

Reversed-phase high-performance liquid chromatographic behaviour of some metal 1-(2-pyridylazo)-2-naphthol chelates

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ABSTRACT

The HPLC behaviour of Cu(II), Co(III), Ni(II) and Fe(II) chelates with 1-(2-pyridylazo)-2-naphthol (PAN) on alkyl-bonded silica gel stationary phase was studied in detail. Peaks of $[\text{Ni}^{\text{II}}(\text{PAN})_2]$ and $[\text{Fe}^{\text{II}}(\text{PAN})_2]$ were observed with the mobile phase acetonitrile–water (70:30, v/v). The cationic chelates, $[\text{Cu}^{\text{II}}(\text{PAN})]^+$ and $[\text{Co}^{\text{III}}(\text{PAN})_2]^+$, were found to be adsorbed on the stationary phase as a result of interaction with the residual silanol groups. A sharp peak of $[\text{Co}^{\text{III}}(\text{PAN})_2]^+$ chelate was obtained by adding one of several organic or inorganic salts to the mobile phase, but a $[\text{Cu}^{\text{II}}(\text{PAN})]^+$ chelate peak could only be obtained on addition of ammonium thiocyanate. The retention of the Co(III) chelate depended to a large extent on the alkyl chain length of the stationary phase and the species of an organic modifier in the mobile phase.

INTRODUCTION

2-Pyridylazo compounds, which form stable chelates with high molar absorptivity in the visible regions with many metal ions, are very useful analytical reagents and have been widely used for spectrophotometric determination and complexometric titration of metals [1]. Moreover, these reagents form different charged chelates depending upon the central metal ions because of tridentate ligands. In reversed-phase high-performance liquid chromatography (RP-HPLC), precolumn chromogenic chelating reagents must contribute not only to the detection but also to the separation of metal ions, in contrast to post column chelating reagents. Thus the reagents are required to form chelates with different partition characteristics between stationary and mobile phases.

Recently some 2-pyridylazo compounds with various functional groups have been synthesized and applied to RP-HPLC as precolumn chelating reagents for the simultaneous determination of trace metals because of their excellent analytical properties. Most of them have been used in the form of water-soluble anionic chelates, and ion-pair RP-HPLC using hydrophobic cations such as tetrabutylammonium has usually been employed [2–5].

1-(2-Pyridylazo)-2-naphthol (PAN) is widely used for the separation and determination of various metals as a colour-forming and extraction reagent, because the reagent itself is rather unselective. A large number of studies describing the use of this reagent and chelation with individual metals have been reported [1]; nevertheless few papers on the RP-HPLC behaviour of PAN chelates have been published [6,7].

Although a few studies on the RP-HPLC separation of Cu(II), Co(III), Ni(II) and Fe(II) chelates have been reported [6], good resolution

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between Ni(II) and Fe(II) chelates was not obtained.

In the present study, the RP-HPLC behaviour of PAN and the chelates of Cu(II), Co(III), Ni(II) and Fe(II) on alkyl-bonded silica gel stationary phase was extensively studied. The adsorption of cationic chelates, $[\text{Cu}^{\text{II}}(\text{PAN})]^+$ and $[\text{Co}^{\text{III}}(\text{PAN})_2]^+$, on residual silanol groups and the retention of these as well as neutral chelates, $[\text{M}(\text{PAN})_2]$ [M: Ni(II), Fe(II)], are discussed.

EXPERIMENTAL

Apparatus

The HPLC system consisted of a Model CCPD dual pump (resin type, Tosoh, Tokyo, Japan), a Model SVM-6M2L ceramic valve injector (dead volume 2 μl , sample loop 8 μl , Sanuki, Tokyo, Japan) and a Model Multi-330 multiwavelength UV-Vis detector (wavelength range 200–800 nm, flow cell 4 μl , 1 mm I.D., optical pathlength 5 mm, Jasco, Tokyo, Japan) equipped with an NEC personal computer. Data acquisition and data analysis were performed using a Jasco Model DP-L320/98 data-processing system (Jasco). An Inertsil ODS-2 packed column (250 mm \times 4.6 mm I.D., particle diameter 5 μm , GL Sciences, Tokyo, Japan) was used unless otherwise noted. The temperature of the column was controlled by a Model 860-CO column oven (Jasco). By using a metal-free pump, injector, column and tubing, contamination by metal ions from the HPLC apparatus was minimized.

Reagents

PAN was synthesized as described previously [1] and dissolved in ethanol. Metal stock solutions (10^{-2} M) were prepared from analytical reagent-grade metallic copper, cobalt(II) and nickel(II) nitrates and iron(II) sulphate, containing 0.01–0.1 M acid. Organic or inorganic salts added to the mobile phase were of analytical-reagent grade or reagent grade. All the organic solvents were of analytical reagent grade. The water used was purified by means of a Toraypure LV-10T ultrapure water manufacturing device (Toray, Tokyo, Japan).

Procedure

A 1-ml volume of an aqueous solution (pH ca. 4) containing 10^{-4} M each of copper(II), cobalt(II), nickel(II) and iron(II) was added to 4 ml of $2.5 \cdot 10^{-4}$ M PAN ethanol solution, and the solutions were allowed to stand for at least 30 min, or were heated to 80°C for the quantitative chelation. The resulting solution was loaded into the sample loop by suction and injected into the column. Mobile phase was filtered through a membrane filter (pore size 0.2 μm , Toyo Roshi) and degassed before use. The flow-rate of a mobile phase was adjusted to 0.8 ml/min. The column was equilibrated with each mobile phase for at least 30 min before sample injection. The capacity factors (k') of PAN and its chelates were calculated using the retention time of sodium nitrite as a measure of the void volume.

RESULTS AND DISCUSSION

Chromatographic behaviour of PAN chelates

Copper and cobalt form cationic chelates of $[\text{Cu}^{\text{II}}(\text{PAN})]^+$ and $[\text{Co}^{\text{III}}(\text{PAN})_2]^+$, while nickel and iron form neutral chelates $[\text{M}^{\text{II}}(\text{PAN})_2]$ under the conditions described in the Procedure section (pH ca. 4). The RP-HPLC behaviour of analytes is often estimated from extractive behaviour because the retention is mainly based on the partition between stationary phase and mobile phase. The extractability of metals with PAN into chloroform was examined. The results are shown in Fig. 1. The cationic Cu(II) and Co(III) chelates, which were minimally extracted without appropriate anions, could be extracted as $[\text{Cu}(\text{PAN})]^+\text{Cl}^-$ and $[\text{Co}(\text{PAN})_2]^+\text{Cl}^-$. At pH 4–10, Cu(II), Co(III), Ni(II) and Fe(II) chelates were quantitatively extracted. Their absorption spectra and the wavelength of their absorption maxima were not changed over this range of pH, although the spectrum of Cu(II)–PAN changed at pH > 7 as a result of the formation of $[\text{Cu}^{\text{II}}(\text{PAN})_2]$ with excess PAN. These results suggest that neutral chelates are retained more strongly than cationic chelates, if secondary effects on the retention other than the partition do not exist.

A solution containing Cu(II)–, Co(III)–, Ni(II)– and Fe(II)–PAN chelates and excess

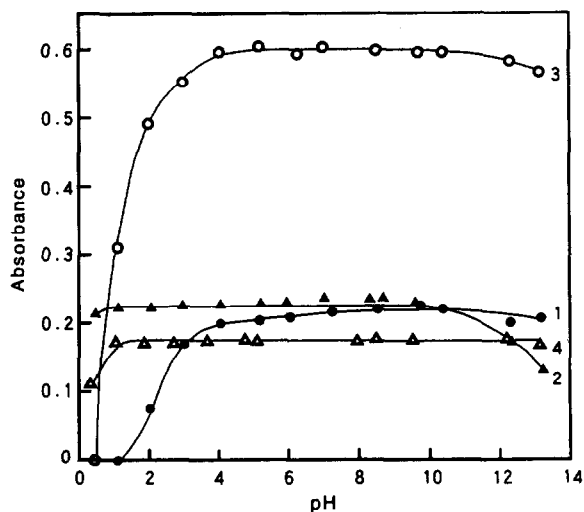


Fig. 1. Effect of pH on the extraction of PAN chelates. Aqueous phase (10 ml) concentrations: metal $1.0 \cdot 10^{-5}$ M; PAN = $1.25 \cdot 10^{-5}$ M (Cu) or $2.5 \cdot 10^{-5}$ M (others); buffer = 0.01 M; KCl = 0.1 M; ascorbic acid = $2.0 \cdot 10^{-4}$ M (Fe); KIO_4 = $1.0 \cdot 10^{-3}$ M (Co). Organic phase (10 ml): chloroform. Metal, wavelength: 1 = Cu, 565 nm; 2 = Co, 583 nm; 3 = Ni, 569 nm; 4 = Fe, 765 nm.

PAN was injected into a column. Fig. 2a shows a chromatogram obtained using acetonitrile–water (70:30, v/v) not containing any salts as a mobile phase. Only three peaks were observed for PAN and neutral chelates of $[\text{Ni}^{\text{II}}(\text{PAN})_2]$ and $[\text{Fe}^{\text{II}}(\text{PAN})_2]$, but not for the cationic chelates of $[\text{Cu}^{\text{II}}(\text{PAN})]^+$ and $[\text{Co}^{\text{III}}(\text{PAN})_2]^+$.

It has been reported that silanol groups remain at the surface of alkyl-bonded silica gel after end-capping and influence the chromatograms of basic or polar compounds [8–10]. As the pK_a of silanol groups is around 7 [11], the cationic chelates of Cu(II) and Co(III) were probably adsorbed to the dissociated silanol ($-\text{SiO}^-$) at $\text{pH} > 7$, or exchanged with a proton of silanol at lower pH.

Effect of eluent pH

The effect of mobile phase pH (ca. 1–7) on the retention of PAN and its chelates, such as Cu(II), Co(III), Ni(II) and Fe(II), was examined with acetonitrile–water (70:30, v/v) containing sulphuric acid (Fig. 2b and c). The lower peaks for Co(III) and Cu(II) chelates were obtained at $\text{pH} < 5$ and < 3 , respectively. The

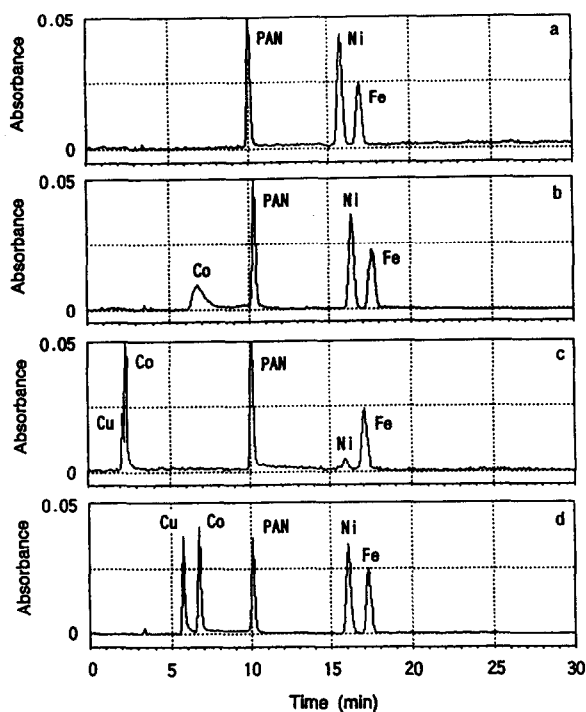


Fig. 2. Chromatogram of PAN chelates. Column: Inertsil ODS-2. Mobile phase: acetonitrile–water (70:30, v/v) containing (a) no additives; (b) $1.0 \cdot 10^{-5}$ M H_2SO_4 , pH 5.4; (c) $1.0 \cdot 10^{-4}$ M H_2SO_4 , pH 3.3; (d) $1.0 \cdot 10^{-2}$ M NH_4SCN , pH 5.3. Flow-rate: 0.8 ml/min. Column temperature: 40°C. Metal, amounts injected (μg), detection wavelength (nm): Cu, 0.013, 555; Co, 0.012, 447; Ni, 0.012, 560; Fe, 0.011, 466.

retention of Cu(II) and Co(III) chelates at $\text{pH} < \text{ca. } 3$ was almost equal to that of sodium nitrite, which was used as a standard for measuring the void volume. It is clear that the residual silanol groups may be negligible at pH 3. At this pH, however, deterioration in the peak heights caused by dissociation of chelates, as seen in Fig. 2c, was also observed for Cu(II) and Ni(II) chelates.

Effect of eluent additives

Various organic and inorganic salts (total concentration adjusted to 0.01 M) were added to the acetonitrile–water (70:30, v/v) mobile phase, and their effect on the retention of PAN chelates was examined. Fig. 2d shows a chromatogram obtained with mobile phase containing 0.01 M ammonium thiocyanate. The capacity factors

TABLE I
EFFECT OF SALTS ON CHROMATOGRAPHIC BEHAVIOUR OF CATIONIC PAN CHELATES

Salt (0.01 M)	Capacity factor (k')		Theoretical plate number (N) ^a	
	Cu(II)	Co(III)	Cu(II)	Co(III)
Sodium 2-naphthalenesulphonate	–	3.80	–	6500
Sodium 1-pentanesulphonate	–	2.37	–	7200
Acetic acid–sodium acetate (pH 5) ^b	–	3.12	–	4800
Citric acid–sodium citrate (pH 5) ^b	–	1.85	–	9200
Tetra- <i>n</i> -butylammonium bromide	–	1.60	–	4300
Sodium bromide	–	1.90	–	7600
Ammonium bromide	0.88	1.60	300	9600
Sodium azide	1.90	3.42	100	7000
Sodium thiocyanate	1.32	2.28	1500	6800
Ammonium thiocyanate	1.15	1.53	11700	5800

^a $N = 5.54 (t_R/W_{1/2})^2$.

^b The sodium ion concentration is less than 0.01 M.

(k') and theoretical plate numbers (N) of cationic PAN chelates are given in Table I together with other salts. Retention of neutral Ni(II) and Fe(II) chelates was not altered by the addition of any salts.

The Co(III) chelate was eluted to give a sharp band with mobile phases containing any combination of a cation and an anion, although the capacity factor varied from 1.6 to 3.8. The Cu(II) chelate, on the other hand, could be eluted only by four salts which contained anions with stronger affinities for Cu(II). The theoretical plate numbers with thiocyanates were much larger than those with azide and bromide (NaSCN compared with NaN₃, and NH₄SCN compared with NH₄Br). Moreover, ammonium salts gave sharp peaks with less retention than sodium salts (NH₄SCN compared with NaSCN).

Thus the combination of ammonium and thiocyanate was found to be most effective as an additive (Fig. 2d). The ammonium ion effectively reduced the adsorption of these cationic chelates on dissociated silanol, and the thiocyanate ion strongly neutralized the chelate to form [Cu^{II}(PAN)]⁺SCN⁻. As a result, the interaction between the chelate and silanol is reduced to distribute favourably on the stationary phase.

Effect of stationary phase

The retention behaviour of PAN chelates was examined with eight kinds of alkyl-bonded silica gel columns commercially available. The substituent groups were octadecyl (–C₁₈; six manufacturers), octyl (–C₈; one manufacturer) and methyl (–C₁; one manufacturer). With mobile phases containing no salts, cationic chelates were not eluted from any column described above; only Kaseisorb ODS-300-5 gave a peak of Co(III) chelate, although the capacity factor was not reproducible. In the presence of 0.01 M ammonium thiocyanate, on the other hand, all the chelates were eluted from all columns. The capacity factors (k') of PAN chelates are listed in Table II. Some differences were found among the ODS columns used. For example, strong retention of Cu(II) and Co(III) chelates with TSK-gel ODS-120T suggests that ammonium thiocyanate of higher concentration was required because of the presence of more residual silanol groups on the column. The elution order, however, was the same and the ratios of capacity factors among chelates were similar to each other.

Alkyl chain length had a great effect on the retention of PAN chelates. With a decrease in

TABLE II
EFFECT OF COLUMNS ON CHROMATOGRAPHIC BEHAVIOUR OF PAN CHELATES^a

Column	CH ₃ CN–water	Capacity factor (<i>k'</i>)				
		Cu(II)	Co(III)	PAN	Ni(II)	Fe(II)
Inertsil ODS-2 (GL Sciences)	70:30	1.23	1.78	2.85	5.32	5.82
Shim-pack CLC-ODS (Shimadzu)	70:30	1.28	2.02	2.68	5.08	5.50
TSK-gel ODS-80Ts (Tosoh)	70:30	1.50	2.27	2.98	6.32	6.86
TSK-gel ODS-120T (Tosoh)	70:30	1.04	2.39	2.53	3.68	3.97
Kaseisorb ODS-SUPER (Tokyo Kasei)	70:30	1.08	1.33	2.47	4.73	5.17
Kaseisorb ODS-300-5 (Tokyo Kasei)	70:30	0.23	0.23	0.54	1.03	1.13
	60:40	0.45	0.56	0.97	2.42	2.70
Unisil Q C8 (GL Sciences)	70:30	1.06	3.50	1.99	3.05	3.24
	60:40	1.98	8.28	3.33	6.71	7.23
Finepak SIL C1-5 (Jasco)	70:30	0.21	0.71	0.24	0.32	0.32
	50:50	0.95	2.69	0.75	1.29	1.33

^a Mobile phase: acetonitrile–water containing 0.01 M NH₄SCN.

the carbon number, the retention of PAN and Cu(II), Ni(II) and Fe(II) chelates dwindled, whereas that of Co(III) chelates showed the maximum at C₈ (Fig. 3). As a result, the retention of [Co^{III}(PAN)₂]⁺SCN⁻ was higher than that of [Ni^{II}(PAN)₂] and [Fe^{II}(PAN)₂],

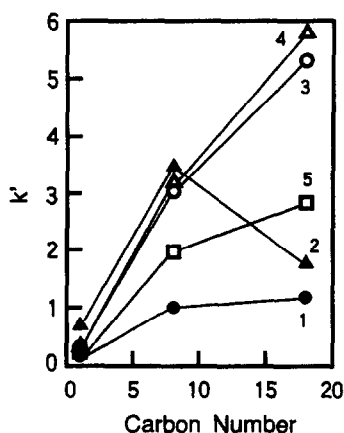


Fig. 3. Effect of carbon number on capacity factors (*k'*). Columns: C₁₈, Inertsil ODS-2; C₈, Unisil Q C₈; C₁, Finepak SIL C1-5. Mobile phase: acetonitrile–water (70:30, v/v) containing 1.0 · 10⁻² M NH₄SCN. 1 = Cu; 2 = Co; 3 = Ni; 4 = Fe; 5 = PAN.

using C₁ and C₈ columns. In the case of C₁ columns, [Cu^{II}(PAN)]⁺SCN⁻ was retained more strongly than PAN with acetonitrile–water (50:50, v/v) (Table II). Thus separation and even elution order could be modified by the hydrophobicity of the stationary phase.

Effect of organic modifiers

The effect of organic modifiers such as acetonitrile, acetone, methanol or ethanol in the mobile phase on the elution of chelates was examined. A Kaseisorb ODS-300-5 packed column (250 mm × 4.6 mm I.D., particle diameter 5 μm, Tokyo Kasei, Tokyo, Japan) was used because of low pressure loss. The results are shown in Fig. 4. With acetonitrile–water containing 0.01 M NH₄SCN, the decrease in retention of Co(III), Ni(II) and Fe(II) chelates caused by a decrease in polarity of the mobile phase was larger than that of Cu(II) chelate and PAN. The slope for [Cu^{II}(PAN)]⁺SCN⁻ is almost the same as that for PAN, whereas those for [Co^{III}(PAN)₂]⁺SCN⁻, [Ni^{II}(PAN)₂] and [Fe^{II}(PAN)₂] are twice as steep. The ratio agrees with the number of PAN ligands involved in each chelate. The intercepts, on the other hand, are smaller for cationic species than for neutral ones:

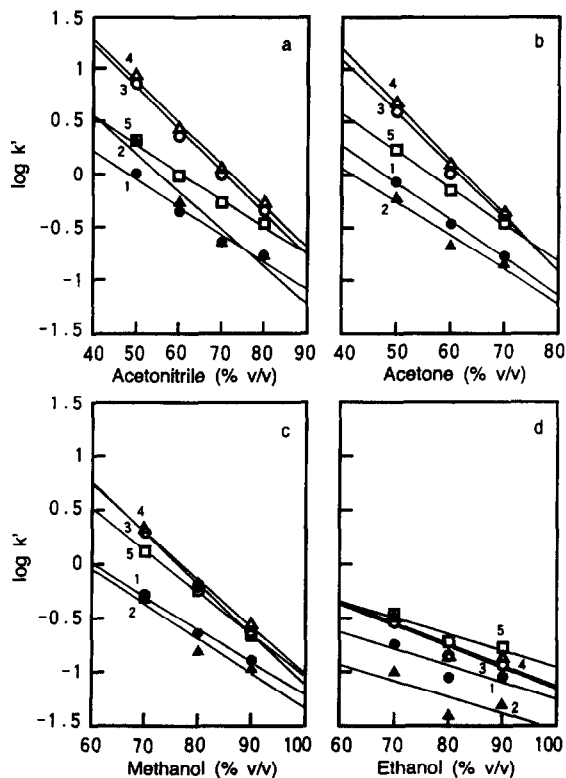


Fig. 4. Effect of organic modifier on capacity factors (k'). Column: Kaseisorb ODS-300-5. Mobile phase: (a) acetonitrile-water; (b) acetone-water; (c) methanol-water; (d) ethanol-water containing $1.0 \cdot 10^{-2} M NH_4SCN$. 1 (●) = Cu; 2 (▲) = Co; 3 (○) = Ni; 4 (△) = Fe; 5 (□) = PAN.

$[Co^{III}(PAN)_2]^+SCN^-$ against $[Ni^{II}(PAN)_2]$ or $[Fe^{II}(PAN)_2]$, and $[Cu^{II}(PAN)]^+SCN^-$ against PAN. Thus an increase in acetonitrile content caused a deterioration in the separation between Ni(II) or Fe(II) chelates and PAN, and that between Co(III) and Cu(II) chelates. The elution order of Co(III) and Cu(II) chelates is reversed in less polar solvent systems such as acetone-water, methanol-water and ethanol-water (Fig. 4b–d). With ethanol-water, even Ni(II) and Fe(II) chelates were eluted earlier than PAN.

Effect of column temperature

Fig. 5a shows the effect of column temperature on the logarithmic k' values for PAN and its chelates. By increasing column temperature,

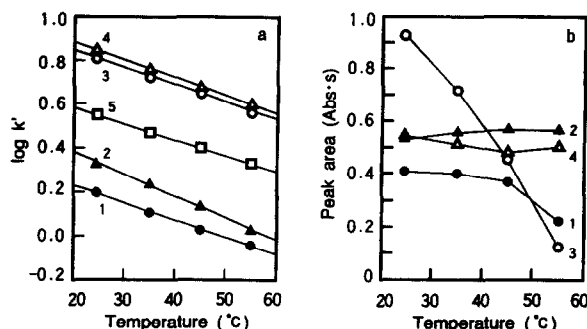


Fig. 5. Effect of column temperature on capacity factors (k') (a) and peak areas (b). Column: Inertsil ODS-2. Mobile phase: acetonitrile-water (70:30, v/v) containing $1.0 \cdot 10^{-2} M NH_4SCN$. Other conditions are the same as in Fig. 2. 1 = Cu; 2 = Co; 3 = Ni; 4 = Fe; 5 = PAN.

elution time was reduced while maintaining good resolution of PAN and chelates because the slopes for all chelates were almost the same. As mentioned above, the increase in organic modifier concentration also reduced elution time, but the separation between Cu(II) and Co(III) chelates deteriorated (Fig. 4a).

The peak areas for Cu(II) and especially for Ni(II) were decreased with the increase in temperature (Fig. 5b). Since such phenomena were not observed without thiocyanate, displacement of PAN on Ni(II) by thiocyanate might be enhanced at a higher temperature.

CONCLUSIONS

The effect of several variables, such as hydrogen ion concentration, additives and organic modifiers in the mobile phase, alkyl chain length of the stationary phase, and column temperature, on the RP-HPLC of PAN chelates were examined.

It was possible to eliminate the adsorption of cationic chelates by using ammonium thiocyanate as an additive, to alter the elution order by altering the length of the alkyl chain and the polarity of the organic modifier, and to reduce the elution time while maintaining good resolution by increasing the column temperature.

These results provide fundamental information on the RP-HPLC of an analyte containing cationic and neutral chelates.

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