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HIGHLY SELECTIVE DETERMINATION OF TRACE AMOUNTS OF COPPER(II), NICKEL(II) AND VANADIUM(V) IONS WITH TETRADENTATE SCHIFF-BASE LIGANDS BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND SPECTROPHOTOMETRIC DETECTION

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SUMMARY

The highly selective determination of trace amounts of metal ions by reversed-phase high-performance liquid chromatography (HPLC) and spectrophotometric detection has been accomplished without the addition of a chromogenic reagent to the eluent. Six tetradentate Schiff-base ligands, all N,N′-*o*-phenylenebis(salicylaldimine) (PBS) derivatives, were synthesized and made to react with the metal ions. These reagents have a high selectivity towards Co^{2+}, Cu^{2+}, Ni^{2+} and V^{5+} ions among eleven metal ions (these plus Al^{3+}, Cr^{3+}, Fe^{3+}, Ga^{3+}, Mn^{2+}, Mo^{6+} and Zn^{2+}). By chromatographing the derivatized metals in the HPLC system, highly sensitive and selective spectrophotometric methods for trace amounts of Cu^{2+}, Ni^{2+} and V^{5+} have been established. For example, 4,4′-di-N,N-diethyl-PBS was suitable for the determination of trace amounts of V^{5+}. In this system, the V^{5+} ion can be determined selectively at the ppb level without any preliminary concentration and separation. The detection limit of the V^{5+} ion was $6 \cdot 10^{-9}$ mol/l (0.3 ppb) at a signal-to-noise ratio of 2.

INTRODUCTION

Recently, reversed-phase (RP) high-performance liquid chromatography (HPLC) with a spectrophotometric detection system has achieved widespread acceptance in inorganic trace metal analysis[1–3]. The highly sensitive spectrophotometric detector (0.005 a.u.f.s. range) permits the determination of trace amounts of metals at sub-ppb* to ppb levels. We have demonstrated the feasibility and versatility of a pre-column derivatization method in which metal ions are converted into coloured chelates by a complexation reaction, the chelates subsequently being chromatographed with a mobile phase containing no chromogenic chelating reagent[4,5]. Under these conditions, kinetically labile chelates are probably decomposed on the column,

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* Throughout this article the American billion (10^9) is meant.

because each chelate migrates completely apart from the reagent zone, thus being forced to dissociate during elution. Apparently, the HPLC column has two functions in this system: destruction of or discrimination against labile complexes and ordinary separation. This provides the HPLC–spectrophotometric method with a unique selectivity toward metal ions.

In our continuing studies on analytical reagents suitable for this chromatographic system, chelating reagents such as maleonitriledithiol (Pd and Pt)[6], 4-(2-pyridylazo)resorcinol (Co, Cr, Fe^{3+}, Ni, Pt and Pd)[5,7–9], 2,2′-dihydroxyazobenzene (Al, Co, Fe^{3+} and V^{5+})[4], and porphyrin compounds (Cu and Zn)[10,11] have been found to have attractive features for the determination of the metal ions indicated in parentheses at sub-ppb to ppb levels.

In addition to these reagents, Schiff-base compounds have been studied as chromogenic reagents for the pre-column derivatization of metal ions in RP-HPLC–spectrophotometry. As reported here, tetradentate Schiff-base ligands with oxygen and nitrogen as donor atoms, forming six- and/or five-membered chelate rings, were found to satisfy all criteria[4] for the analytical system mentioned. Although several workers have reported the HPLC of metal chelates of such Schiff bases or analogous β-ketoamines[12–14], they have only demonstrated the applicability of the reagents to HPLC by using the isolated (purified) chelate compounds as models, chosen by analogy with the gas chromatographic separation[15].

In this paper, a highly sensitive RP-HPLC–spectrophotometric method for Cu^{2+}, Ni^{2+} and V^{5+} ions with the use of tetradentate Schiff-base reagents is described and its practical applicability is suggested. The unique selectivity of this system is emphasized.

EXPERIMENTAL

Apparatus

The HPLC system consisted of an LC-5A pump unit, an SPD-2A spectrophotometric detector (both from Shimadzu, Kyoto, Japan) and a Rheodyne 7125 loop injector (100 μl). A Shim-pack FLC-ODS column (50 mm × 4.6 mm I.D., 3 μm) from Shimadzu, a Shimadzu UV-365 recording spectrophotometer and a Hitachi-Horiba M-7E pH meter (Hitachi, Kyoto, Japan) were used.

Preparation of ligands

Six tetradentate Schiff-base ligands, all N,N′-*o*-phenylenebis(salicylaldimine) (PBS) derivatives (Fig. 1), which are not commercially available, were synthesized[16]: PBS, 3,3′-di-CH_3O-PBS, 3,3′,5,5′-tetra-Cl-PBS, 4,4′-di-$N(C_2H_5)_2$-PBS, 4,4′-di-OH-PBS and 5,5′-di-NO_2-PBS. Stoichiometric amounts of the diamine (0.02 mol in 20 ml of ethanol) and aldehyde (0.04 mol in 40 ml of ethanol) were mixed and refluxed for 1 h. After cooling, the resulting precipitate was collected by filtration and recrystallized twice from hot ethanol. The ligand compounds were characterized by elemental analysis and infrared spectrometry.

Solutions

Standard stock solutions (0.01 *M*) of V^{5+} and Mo^{6+} ions were prepared by dissolving ammonium metavanadate and ammonium molybdate respectively, in 0.1

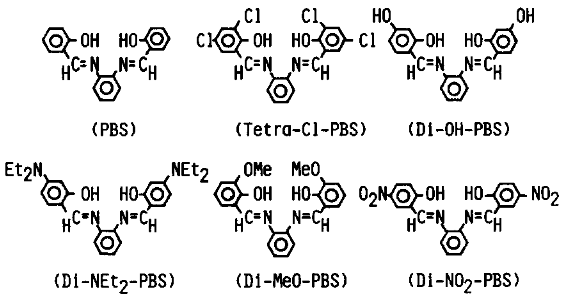


Fig. 1. Schiff-base ligands used. PBS = N,N′-*o*-phenylenebis(salicylaldimine). Et = ethyl, Me = methyl.

M hydrochloric acid. Other metal stock solutions (0.01 *M*) were prepared from appropriate salts and standardized by complexometric titration with disodium ethylenediaminetetraacetate (EDTA). Working solutions of metal ions of appropriate concentrations were prepared by accurate dilution of the stock solutions. The reagent solutions were prepared by dissolving an appropriate amount of the solid in N,N-dimethylformamide (DMF). Tetra-*n*-butylammonium bromide (TBAB) of analytical-reagent grade was used as an ion-interaction agent. All other reagents and solvents were of guaranteed reagent grade from Tokyo Kasei (Tokyo, Japan).

Mobile phase

The mobile phase was an aqueous methanol or acetonitrile solution containing $5 \cdot 10^{-3}$ mol/kg of sodium acetate and $1 \cdot 10^{-4}$ mol/kg of disodium EDTA at a flow-rate of 0.5–1.0 ml/min. The mobile phase was buffered at pH 7.5 ± 0.5 with sodium acetate and disodium EDTA. The latter acts also as a masking agent for metal ion contaminants in the HPLC system. Detailed HPLC conditions are given in the figure captions.

Procedure

To 1 ml of sample solution containing 10^{-7} mol of each metal ion (Al^{3+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Ga^{3+}, Fe^{3+}, Mn^{2+}, Mo^{6+}, Ni^{2+}, V^{5+} and Zn^{2+}), 1 ml of 10^{-2} *M* reagent solution and 2 ml of 1 *M* sodium acetate buffer (pH 5.0) were added. In this system, on account of consumption of the reagent by the diverse ions, the amount of the reagent added was set at about a 10-fold molar excess over the total concentration of the metal ions. The solution was diluted to 50 ml with water and DMF to give a 50% aqueous DMF solution. The solution was heated in a water-bath at 60°C for 30 min and then cooled. An aliquot (100 µl) of the solution was injected with a 100-µl loop injector. The amounts of metal ions eluted were determined from the peak heights.

RESULTS AND DISCUSSION

Spectral and chelate formation studies

The spectral and complexation behaviour of PBS with the above eleven metal ions was extensively studied, as PBS is the simplest member of the tetradentate Schiff bases. The composition of all the PBS chelates studied was found to be Me:PBS = 1:1

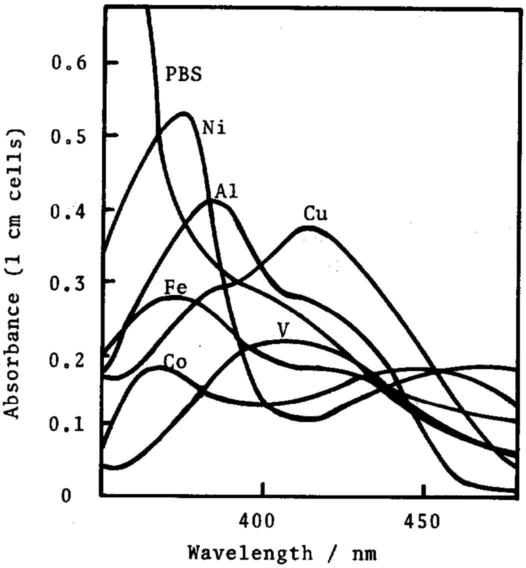


Fig. 2. Absorption spectra of PBS and its metal chelates in 50% aqueous. DMF solution. Metal concentration, $2.0 \cdot 10^{-5}$ *M*; PBS concentration, $2 \cdot 10^{-4}$ *M*.

by the molar ratio method. The absorption spectra of the PBS chelates are shown in Fig. 2; it can be seen that the spectra of the chelates not only overlap but also are scarcely shifted from that of PBS reagent itself. For the other reagents, the spectral properties are essentially similar to those for the PBS system. Although these properties of the Schiff-base ligands are a disadvantage in conventional spectrophotometry, they are desirable for the simultaneous detection of eluted metal chelates at a fixed wavelength in HPLC–spectrophotometry. Neither colour changes on complexation nor spectral differences among the metal chelates are essential for the reagents utilized in HPLC.

The solutions of these Schiff-base ligands are usually unstable. While such a

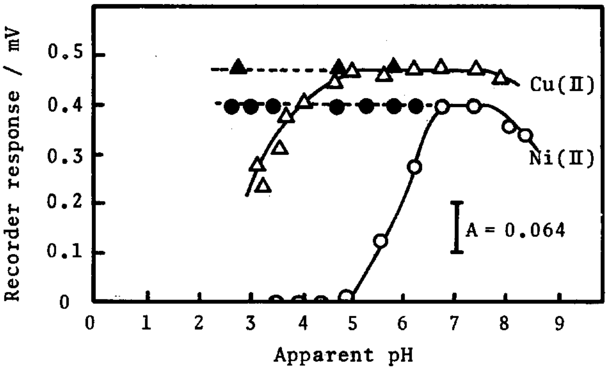


Fig. 3. Effect of reaction pH on the complexation of Cu^{2+}– and Ni^{2+}–PBS chelates in 50% aqueous DMF solution. Cu concentration, $1.99 \cdot 10^{-5}$ *M*; Ni concentration, $2.01 \cdot 10^{-5}$ *M*; PBS concentration, $2 \cdot 10^{-4}$ *M*.

degradation of the reagent solutions is troublesome in conventional absorbance measurements, this problem can be avoided in spectrophotometry coupled with HPLC, provided that the reagent gives stable (inert) chelates that can be separated from the reagent or the degradation products.

The effects of pH on the complexation of Cu^{2+} and Ni^{2+} ions with PBS are illustrated in Fig. 3. The absorbance *versus* pH curves have maximal and constant values at pH 4.7–7.8 and 6.4–7.6 for Cu^{2+} and Ni^{2+}, respectively. As the complexation reaction is slow at room temperature, heating the solution to 60°C is necessary for quantitative formation of the chelates within 30 min. The broken lines in Fig. 3 show that both the chelates, once formed at slightly acidic pH, are stable even in more acidic solution because of their inertness. The ligand-exchange reaction of these chelates with EDTA is also very slow in the medium pH region. On the basis of the data in Fig. 3, the subsequent optimization experiments were carried out in aqueous DMF buffered at pH 6.4–6.8.

HPLC studies

The Schiff-base ligands of PBS, tetra-Cl-PBS and di-$N(C_2H_5)_2$-PBS showed some well resolved peaks for metal ions under certain HPLC conditions, as shown in Figs. 4–6. However, di-OH-PBS, di-NO_2-PBS and di-CH_3O-PBS gave no elution peaks corresponding to the metal chelates under any of the conditions examined. The chelates of the last three Schiff bases seem to be decomposed during HPLC separation. It is interesting that the HPLC behaviour is strongly influenced by the functional groups on the ligand molecules. Further work is planned to rationalize the HPLC separation with the structural features of these ligands.

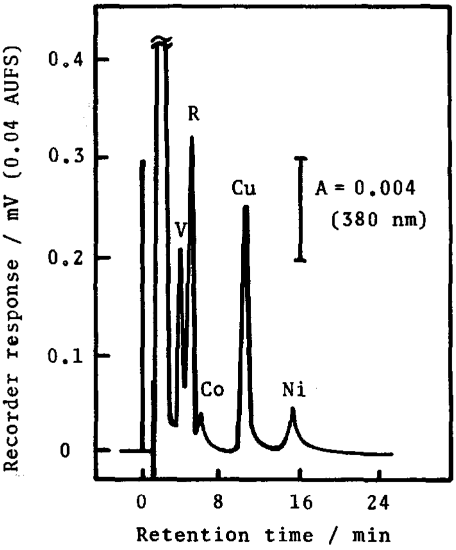


Fig. 4. Chromatogram of PBS system. Eluent: acetonitrile–water (58:42, w/w) containing $5 \cdot 10^{-3}$ mol/kg sodium acetate and $1 \cdot 10^{-4}$ mol/kg EDTA. Flow-rate, 0.5 ml/min. Metal concentration, $2 \cdot 10^{-6}$ *M*; PBS concentration, $2 \cdot 10^{-4}$ *M*.

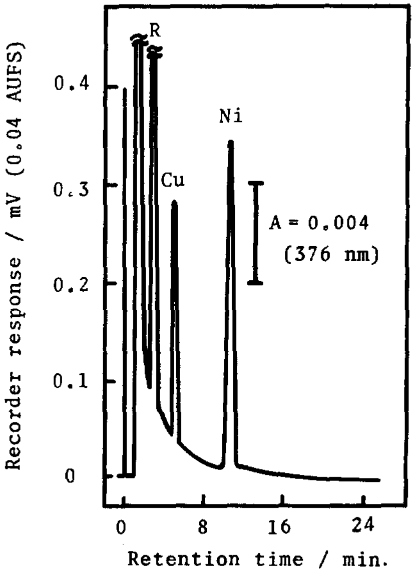
PBS system

A typical chromatogram is shown in Fig. 4. Among the eleven metal ions mentioned above, only V^{5+}, Co^{2+}, Cu^{2+} and Ni^{2+} gave well resolved peaks. The reason why the other metal chelates gave no elution peaks is interpreted in terms of their labile nature, which causes their dissociation during the elution process. In the HPLC system adopted here, the ligand was not added to the eluent, and therefore the kinetically labile chelates were probably decomposed on the column.

A highly sensitive and selective method for the determination of trace amounts of Cu^{2+} using the PBS reagent was devised. The peak-height calibration graph was linear over the copper concentration range $4 \cdot 10^{-8}$–$2 \cdot 10^{-6}$ mol/l at 0.01 a.u.f.s. The detection limit, defined as the concentration that gives a signal twice the noise level, was about $1 \cdot 10^{-8}$ mol/l (0.6 ppb). The relative standard deviation at $2 \cdot 10^{-6}$ mol/l of copper in five replicate analysis was 0.56%.

Tetra-Cl-PBS system

As can be seen from Fig. 5, only Cu^{2+} and Ni^{2+} ions gave peaks in the tetra-Cl-PBS system. Based on these results, a method for the simultaneous determination of Cu^{2+} and Ni^{2+} ions at trace levels was established. The calibration graphs are linear over the ranges $1 \cdot 10^{-7}$–$2 \cdot 10^{-6}$ mol/l and $4 \cdot 10^{-8}$–$2 \cdot 10^{-6}$ mol/l for Cu^{2+} and Ni^{2+} ions, respectively, at 376 nm.



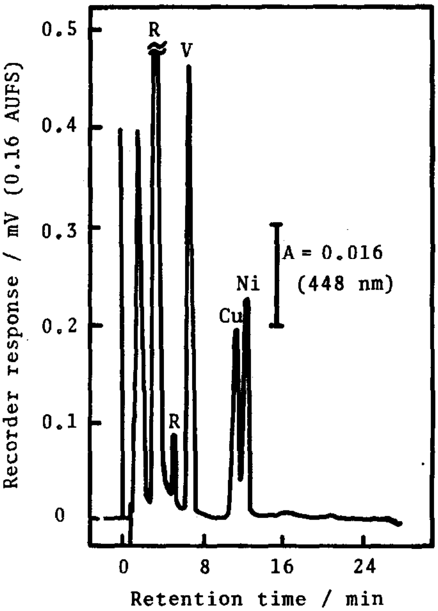


Fig. 5. Chromatogram of tetra-Cl-PBS system. Eluent: acetonitrile–water (65:35, w/w) containing $5 \cdot 10^{-3}$ mol/kg sodium acetate and $1 \cdot 10^{-4}$ mol/kg EDTA. Flow-rate, 0.8 ml/min. Other conditions as in Fig. 4.

Fig. 6. Chromatogram of di-$N(C_2H_5)_2$-PBS system. Eluent: acetonitrile–water (65:35, w/w) containing $5 \cdot 10^{-3}$ mol/kg sodium acetate, $1 \cdot 10^{-4}$ mol/kg EDTA and $6.2 \cdot 10^{-3}$ mol/kg TBAB. Flow-rate, 0.6 ml/min. Other conditions as in Fig. 4.

Di-N(C_2H_5)$_2$-PBS system

The introduction of diethylamino groups into the two salicylaldehyde moieties resulted in an increase in the molar absorptivities of the chelates. Specifically, this reagent is highly sensitive for the V^{5+} ion, as shown in Fig. 6. Concentrations of V^{5+} ion of $1 \cdot 10^{-8}$–$2 \cdot 10^{-6}$ mol/l can be determined at 0.01 a.u.f.s. quite selectively without any preliminary concentration and separation. Further, Cu^{2+} and Ni^{2+} ions at $4 \cdot 10^{-8}$–$2 \cdot 10^{-6}$ mol/l^{4-1} levels can be determined simultaneously at the same wavelength (448 nm). The detection limit at a signal-to-noise ratio of 2 was $6 \cdot 10^{-9}$ mol/l (0.3 ppb) of V^{5+} ion.

Interferences by diverse ions in the determination of $2.22 \cdot 10^{-6}$ mol/l of V^{5+} were studied. A 100-fold molar excess of Al^{3+}, Cd^{2+}, Cr^{3+} and Pb^{2+} ions and a 50-fold molar excess of Cu^{2+}, Fe^{3+}, Mn^{2+} and Ni^{2+} ions did not interfere in the determination. The interferences encountered at higher concentrations of Cu^{2+} and Ni^{2+} ions can be circumvented by adding the reagent in sufficient excess.

CONCLUSION

PBS, tetra-Cl-PBS and di-N(C_2H_5)$_2$-PBS have been shown to be excellent reagents for the RP-HPLC pre-column derivatization system. These compounds can be applied with reasonable success in ultra-trace analysis using RP-HPLC–spectrophotometric detection. Such a method may be applicable to trace metal analysis in environmental and clinical disciplines. The highly unique selectivity towards metal ions, which is due to the kinetic behaviour of the chelates during elution, provides a novel concept for the design of reagents for HPLC coupled with spectrophotometry.

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