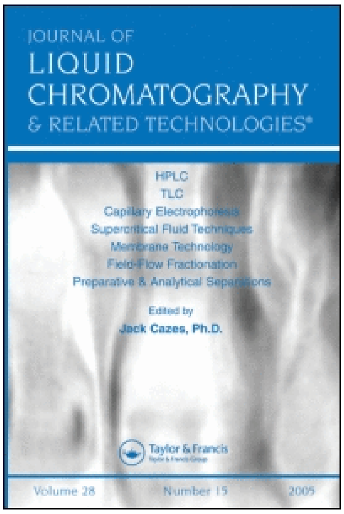

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RETENTION CHARACTERISTICS OF Co^{+3}, Fe^{+3}, and Cu^{+2} 4-(2-PYRIDYLAZO)RESORCINOL (PAR) COMPLEXES ON C-18 AND AMINO SILICA PACKINGS

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ABSTRACT

Chloroform extraction of Co-PAR, Fe-PAR, and Cu-PAR complexes in a pH 6.5 phosphate buffer implied the first two species were primarily monoanions, but the latter was a dianion. Examination of the literature and retention data of these complexes on C-18 and amino columns confirmed the more anionic nature of Cu-PAR. The Co^{+3} and Cu^{+2} complexes were slightly resolved from each other, but the Fe^{+3} complex was retained longer on the C-18 silica. In contrast, the Co^{+3} and Fe^{+3} complexes were not resolved, but the Cu^{+2} complex was well-retained on the weak anion exchange amino silica column. Use of short amino and C-18 columns in series provided a good separation of all three complexes. Detection of the metal complexes at 546 nm instead of 254 nm avoided interference by PAR and good detection limits were still maintained.

INTRODUCTION

A wide variety of reagents have been used to complex metal ions prior to separation by reversed phase HPLC. Good reviews of this subject are available (1,2,3). Complexing agents such as

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β-diketones (4), 8-hydroxyquinoline (5), 1,10 phenanthroline (6), and a variety of dithiocarbamates (7-12) in particular have been investigated.

The 2-pyridyl-azo class of metal complexing agents has been well documented in the literature for the colorimetric determination of essentially all metals except the alkali metals (13). In particular, 4-(2-pyridylazo)resorcinol (PAR) has been used typically with chemical masking agents to try to overcome the non-specificity of metal chelation (14). Although PAR has been used extensively for post column HPLC derivatization, only a few studies using this reagent for derivatization of metal ions prior to reversed phase HPLC have been reported (15-17). Various mobile phases have been used, but no systematic studies of mobile phase composition for the reversed phase separation of metal-4-(2-pyridylazo)resorcinol complexes have been carried out. In addition, alternative column packings to hydrocarbon-modified silica have not been explored.

We have carried out a more thorough study of mobile phase parameters for the separation of Co^{+3}, Fe^{+3}, and Cu^{+2} PAR complexes on two different columns, C-18 and amino silica. These two packings were considered because at a slightly acidic pH, the metal-PAR complexes will exist either as neutral species or anions. The C-18 column should retain any neutral complexes while the amino column could act as a weak anion exchanger. It was found that the C-18 and amino silica columns when connected in series provided better resolution of these metal complexes than either column separately.

EXPERIMENTAL

Apparatus.

The liquid chromatograph used consisted of a Beckman Model 110A pump, a Rheodyne Model 7125 injector equipped with a 20 microliter sample loop, and an Altex Model 153 fixed wavelength (546 nm) detector. The chromatographic data was collected on an Apple computer. Integration of the peak areas was carried out using Audiovideogration software (Heydon & Sons).

The columns used for this study include: 250 mm X 4.5 mm C-18 column, 5 micron (IBM); 250 mm X 4.5 mm methyl column, 5 micron (IBM); 250 mm X 4.5 mm amino column, 5 micron (IBM); house packed 100 mm X 4 mm C-18 column, 10 micron (LiChrosorb); and a house packed 100 mm X 4 mm amino column, 10 micron (Anspec; Ann Arbor, MI). Each house packed column was cut from 300 mm X 4 mm stainless steel tubing obtained through Anspec, and was washed with water and rinsed with methanol prior to packing. The columns were slurry packed in methanol with a pneumatic pump (Hydraulic Engineering Corp.; Los Angeles, CA).

Reagents.

The reagents used were: $(NH_4)_2HPO_4$, reagent (Mallinckrodt); Na_2HPO_4, reagent; $Co(NO_3)_2 \cdot 6H_2O$, reagent (MCB); $Fe(NO_3)_3 \cdot 9H_2O$, certified; $CuSO_4 \cdot 5H_2O$, certified (Fisher); and 4-(2-pyridylazo)-resorcinol, monosodium salt monohydrate (Aldrich). The solvents used were: methanol, HPLC grade (J. T. Baker); acetonitrile, HPLC grade (Mallinckrodt); and water obtained from a Barnstead Nanopure distillation unit.

Procedure.

The metal-PAR stock solutions used were made fresh daily, since discoloration has been observed after 12 hours (16). A metal complex with a concentration greater than 50 ppm showed some problems with solubility in water. In order to increase the solubility of the complexes, all solutions were prepared with 50% water and 50% acetonitrile.

To determine the required excess of PAR for stable complex formation, the metal ion to PAR ratio was varied from 1:2 to 1:10 and the solutions injected into the HPLC for comparison of the metal-PAR peak heights. The optimum ratio was found to be 1:5; a decrease in peak height was observed for lower ratios of PAR while higher PAR ratios caused no change. This result was similar to that found by Nonova and Stoyanov, who found a 1:6 ratio was optimum for Cu^{2+} complexes of PAR determined spectrophotometrically, and above which the absorbance was practically independent of PAR concentration (14). Therefore, standard metal solutions were prepared by dilution from a freshly prepared stock solution, and to this solution was added an aliquot of stock PAR five times the metal concentration.

Some memory effects were noted when using the higher concentration of metal complexes. Therefore, after each injection, the injector loop was washed with a dilute nitric acid solution and then distilled water to clean the loop. After each day's work, the columns were washed with copious amounts of water, and then methanol. This was done to help ensure that no residual uncomplexed metal ions or non-eluting metal complexes remained on the columns.

River water samples were taken from the Seven Mile creek near Oxford, Ohio and filtered before used. The water samples were analyzed the same day they were collected.

RESULTS AND DISCUSSION

Initially, the absorbance and charge characteristics of the Co^{+3}, Fe^{+3}, and Cu^{+2} PAR complexes were investigated at less than pH = 7 to help define promising chromatographic studies using silica based columns. The metal complexes examined have a single absorbance maximum between 510 and 536 nm at pH 6.5 which implies a 1:2 metal:PAR ligand ratio as stated by Shibata (13). Table I compares the molar extinction coefficient for PAR with the molar extinction coefficients for the Co^{3+}, Fe^{3+}, and Cu^{2+} PAR complexes at 254 and 546 nm. This latter wavelength value represented the closest available filter to the optimum absorbance range of the metal-PAR complexes. The molar extinction coefficients for the Fe^{3+} and Cu^{2+} complexes show a definite increase when switching to

TABLE 1.

Comparison of Molar Extinction Coefficients for PAR and PAR Chelates.

	PAR	Co^{3+}	Fe^{3+}	Cu^{2+}
ε @ 254 nm	5,465	17,881	16,265	11,935
ε @ 546 nm	240	16,903	23,634	13,806

@Measured in 50% Methanol, 50% 0.005 M Na_2HPO_4 at pH 6.5

546 nm from 254 nm, but the Co^{3+} complex shows a small decrease in its molar extinction coefficient. The UV-visible spectrum of PAR shows an absorbance maximum at 410 nm, which is confirmed by its yellow color in dilute solutions. However, the extinction coefficient for PAR is reduced about 95% when switching from 254 nm to 546 nm. Thus, working at 546 nm should discriminate against the uncomplexed PAR signal, while not lowering the height of the metal complex peaks. In addition, when studying metal ions in complex matrices the chromotograms should be simpler since fewer substances will absorb at 546 nm as opposed to 254 nm.

To determine the charge of the complexes in weak acid solution, chloroform extractions of the metal complexes in a 0.05 M phosphate buffer solution were performed at pH 6.5. The Co-PAR and Fe-PAR complexes could both be slightly extracted into the chloroform layer as indicated by a pink color in the organic phase and a red color in the aqueous phase. However, the extraction of both complexes could be greatly enhanced by addition of the ion pairing agent tetrabutylammonium hydrogen sulfate. Now the aqueous layer was pink and the chloroform layer was dark red. Therefore, it is likely the Co-PAR and Fe-PAR complexes exist primarily in the form of anions and to a lesser extent as neutral species. The Cu-PAR complex on the other hand would not be extracted at all from the buffer solution; the chloroform layer remained colorless. Addition of tetrabutylammonium hydrogen sulfate to the Cu-PAR solution permitted slight extraction as evidenced by the light red chloroform layer. This data implies Cu-PAR exists only in the anionic form.

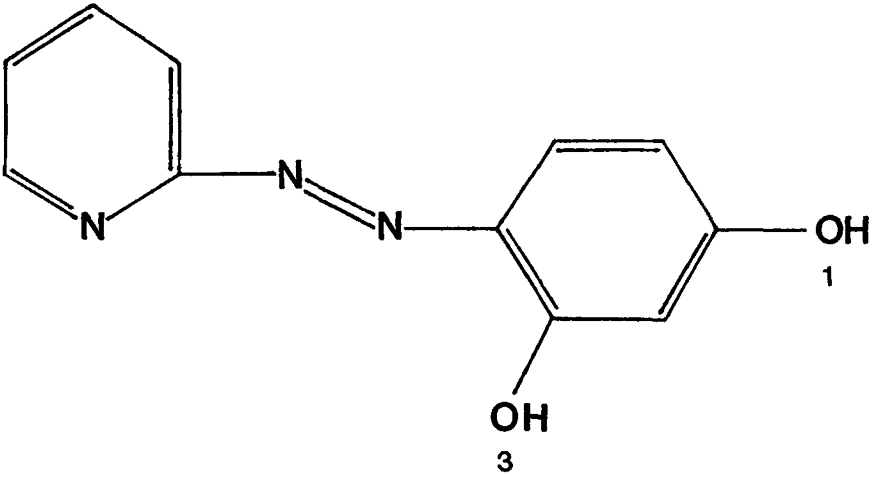

FIGURE 1. Structure of 4-(2-pyridylazo)resorcinol (PAR).

Examination of the reported literature also supported the previous results. The pK_a values for the 1 and 3-hydroxy protons of PAR (Figure 1) are 7.0 and 12.0 respectively. Therefore at pH 6.5, a reasonable amount of H_2L as well as HL^-, where L = unprotonated PAR ligand, is present. It has been reported that upon chelation of a metal (M) with PAR, the 3-hydroxy protons are released forming $M(HL)_2$ (18). Bonding occurs through the two azo nitrogens and the unprotonated 3-hydroxy group.

Although the acid strength of the 1-hydroxy protons does vary depending on the metal-PAR chelate, it is likely that at pH 6.5 M(HL)(L) or $M(L)_2$ are formed. Therefore the dominant Fe-PAR species is $Fe(III)(L)_2^-$ with Fe(III)(HL)L present in lesser amounts. Because it has been reported that Co(II) can oxidize easily to Co(III) (19,20), the dominant Co-PAR species is probably Co(III) $(L)_2^-$ with Co(III)(HL)L present in a small amount. It has been reported that between the pH range of 6-8, Cu-PAR exists both as $Cu(HL)L^-$ and $Cu(L)_2^{-2}$. However, only the monovalent species is easily extracted in most ion-association systems (21). Based on our extraction data, the dominant Cu-PAR species is $Cu(II)(L)_2^{-2}$ with $Cu(II)HL(L)^-$ present only to a small degree. Therefore, both a C-18 reversed phase column for retention of the Co-PAR and Fe-PAR complexes and an amino weak anion exchange column for retention of the more highly charged Cu-PAR complex were considered.

Investigation of the mobile phase composition as a function of metal complex retention was first carried out on hydrocarbon-derivatized silica columns. Figure 2 shows a k′ plot of Co-PAR, Cu-PAR, Fe-PAR, and PAR as a function of methanol concentration using a methyl column. A 0.05 M $(NH_4)_2HPO_4$ buffer was used instead of water to minimize peak tailing as reported previously (16). The higher k′ values for the Co-PAR and Fe-PAR complexes were reasonable considering the complexes were not highly charged species. On the other hand, Cu-PAR having more anionic character and PAR itself showed a lesser hydrophobic effect and a lower k′ trend. At 35% methanol, all the k′ data were rapidly converging. A comparative study was carried out using a C-18 column (Figure 3) and a about a factor of two increase in retention at a given mobile phase composition was found. Interestingly, the Co-PAR complex shows a marked affinity for the mobile phase at organic compositions above 35% methanol. However, using the same mobile phase

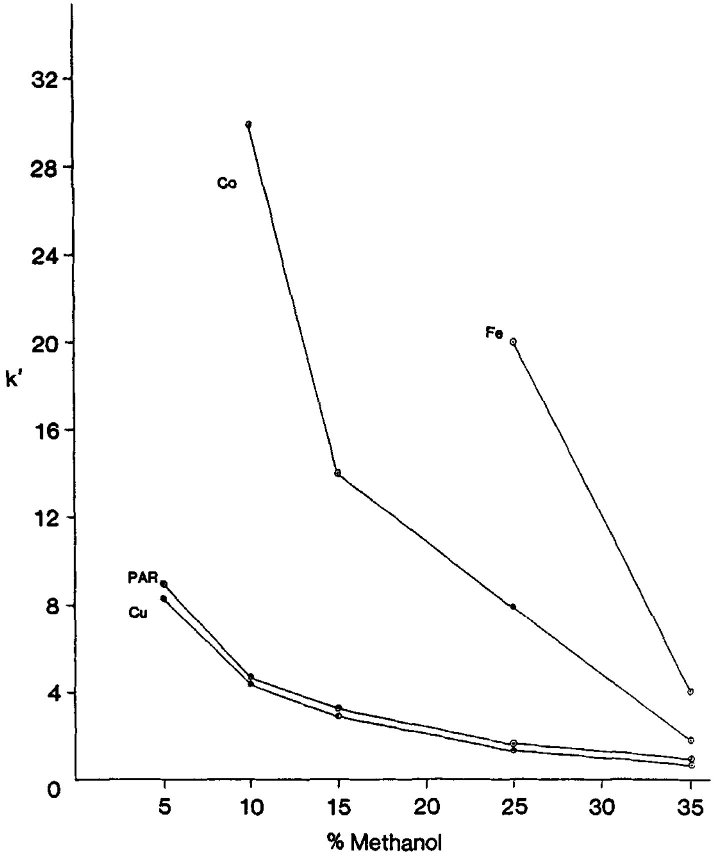

FIGURE 2. Study of the effects of methanol concentration upon k' on a methyl column. The $(NH_4)_2HPO_4$ concentration was held constant at 0.050 M at pH 6.5. Co^{3+}, Fe^{3+}, and Cu^{2+} complexes were approximately 10 ppm each, and PAR was 1.2×10^{-5} M. Conditions: 250 mm X 4.5 mm methyl column from IBM, 5 micron particles; flow rate, 1.0 mL/min.; visible detection, 546 nm.

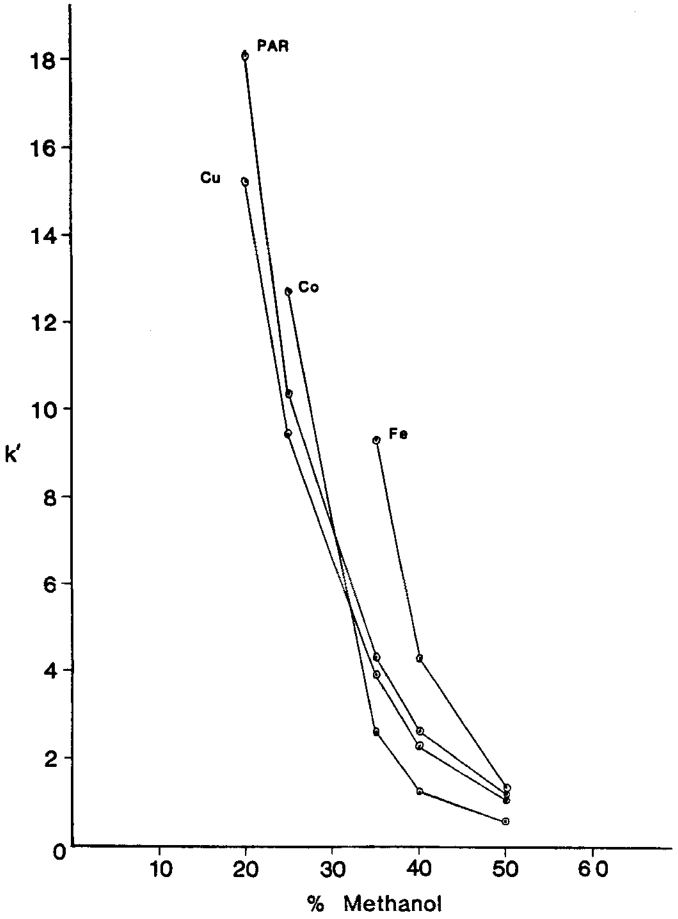

FIGURE 3. Study of the effects of methanol concentration upon k' on a C-18 column. The $(NH_4)_2HPO_4$ concentration was held constant at 0.050 M at pH 6.5. Co^{3+}, Fe^{3+}, and Cu^{2+} complexes were approximately 10 ppm each, and PAR was 1.2×10^{-5} M. Conditions: 250 mm X 4.5 mm C-18 column from IBM; 5 micron particles; flow rate, 1.0 mL/min.; visible detection, 546 nm.

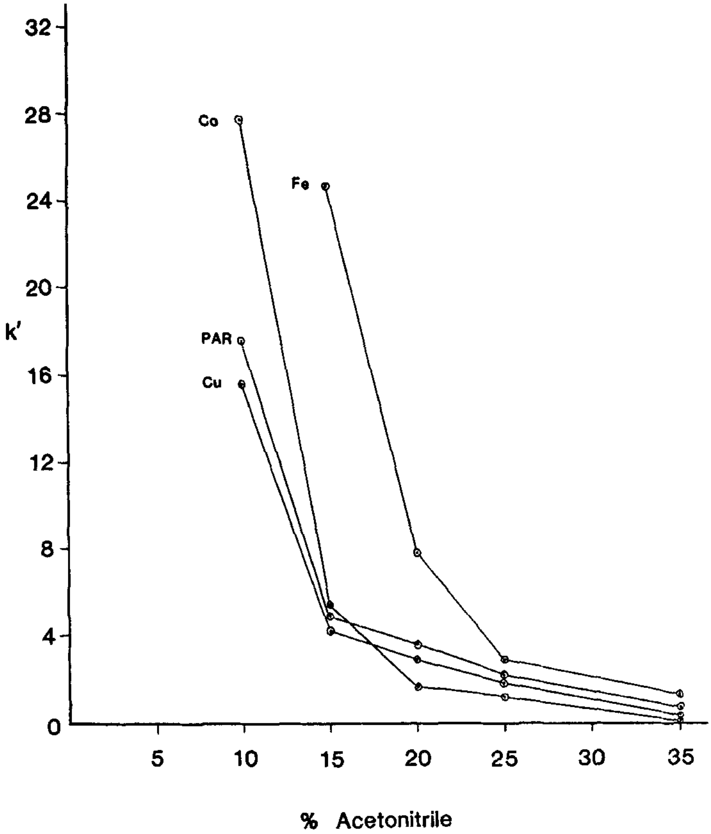

FIGURE 4. Study of the effects of acetonitrile concentration upon k′ on a C-18 column. The $(NH_4)_2HPO_4$ concentration was held constant at 0.050 M at pH 6.5. The metal concentrations and column parameters were the same as in Figure 3.

with 35% methanol, Roston (16) found that the Cu-PAR complex eluted before the Co-PAR complex. This observation may be due to interaction of the metal-PAR complexes with exposed silanols caused by use of C-18 columns from different suppliers. At 50% methanol, the k′ values for all the complexes have converged. The effect of k′

for the same solutes as a function of acetonitrile in the mobile phase was studied on the C-18 column as shown in Figure 4. The trend is similar to that for methanol as expected. Rapid convergence of the k′ values at about 35% acetonitrile was found. Methanol was used for all future work due to better solubility with the buffer mobile phase component.

The buffer concentration and organic content of the mobile phase will both effect retention in the ion exchange process. By varying the phosphate buffer concentration, the effect of ionic strength with ion retention on the amino column was first examined. The anionic PAR and Cu-PAR show a similar and marked decrease in k′ as the phosphate concentration is varied between 10 and 50 mM (Figure 5). The complexes of Co-PAR and Fe-PAR have little affinity for the amino column and the k′ values are essentially constant throughout the same buffer concentration range. To enhance the retention of Cu-PAR, the aqueous mobile phase component used for all future work was 5 mM Na_2HPO_4, pH 6.5. The absence of NH_4^+ ions and the lowered buffer concentration had the added benefit of extending column lifetime of both the C-18 and amino columns. Figure 6 shows the effect of increasing methanol concentration in the mobile phase on the k′ values of the four solutes using the amino column. The retention of the anions PAR and Cu-PAR increase likely due to the decrease in ionic strength of the mobile phase. The k' values for Co-PAR and Fe-PAR are largely unaffected until the organic content of the mobile phase has dropped to 25%.

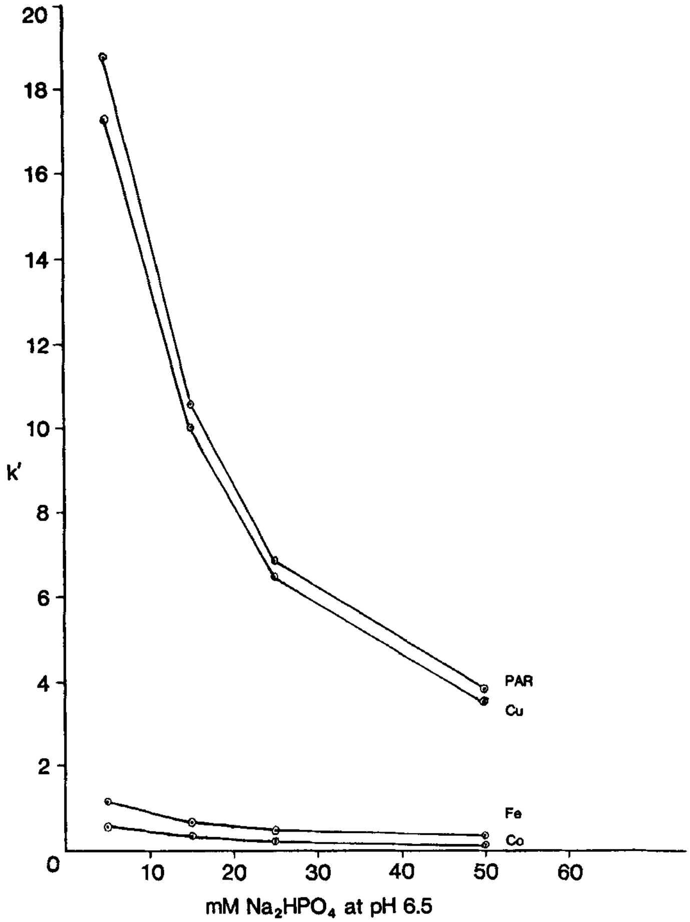

FIGURE 5. Study of the effects of Na_2HPO_4 concentration upon k' on an amino column. The methanol concentration was held constant at 50%, and the pH was adjusted to 6.5. Co^{3+}, Fe^{3+}, and Cu^{2+} complexes were approximately 10 ppm each, and PAR was 1.2 x 10^{-5} M. Conditions: 250 mm X 4.5 mm amino column from IBM; 5 micron particles; flow rate, 1.0 mL/min.; visible detection, 546 nm.

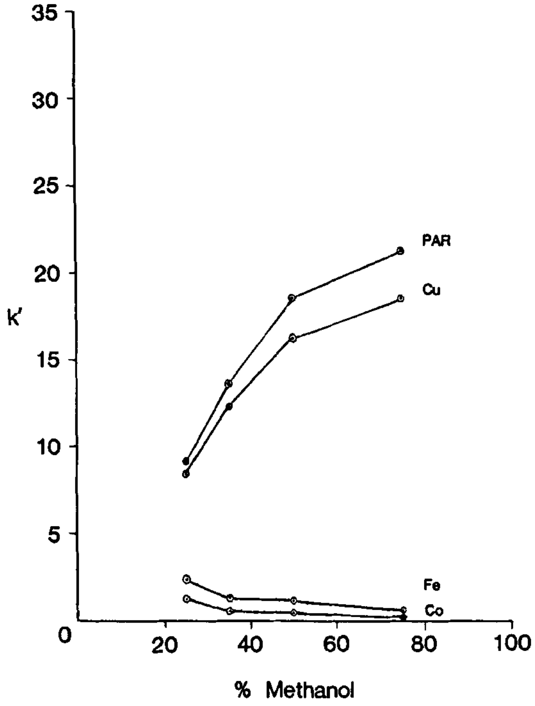

FIGURE 6. Study of the effects of methanol concentration upon k' on an amino column. The Na_2HPO_4 concentration was held constant at 0.005 M at pH 6.5. The metal concentrations and column parameters were the same as in Figure 5.

Because the C-18 column showed retention of the Co-PAR and Fe-PAR complexes and the amino column selectively retained Cu-PAR, use of the two columns in series should provide a good separation of all three complexes. Figure 7 shows a comparison separation for the 10 cm amino, 10 cm C-18, and the dual 10 cm amino and C-18 columns. Note that the Co-PAR and Fe-PAR complex peaks are just resolved enough to be identified as two components on the amino column alone, while Cu-PAR was well resolved. The chromatogram

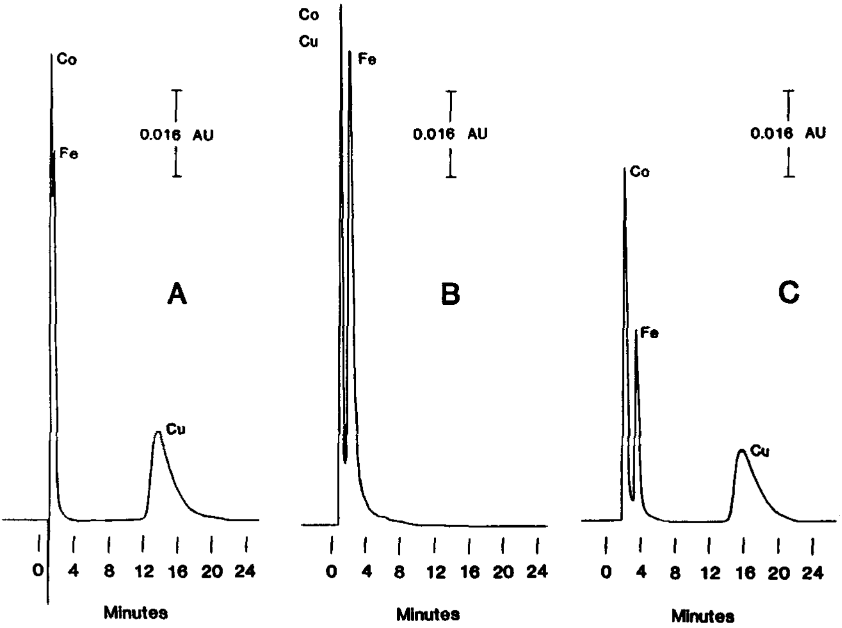

FIGURE 7. Comparative separations of 1.1 ppm Co^{3+}, 3.0 ppm Fe^{3+}, and 5.1 ppm Cu^{2+} complexes of PAR on three different columns. (A) 100 mm X 4 mm house packed amino column, 10 micron; (B) 100 mm X 4 mm house packed C-18, 10 micron; (C) Column (A) and column (B) combined in series. All mobile phases were 50% methanol, 50% 0.005 M Na_2HPO_4 at pH 6.5; flow rate, 1.0 mL/min.; visible detection, 546 nm.

for the C-18 column shows Co-PAR and Cu-PAR complexes eluting as the first peak while Fe-PAR was the second resolved peak. PAR does not appear in any of the chromatograms because H_2L has a low molar absorptivity and the PAR concentration normally used to complex the metal ions was too dilute to be seen at 546 nm. The dual column chromatogram shows relatively good peak shape and resolution for all three components. Because both the C-18 and amino columns are substantially shorter, excessive peak broadening for Fe-PAR and Cu-PAR is avoided.

TABLE 2.

Linear Range and Detection Limit Data for Metal PAR Chelates.

	Co^{3+}	Fe^{3+}	Cu^{2+}
Linear Range, ppm	0.008-45	0.075-75	0.12-75
Detection Limits, ppm	0.017	0.21	0.018
Amount Injected, ng	0.34	4.2	0.36
Slope[a] ± S.D.[b]	77,737±77	17,666±54	24,726±103

[a] X = metal ion concentration, ppm; y = peak area, arbitrary units (1mm^2 = 25 arbitrary units). Slope = y/x.

[b] S.D. = Standard Deviation

Linear range and detection limit data for the three metal-PAR complexes is presented in Table 2.

The precision of the data based on five trials of integrated peak areas were 1.4%, 1.9%, and 2.5% for 1.1 ppm Co^{3+}, 3.0 ppm Fe^{3+}, and 5.1 ppm Cu^{2+}, respectively. The plotted points were found to have a correlation coefficient greater than 0.9999 for all the metals complexed. The detection limits for the metal complexes were calculated as three times the y-intercept value. Detection limits for these metal complexes are comparable to those previously attained (15,16). Figure 8 shows the chromatogram of a river water sample (A) and a chromatogram of another aliquot of this sample spiked with Co^{+3}, Fe^{+3}, and Cu^{+2} ions after PAR complexation (B). Clearly, matrix effects from this sample pose no

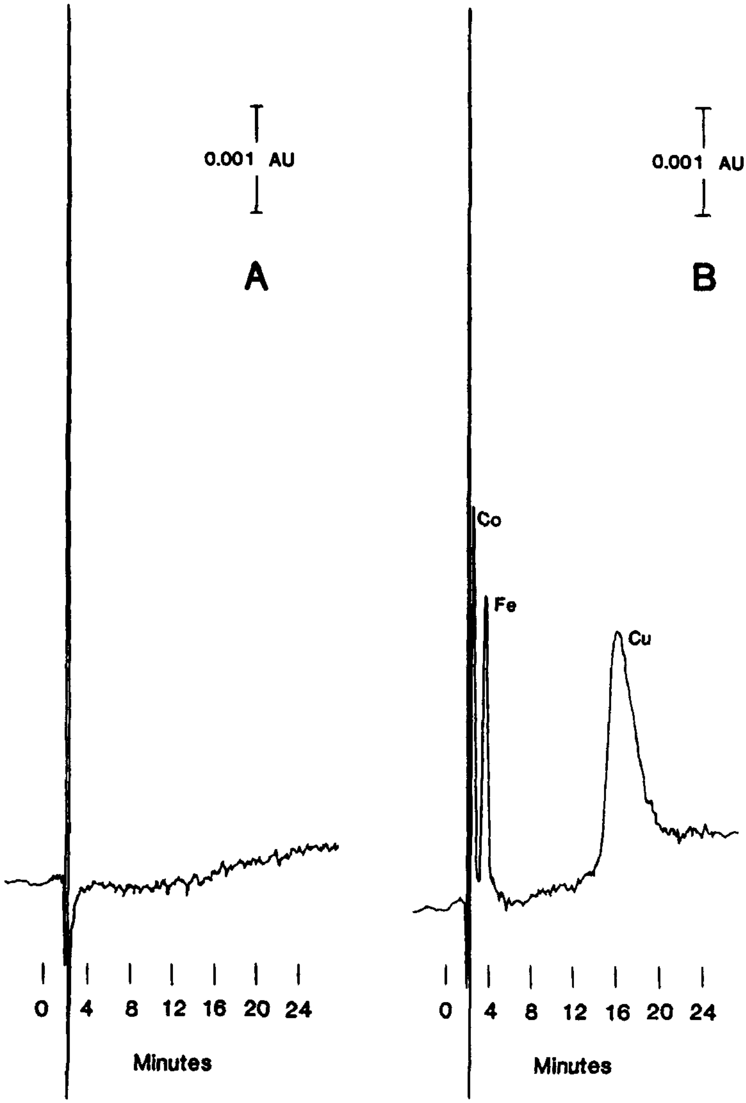

FIGURE 8. Chromatograms of a river water sample and a river water sampled spiked with metal PAR Complexes. (A) Filtered river water; (B) Filtered river water with 55 ppb Co^{3+}, 273 ppb Fe^{3+}, and 550 ppb Cu^{2+} PAR complexes. Mobile phase parameters were the same as Figure 7, and were separated on the coupled columns as described in Figure 7C.

potential problem in part because detection is at 546 nm. This dual column approach should be particularly useful for the determination of Cu^{+2} in the presence of certain other metal ions, such as Ni^{+2} which is also expected to be uncharged or monoanionic at pH 6.5 (22).

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