

# New thiohydrazones for complexation and chromatographic determination of metal ions

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## ABSTRACT

A general scheme is outlined for rapid determination of metal cations by complexation and subsequent high-performance liquid chromatographic separation. The synthesis and general properties are described for several new thiohydrazone complexing reagents. Solubility considerations suggest that the metal complexes have a positive charge. Excellent chromatographic separations are obtained for mixtures of up to seven metal ion complexes. Addition of a positively charged additive to the eluent is shown to have a significant effect on both the retention times and sharpness of the chromatographic peaks. Separation of the metal complexes on resins with a permanent charge is also shown to be feasible.

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## INTRODUCTION

Reaction of a thiosemicarbazide or a substituted thiosemicarbazide with a carbonyl compound results in a product with strong chelating properties for metal ions that have an affinity for sulfur. Especially strong chelating reagents are formed by reaction of two molecules of the semicarbazide with a 1,2-dicarbonyl compound. Thiosemicarbazones have been used as chemical spray reagents for metal ions separated by thin-layer chromatography [1] and as color-forming reagents for spectrophotometric determination of  $\text{Co}^{2+}$  [2], iron [3],  $\text{Cu}^{2+}$  [4], uranium [5] and other metal ions [6]. Main and Fritz [7] employed a reagent made by reacting a thiosemicarbazide with 2-acetylpyridine for complexation and chromatographic separation of metal ions.

An attractive and rapid way to determine metal ions is to add an appropriate complexing agent to the sample and separate the metal complexes by high-performance liquid chromatography (HPLC). Several review articles have been published on this technique [8–13]. Many of the published methods use reagents that form water-insoluble complexes

and thus require a preliminary solvent extraction step. It is better to employ a reagent that forms complexes with a reasonable solubility in water. It is also necessary to use a reagent that forms complexes with sufficient stability to avoid decomposition during the chromatographic separation. One of the new reagents described in this paper has the necessary properties and has been used for the chromatographic separation of several metal ions.

We first evaluated a reagent made by reaction of semicarbazide with glyoxal that was reported to form highly colored complexes with silver(I) and mercury(II) even in highly acidic solutions [14]. We confirmed this behavior but found both the reagent and its metal complexes to be sparingly soluble in water and water-organic solvent mixtures that are usually employed as eluents in HPLC separation. However, more water-soluble reagents with good chelating and color-forming properties can be prepared by a similar reaction by varying the chemical structures of both reactants. In this paper, the preparation and properties of several new reagents are described. One of the new reagents is chosen for complexation and chromatographic separation of several metal ions.

## EXPERIMENTAL

*Synthesis and characterization of thiohydrazones*

To synthesize reagent IV, 1.55 g of 4-(4-dimethylaminophenyl)-3-thiosemicarbazide (Fairfield) and approximately 250 ml of absolute ethanol were added to a 500-ml round-bottom flask. The solution was heated to 80°C while refluxing in an oil bath to help dissolve the thiosemicarbazide. Then 0.33 ml of diacetyl dissolved in 10 ml hot ethanol was added to the flask dropwise. A few drops of acetic acid were also added to the flask as the catalyst. The whole mixture was refluxed at 70°C for 5 h and the resulting yellow precipitate was washed by hot ethanol and collected.

Complexing reagents I and II were synthesized from their different starting materials in a similar manner. To synthesize reagent III, absolute ethanol was replaced by dimethylformamide as solvent and reaction temperature was further raised to above 100°C owing to the low reactivity of the conjugated 1,2-dicarbonyl compound.

Since reagent IV turned out to be the best overall (see Results and Discussion section), later experiments were all based on reagent IV.

The following procedures were used to characterize reagent IV. The melting point was measured by a Thomas "Uni-Melt" melting point unit with thermometer reader and illuminator. The infrared spectrum was taken on an IBM IR/98 (film). The NMR spectrum was obtained on a Nicolet NT-300 instrument using dimethyl sulfoxide as the solvent and a chemical shift standard. The mass spectrum was taken on a Kratos MS-50 employing a high-resolution mass spectrometer. Results for characterization of this reagent are summarized in Table I.

*Preparation of solutions*

Metal ion stock solutions were prepared from their chloride or nitrate metal salts and kept in HCl or H<sub>2</sub>SO<sub>4</sub> media. Chromatographic samples were prepared by taking an aliquot of stock metal solution and adding enough reagent. The pH value of the solution is controlled either at 2.0 (0.01 M HClO<sub>4</sub>) or 4.6 (160 mM acetate). Finally, the solution was diluted by deionized water and HPLC-grade acetonitrile to make the metal complex concentration 10<sup>-5</sup> – 10<sup>-4</sup> M. Whenever possible, the volume percentage of acetonitrile in sample solu-

TABLE I

CHARACTERIZATION OF DIACETYL DI-(4-DIMETHYLAMINOPHENOL)-3-THIOSEMICARBAZONE

Melting range (°C)	IR maxima (cm <sup>-1</sup> )	Major mass spectral lines ( <i>m/z</i> )	Proton shift of NMR (ppm)
232–234	3287	471.2	9.86
	3194	261.1	7.34–7.38
	1653	235.1	6.78–6.81
	1605	196.1	3.43
	1578	179.1	2.98
	1526	137.1	2.58
	1475	121.1	2.38
	1337		
	1256		
	1178		
	1132		
	810		

tion was made the same as the percentage in the eluent.

The UV–VIS spectra of product VI and its three metal complexes were taken at a pH 5.0 in a 50 mM acetate buffer. Both spectra were blanked with same buffer. The concentration of metals ranged from 1.0 · 10<sup>-6</sup> M to 1.0 · 10<sup>-5</sup> M.

*HPLC studies*

The chromatographic system consisted of a LKB 2150 HPLC pump, LKB 2156 HPLC controller, Rheodyne 7125 injector (20-μl injection loop), and a Kratos Spectroflow 783 UV–VIS detector. Most of the separations were performed on columns from Sarasep of different dimensions, packed with 10-μm polystyrene–divinylbenzene resins (RP-80). An anion-exchange column (Sarasep AN-1, 50 × 4.6 mm) was used for one group of separations. Other columns used were a PLRP-S 100A 5-μm polystyrene–divinylbenzene (150 × 4.6 mm) column from Polymer Labs. and a C<sub>18</sub> silica (250 × 4 mm) column from E. Merck.

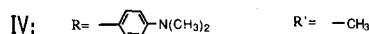
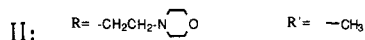
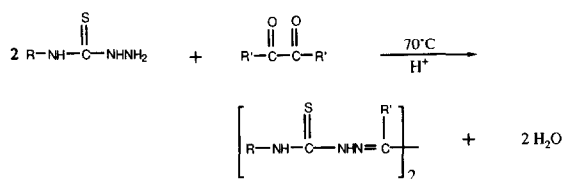
Eluents were prepared from Fisher HPLC-grade acetonitrile and water purified with a Barnstead Nanopure II system on a percent volume basis. Acids and organic amine salts were all reagent grade or better. The pH values of eluents were controlled by 0.01 M HClO<sub>4</sub> or acetate buffer. The eluent components were mixed, filtered with 0.2-μm nylon 66 filters (Rainin) and degassed before use in

the chromatographic system. The flow-rate was 1 ml/min. The detection wavelength was 474 nm.

## RESULTS AND DISCUSSION

### Preparation and properties of chelating reagents

The synthetic method used is given by the following equation:



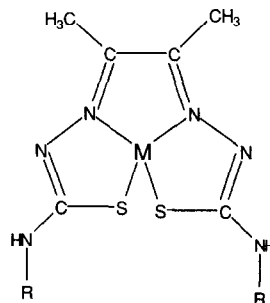
Four different reagents of this type were prepared and tested. Reagent I gave strongly colored complexes with several metal ions. Its solubility in water-organic solvent mixtures was much better than the reagent where both R and R' = H but a rather high percentage of organic solvent was still needed to form soluble metal complexes.

Reagent II had very good solubility but most of the metal complexes were colorless which could be caused by the loss of conjugation from benzene ring. Preparation of a reagent III was attempted but apparently failed because of the low reactivity of the conjugated 1,2-dicarbonyl compound.

Reagent IV was the best of the reagents. It formed stable, colored complexes with several metal ions. Both the reagent and the metal complexes were very soluble in acidic aqueous solution owing to protonation of the dimethylamino groups. However, in basic solution the acidic hydrogens were lost and the complexes became less soluble than those of reagent I.

The structure of similar metal complexes has been studied [15]. With divalent metal ions a hydrogen atom is displaced from each of the two -NH

groups, thus forming neutral complexes of the following structure.



However, the diphenylamino groups in reagent IV are protonated in acidic solutions, leading to charged metal complexes (probably 2+). According to Budesinsky and Svec [14], in the case of the Ag<sup>+</sup> complex, coordination with only the sulfur atoms is likely.

The qualitative color-forming properties of reagent IV are summarized in Table II. Spectra of reagent IV and three of its metal complexes are portrayed in Fig. 1. The molar absorptivities and optimum analytical wavelengths of several metal complexes are listed in Table III. All of the molar absorptivities are high enough to detect very low concentrations of metal ions.

### Chromatographic separations

Spectrophotometric determination of a metal ion is apt to be subject to numerous interferences unless

TABLE II  
COLOR-FORMING PROPERTIES OF REAGENT IV

Metal ions	Acidic (pH 2-3)	Neutral (pH 5-6)
Hg <sup>2+</sup>	Yellow	Yellow
Ag <sup>+</sup>	Yellow	Golden
Cu <sup>2+</sup>	Rust	Rust
Co <sup>2+</sup>	Rust	Brown
Ni <sup>2+</sup>	Yellow	Golden
Pd <sup>2+</sup>	Green	Brown
Bi <sup>3+</sup>	Yellow	Purple
Fe <sup>2+</sup>	Purple	Golden
Fe <sup>3+</sup>	Purple-> golden	Golden
In <sup>3+</sup>		Yellow
Zn <sup>2+</sup>		Yellow
Cd <sup>2+</sup>		Yellow

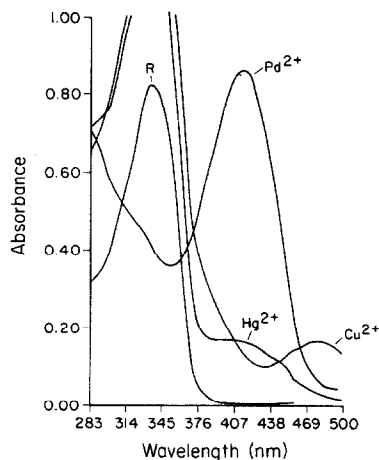


Fig. 1. Spectra of reagent IV and some of its metal complexes. Hg(II) and Pd(II) complexes were  $1.0 \cdot 10^{-5} M$ . The Cu(II) complex was  $1.7 \cdot 10^{-6} M$ .

the color-forming reagent is highly selective for that particular metal ion. Complexation of a group of metal ions, followed by HPLC separation of the individual metal complexes, can be an attractive alternative approach.

We first tried the separation of metal complexes of reagent IV on a short polymeric resin column using an acetonitrile-water eluent containing some perchlorate ion to form an ion pair with the positively charged complexes. It was difficult to adjust the acetonitrile content of the eluent so that good separations were obtained. Sharp but early and unresolved peaks were obtained for a mixture of several metal ions. Lowering the acetonitrile content of

TABLE III

ANALYTICAL WAVELENGTHS AND MOLAR ABSORPTIVITIES FOR METAL COMPLEXES OF REAGENT IV IN AQUEOUS SOLUTION AT pH 5.0

Metal ion	Analytical wavelength (nm)	Molar absorptivity
Ag <sup>+</sup>	410-420	$2 \cdot 10^4$
Cd <sup>2+</sup>	450	$2.8 \cdot 10^4$
Cu <sup>2+</sup>	474	$9.1 \cdot 10^4$
Hg <sup>2+</sup>	404	$1.9 \cdot 10^4$
Pd <sup>2+</sup>	420	$7.3 \cdot 10^4$
Zn <sup>2+</sup>	410-420	$2 \cdot 10^4$

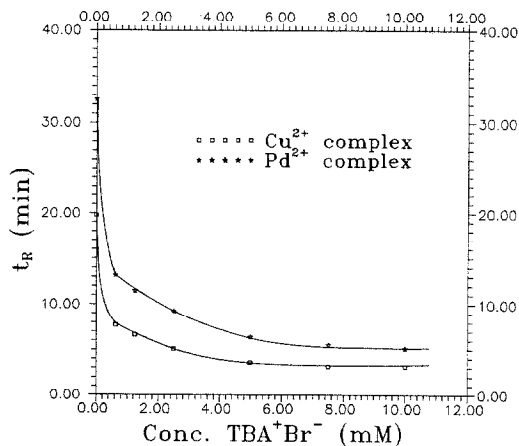


Fig. 2. The effect of tetrabutylammonium (TBA) concentration on the retention time of metal complexes. The eluent was acetonitrile-water (25:75) containing 0.01 M perchloric acid. A  $50 \times 4.6$  mm RP-80 column was used. The sample contained  $1.0 \cdot 10^{-5} M$  Cu(II) or  $3.8 \cdot 10^{-5} M$  Pd(II) plus an excess of reagent.

the eluent resulted in later and better resolved peaks, but the peaks were too broad for really good separations.

The addition of a quaternary ammonium salt ( $Q^+$ ), such as tetraethylammonium bromide or tetrabutylammonium chloride, to the eluent resulted in lower retention times and substantially narrower peaks. Good resolution of several metal ions in a mixture was possible. These effects are shown in Figs. 2 and 3 in which retention time ( $t_R$ ) and peak

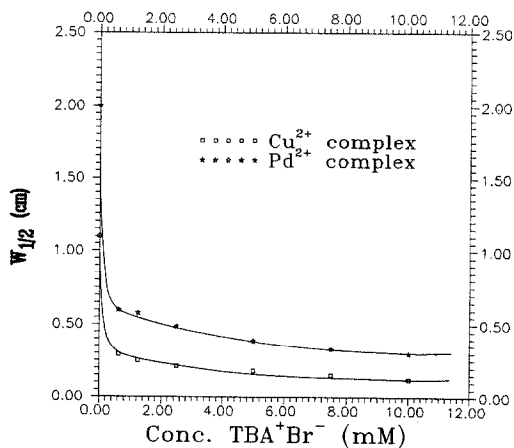


Fig. 3. The effect of tetrabutylammonium concentration on the peak width of metal complexes. Conditions same as in Fig. 2.

width ( $W_{1/2}$ ) respectively, are plotted against the concentration of  $Q^+$  added to the eluent.

The chain length of the  $R_4N^+$ , as well as the concentration added, also affects the chromatographic behavior of the positively charged metal complexes. Incorporation of a tetraethylammonium salt in the eluent has a substantially lower effect on retention time and peak width than does a tetrabutylammonium salt at the same concentration.

In HPLC the addition of an ion *opposite* in charge to a charged sample ion has often been used to increase retention via an ion-pair or ion-interaction mechanism. However, the addition of an ion of the same charge as the sample ion to the eluent has rarely been used. Miura [16] added tetrabutylammonium bromide to assist the separation of vanadium and other transition metal ion complexes by azo dyes on a silica column. Bidlingmeyer *et al.* [17] and others [18] demonstrated the effect of an ionic additive to the eluent for HPLC separations of simple, charged organic compounds.

The principle involved is also explained briefly by Haddad and Jackson [19]. The added  $Q^+$  undergoes an equilibrium between the liquid mobile phase and the solid, stationary phase. Increased  $Q^+$  concentration in the mobile phase or use of  $Q^+$  with more carbon atoms would both increase the amount of  $Q^+$  on the surface of the stationary phase. The presence of  $Q^+$  on the solid surface tends to repel the positively charged sample ions and thereby reduce

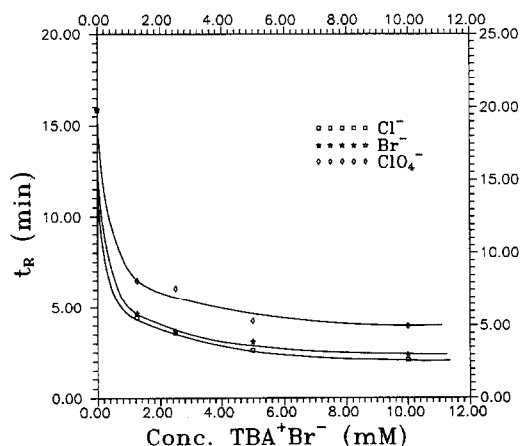


Fig. 4. The effect of different counter ions on the retention time of copper(II). Conditions as in Fig. 2 except for varying the counter ion.

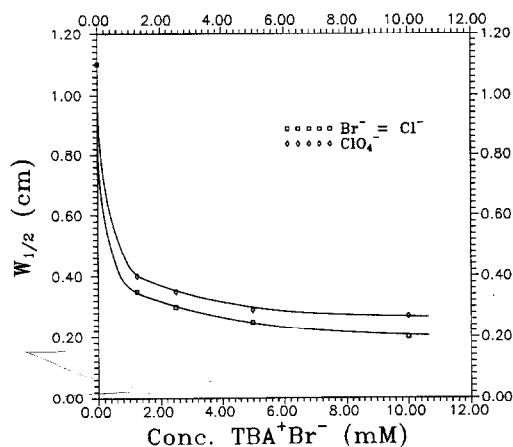


Fig. 5. The effect of different counter ions on the peak width of copper(II). Conditions as in Fig. 4.

their retention times. However, this repulsion effect is less than the attraction of the organic sample ions for the porous resin, thus ensuring that the sample ions are still retained sufficiently by the solid resin phase. For a neutral analyte, adding  $Q^+$  in the eluent essentially has no effect on the retention time and peak width.

Since the sample complexes are cationic in the pH range used, it follows that the chemical nature of the counter anion would have an effect on the HPLC separation. To test this, different anions were added to the eluent. Fig. 4 shows that retention times are longer with perchlorate than with bromide or chloride. However, Fig. 5 shows that the peak width is slightly greater with perchlorate.

If the mechanism postulated for the addition of  $Q^+$  to the eluent is correct, it would follow that incorporation of a fixed positive charge on the resin surface would have a similar effect. Fig. 6 shows an excellent separation of several metal complexes on a low-capacity anion-exchange resin (containing permanent  $Q^+$  groups). The retention times are shorter and the peaks are sharper than when the same separation is attempted on a neutral resin column.

The chromatographic separation shown in Fig. 7 was obtained on a column only 5 cm long and packed with 10- $\mu$ m neutral resin. The eluent contained an added quaternary ammonium salt. Separations with longer columns did not show very much improvement over those obtained with shorter columns. Fig. 8 shows a separation obtained with

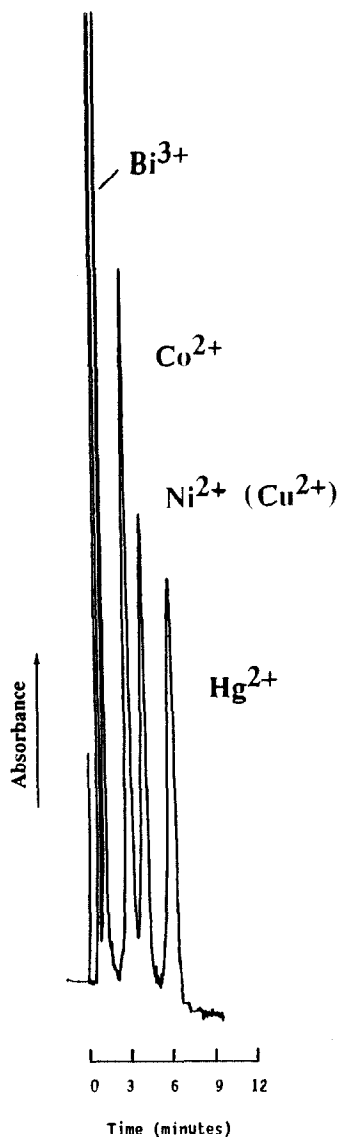


Fig. 6. Separation of metal complexes of reagent IV on a  $50 \times 4.6$  mm AN-1 anion-exchange column. Eluent is acetonitrile-water (25:75) containing  $0.01 M$  perchloric acid. Detection at  $474$  nm.

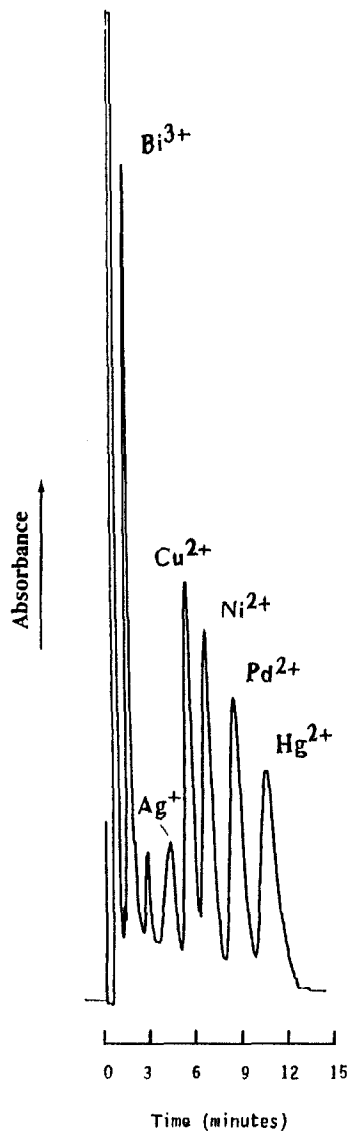


Fig. 7. Separation of metal complexes on a  $50 \times 4.6$  mm neutral RP-80 polymeric resin column with  $2.5 M$  tetraethylammonium bromide added to the eluent. Other separation conditions as in Fig. 6.

a  $250 \times 4.6$  mm column using  $10 mM$  tetraethylammonium chloride as the additive.

Separations with a reversed-phase silica  $C_{18}$  column were reasonable, but generally not as good as those obtained with a polymeric resin column. Fig.

9 shows the separation of complexes of indium(III), copper(II) and mercury(II) using a  $250 \times 4$  mm  $C_{18}$  column. This separation was at a somewhat higher pH than the other separations, and a much higher acetonitrile content was required in the eluent.

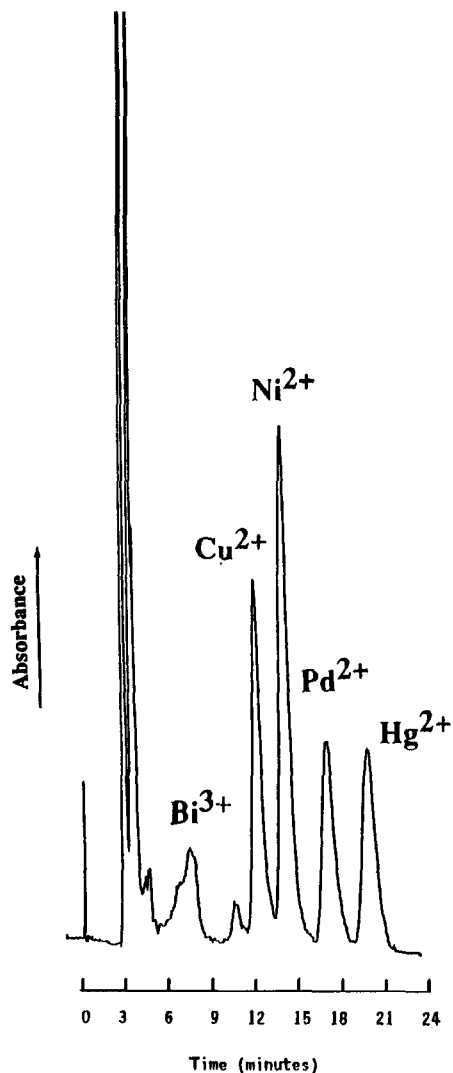


Fig. 8. Separation of metal complexes on a  $250 \times 4.6$  mm RP-80 column using acetonitrile-water (30:70) containing 0.01 *M* perchloric acid and 10 *mM* tetraethylammonium chloride.

#### Scope of chromatographic separations

Chromatographic separations were obtained for most of the elements that form stable complexes with our thiosemicarbazone reagent (reagent IV) at an acidic pH values. Ions such as zinc(II) and cadmium(II) form thiosemicarbazone complexes, but a more alkaline pH is required. Unfortunately, reagent IV and its complexes are deprotonated at higher pH values and are much less soluble in water

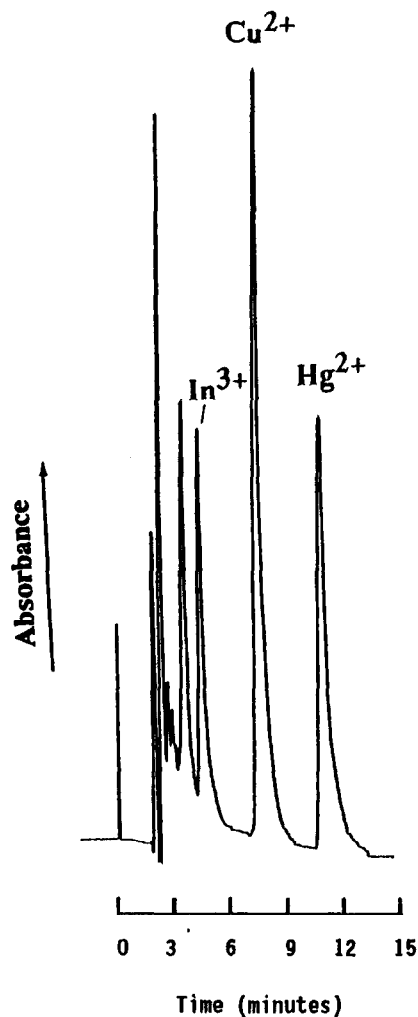


Fig. 9. Separation of reagent IV complexes on a  $250 \times 4$  mm  $C_{18}$  silica column. Eluent is acetonitrile-water (75:25) containing 5 *mM* tetrabutylammonium bromide and buffered with 160 *mM* acetic acid-sodium acetate pH 4.6.

and in water-organic solvent mixtures. Reagent I might be a better candidate for separations carried out at a neutral or alkaline pH because the reagent and its complexes both have a reasonable solubility in acetonitrile-water mixtures.

#### CONCLUSIONS

Semicarbazone reagent IV is an excellent color-forming reagent. It forms highly colored complexes

with a number of metal ions and has the added advantage that both the reagent and its metal complexes are very soluble in water at acidic pH values. Complexes of several metal ions with reagent IV are also well separated on a short HPLC column if an appropriate quaternary ammonium salt is added to the eluent. This should be a convenient and practical way to determine certain metal ions in analytical samples. The complexes are easily formed simply by adding the reagent to the aqueous sample and adjusting the pH. The chromatographic step takes only a few minutes. At acidic pH values, reagent IV forms complexes with only a few metal ions, thereby imparting considerable selectivity to the chromatographic determination of these ions.

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