBHDPB, 5×10^{-4} M.

The concentration of acetonitrile was varied in the range 30–55% on the addition of TBPB and 60–80% on the addition of TBHDPB. These ranges were selected because of the following factors: at lower concentrations, only lead unithiolate was eluted from the chromatographic column, whereas the other complexes were irreversibly sorbed on the stationary phase; at higher concentrations of acetonitrile, complexes were virtually not retained in the column ($k' < 0.2$). Figure 2 presents the dependences of k' on the concentrations of acetonitrile in the mobile phase at the constant concentration of the ion-pair reagent. As seen from these data, the retention of metal complexes decreases with increasing concentration of acetonitrile in the mobile phase, which is in agreement with the main regularities of retention in ion-pair chromatography. Note that in the presence of TBPB the decrease in retention upon increasing the concentration of acetonitrile is less pronounced than in the presence of TBHDPB. Possibly, this is due to the different hydrophobicity of the ion-pair reagents and the effect of acetonitrile on their adsorption at the hydrophobic surface of silica gel.

It was demonstrated that, at low concentrations of acetonitrile, the retention of complexes in the presence of TBPB and TBHDPB is much higher than in systems with quaternary ammonium salts. For example, when TBPB is used at the concentration of acetonitrile in the mobile phase 30%, only Pb(II) and Fe(II) complexes were eluted from the column. In the presence of TBHDPB, this effect was observed even at the concentration of acetonitrile 60%. As the concentration of the organic modifier is increased, differences in capacity factors become not so large. The best selectivity of the separation of unithiolate complexes was observed at the concentration of acetonitrile 35% (TBPB and TBAB) and 65% (TBHDPB and CTMAB); however, under these conditions, broadening of chromatographic zones is so large that the analysis of multicomponent metal mixtures becomes impossible.

Effect of inorganic salts. It is known that in reversed-phase HPLC the addition of inorganic salts to the mobile phase leads to a decrease in retention because of the effect of the ionic strength. In ion-pair chromatography, the addition of electrolytes has a particularly large effect because added ions can exert a competing action on the retention of complexes by both the dynamic ion exchange mechanism and the mechanism of the formation of ion pairs in the mobile phase. In this work, we studied the effect of two salts: $NaClO_4$ and $NaNO_3$, whose anions, according to the regularities of ion exchange, exhibit different elution strength. The dependences of $\log k'$ on $\log c_{NaA}$ are presented in Fig. 3. It is easily seen that the addition of $NaClO_4$ increases the elution ability of the mobile phase to a larger extent than the addition of $NaNO_3$. Thus, the addition of inorganic salts results in a decrease in the retention of complexes in the chromatographic column. The effect of $NaClO_4$ on the retention of metal unithiolates in the
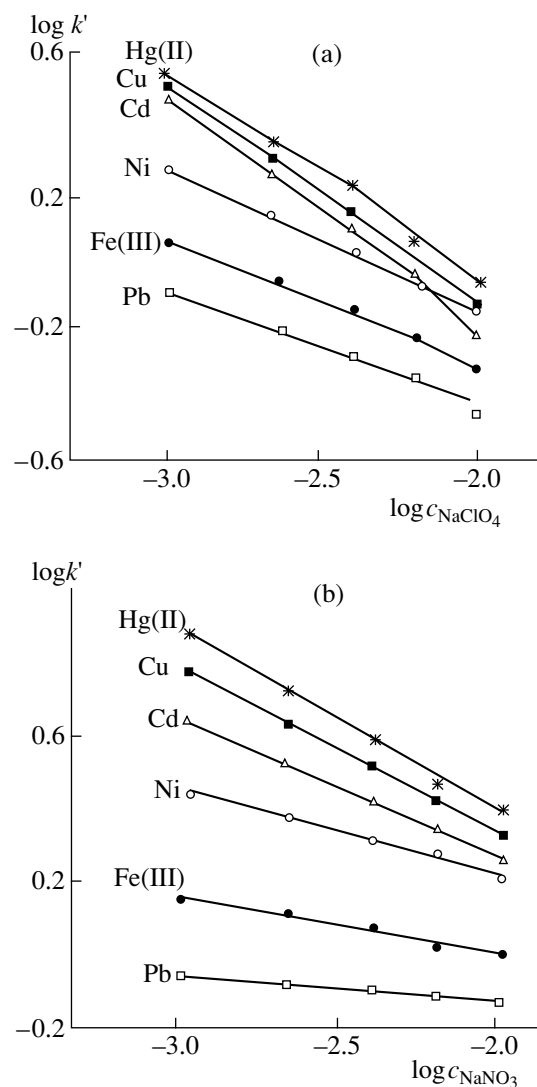

Fig. 3. Effect of the concentration of (a) $NaClO_4$ and (b) $NaNO_3$ on the retention of metal unithiolates; mobile phase: (30 : 70) acetonitrile–acetate buffer solution (pH 6.0), 5×10^{-3} M unithiol and 1×10^{-2} M TBPB.

presence of TBPB and TBHDPB is similar; however, a higher concentration of salt is required for attaining the same values of k' in the system with the more hydrophobic ion-pair reagent. The addition of $NaClO_4$ to the mobile phase made it possible to elute all chelates at the concentration of acetonitrile in the mobile phase 30% in the presence of TBPB and 65% in the presence of TBHDPB within 15 min.

Effect of the pH of the eluent on the retention of complexes. It is known that unithiol and metals form stable complexes of different composition; complexation occurs in weakly acidic, neutral, and alkaline solutions. In strongly acidic solutions, only redox reactions occur between metal ions and unithiol [10, 11]. According to the data reported in the literature, the degree of formation of complexes under study is maxi-

Table 2. Effect of the nature of the ion-pair reagent on the efficiency of the chromatographic column (number of theoretical plates per meter)

Ion-pair reagent	Pb	Cu	Hg	Ni	Cd	Fe
2×10^{-2} M TBAB	1472	2272	3360	2560	2560	1376
1×10^{-2} M TBPB	3220	2316	1975	5395	4026	3876
5×10^{-4} M TBHDPB	1240	300	900	820	500	–
1×10^{-2} M TBPB 1×10^{-3} M NaClO$_4$	4034	2465	6491	5420	1403	4424
2×10^{-3} M TBHDPB 1×10^{-3} M NaClO$_4$	2240	360	1700	1202	290	–

mum at pH 5–7; therefore, this pH range was selected for studies. To adjust the pH of the eluent, we used acetate buffer solutions. In our previous work [6], it was found that, when TBAB was used as the ion-pair reagent, the retention of chelates in the pH range 5.1–6.3 remained nearly constant; however, at lower pH, the shape of peaks was impaired and the height of peaks was decreased. Systems with TBPB and TBHDPB were found to be more sensitive to changes in pH. Chromatographic peaks of complexes were obtained only in the pH range 5.5–6.5. The increase in pH only slightly changed the retention of chelates but affected the shape of peaks. The best results were obtained using

an acetate buffer solution with pH 6.0, which was further used for the preparation of mobile phases.

An important factor determining the separation of a mixture of sorbates on a chromatographic column is the efficiency of the column. We studied the dependence of the number of theoretical plates for the separation of metal unithiolates on the nature of the ion-pair reagent. Efficiency was compared at the optimal concentrations of ion-pair reagents: 2×10^{-2}, 1×10^{-2}, and 5×10^{-4} M for TBAB, TBPB, and TBHDPB, respectively (see Table 2). We see that efficiency is higher in the system with TBPB than with TBAB. In our opinion, this is in agreement with the data reported in the literature that TBPB exhibits higher activity in interphase transfer processes [14]. Similarly to the case of quaternary ammonium salts, the replacement of TBPB with more hydrophobic TBHDPB results in broadening of the chromatographic zone and decreases the efficiency of the chromatographic column. The addition of inorganic salts, in particular, NaClO$_4$ increases the efficiency of the chromatographic system.

Summarizing the obtained results, we can conclude that bromides of quaternary phosphonium bases are efficient ion-pair reagents for the separation of heavy-metal complexes of unithiol. The higher selectivity of separation is attained on the addition of TBPB to the mobile phase. Our studies made it possible to select the optimal composition of the mobile phase for the separation of multicomponent mixtures of metals: (30 : 70) acetonitrile–acetate buffer solution (pH 6.0); concentrations: 5×10^{-3} M unithiol, 1×10^{-2} M TBPB, and 1×10^{-2} M NaClO$_4$.

The chromatographic parameters of the separation of a mixture of metals under study are presented in Table 3. As seen from the table, in spite of high selectivity, the resolution of peaks is not quite satisfactory.

Table 3. Chromatographic parameters of the separation of a mixture of metal unithiolate complexes; mobile phase, (30 : 70) acetonitrile–acetate buffer solution (pH 6.0) containing 5×10^{-3} M unithiol, 1×10^{-2} M TBPB, and 1×10^{-2} M NaClO$_4$

Characteristics of the column	Metal	Capacity factor	Selectivity factor	Resolution
Size 120 × 2 mm,	Pb	0.66		
flow rate 100 µL/min,	Ni	0.71	1.08	1.00
	Cd	1.85	2.60	0.98
dead time 2.26 min*	Cu	1.94	1.08	0.74
	Hg	2.83	1.45	0.40
Size 150 × 3 mm,	Pb	0.89		
flow rate 500 µL/min,	Ni	1.44	1.62	1.14
	Cd	2.19	1.52	1.71
dead time 1.27 min**	Cu	3.17	1.45	2.00
	Hg	3.57	1.13	1.00

 * Separation was performed on a Milikhrom-4 chromatograph.
** Separation was performed on a Kontron chromatograph.
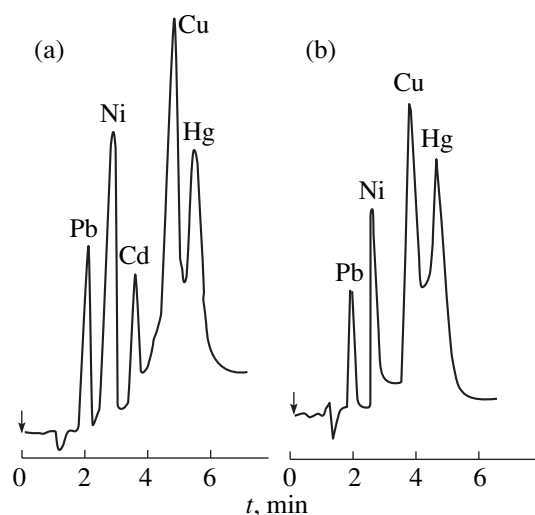
Fig. 4. Chromatograms of (a) a model mixture of metal complexes of unithiol and (b) waste water from a electroplating plant; mobile phase: (30 : 70) acetonitrile–acetate buffer solution (pH 6.0), 5×10^{-3} M unithiol, 1×10^{-2} M TBPB, and 1×10^{-2} M NaClO$_4$.

To improve the resolution of peaks, the efficiency of the column was increased by increasing its length to 150 mm. However, a Milikhrom-4 chromatograph is unsuitable for operation with large columns; therefore, further studies were performed on a Kontron chromatograph. The higher sensitivity of the detector of this chromatograph made it possible to decrease the detection limit of mixture components. The chromatographic parameters of the separation of a metal mixture are compared in Table 3. The obtained values of R_s indicate that metals can be successfully separated.

We studied the use of the chromatographic separation of metal unithiolates by ion-pair chromatography for the determination of metals in waste and process waters. For this purpose, we obtained chromatograms of several mixtures of Pb, Cd, Ni, Cu, and Hg in the concentration range 6×10^{-3}–1×10^{-1} μg/mL (at the ratio of metals 1 : 1). Using these chromatograms, we constructed calibration plots of the dependence of the peak area on the concentration of the metal in the solution. These plots are described by the following equations: $y = 0.51x$ for cadmium, $y = 9.8x$ for copper, $y = 0.43x$ for mercury, $y = 2.8x$ for lead, and $y = 4.9x$ for nickel. It was found that for the ratio of metal concentrations within 1 : 5, the mutual interfering effect of

determined metals is absent. The determination limits calculated from the signal-to-noise ratio equal to 2 were 0.05, 0.005, 0.07, 0.01, and 0.01 μg/mL for Cd, Cu, Hg, Pb, and Ni, respectively. The accuracy of the procedure was verified by the added–found method using a model metal mixture (Fig. 4a) containing 0.10 μg/mL Pb, Cu, and Cd; 0.05 μg/mL Hg; and 0.03 μg/mL Ni; relative standard deviations in the determination of all metals were no higher than 10%. The procedure was used for the analysis of process water in electroplating industry (Fig. 4b). Found in a sample (μg/mL): Pb, 32 ± 2; Ni, 10 ± 1; Cu, 15 ± 2; Hg, 42 ± 5. The accuracy of the results was verified by inductively coupled plasma atomic emission spectrometry. Satisfactory agreement of the results was obtained.

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