

Reversed-Phase Ion-Pair High-Performance Liquid Chromatography of Metal Chelates with Sulfonated 2-(2-Pyridylazo)-1-naphthols. Positional Effect of Sulfonate Group

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Heterocyclic azo compounds have been widely used as metal indicators and spectrophotometric reagents. Especially the compounds having an *o*-hydroxy group form stable chelates with various metal ions and have been used as precolumn derivatizing reagents for high-performance liquid chromatography (HPLC).

1-(2-Pyridylazo)-2-naphthol (PAN) forms neutral chelates with divalent metal ions and cationic chelates with trivalent ions, which can be separated by reversed-phase HPLC.¹ The chelates with 4-(2-pyridylazo)resorcinol (PAR), on the other hand, have different charges due to the deprotonation of phenol groups at a position *para* to an azo group: 0 to 2- for divalent metal chelates and 1+ to 1- for trivalent metal chelates.² With ion-pairing reagents, these chelates were successfully separated by reversed-phase ion-pair HPLC.³⁻⁵

We developed the sulfonated derivatives of PAN and 2-pyridylazophenol for sensitive spectrophotometry of metal ions^{6,7} and also evaluated their performances as a precolumn derivatizing reagent.^{8,9} In this work, we have examined four isomers of mono-sulfonated 2-(2-pyridylazo)-1-naphthol (α -PAN-*x*S, where *x* denotes the position of a sulfonic group on the naphthol ring; H₂L; Fig. 1) as a derivatizing reagent for reversed-phase HPLC with tetraalkylammonium bromide (NR₄Br) and have studied the effects of the position of a sulfonic group on the chromatographic behavior of three metal (Fe, Co

and Ni) chelates.

Experimental

Apparatus

The HPLC system consisted of a Model PU-980 intelligent pump, a Model 860-CO column oven and a Model MD-915 multiwavelength detector (JASCO, Tokyo, Japan) equipped with a DELL personal computer. Data acquisition and data analysis were performed using a DP-L910/915 UV/VIS multiwavelength detector software.

Reagents

The sulfonated α -PANs were synthesized as described previously^{6,10} and were dissolved in aqueous acetonitrile to give 2.5×10^{-4} M solutions. As an ion-pairing reagent, tetraethylammonium bromide (TEABr), tetrapropylammonium bromide (TPABr) and tetrabutylammonium bromide (TBABr) were used.

Procedure

An aliquot (1 ml) of solution containing 10^{-4} M Fe(II), Co(II) and Ni(II) was added to 4 ml of a reagent solution, followed by the addition of 1 ml of an acetate buffer (pH 5, 0.1 M). The resulting solution was loaded into the sample loop (8 μ l) and injected into the column (Inertsil ODS-2 [4.6 mm i.d. \times 250 mm, 5 μ m, GL Science, Tokyo, Japan]). Acetonitrile-water mixture containing NR₄Br and acetate buffer was used as a mobile phase, which had been filtered through a membrane filter (pore size 0.2 μ m, Toyo Roshi) and degassed before use. Elution was carried out at a flow rate of 0.8 ml/min and at 40°C and absorption at 400–800 nm was monitored. The eluates were collected and subjected to pH-measurement.

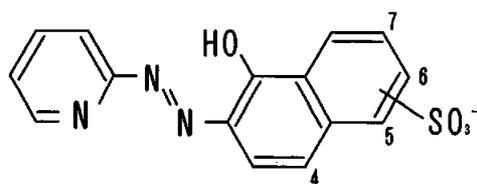


Fig. 1 HL⁻ form of α -PAN-*x*S (*x*=4–7)

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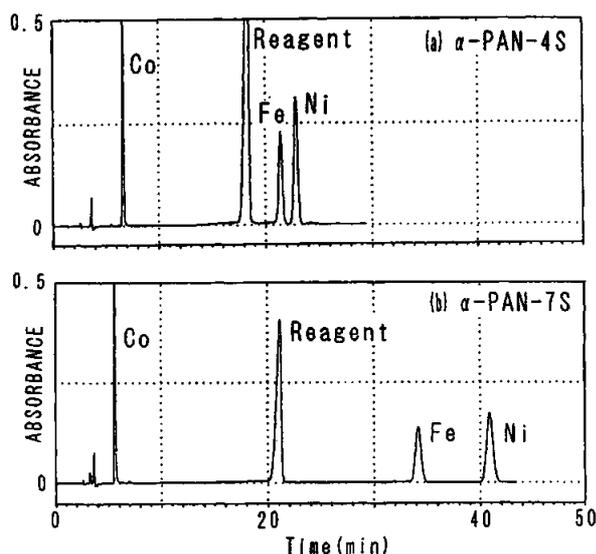


Fig. 2 Chromatograms of α -PAN-4S and α -PAN-7S chelates. Mobile phase: acetonitrile-water (40:60, v/v) containing 10^{-2} M TBABr and 10^{-3} M aqueous acetate buffer of pH 5.0.

Results and Discussion

Preliminary experiments

Complexation reactions of metal ions with sulfonated α -PANs were qualitatively studied from the changes in visible absorption spectra measured by batchwise experiments.^{6,10} In a neutral pH range, all the reagents react with Fe^{2+} or Ni^{2+} to form only an anionic chelate of FeL_2^{2-} or NiL_2^{2-} . The reaction with Co^{2+} first forms $\text{Co}^{\text{II}}\text{L}_2^{2-}$, which is slowly oxidized by the dissolved oxygen to give $\text{Co}^{\text{III}}\text{L}_2^-$.^{6,11} When sample solutions containing these metal chelates were injected in the chromatographic system, Fe, Co and Ni each gave one sharp peak. The complex of $\text{Co}^{\text{II}}\text{L}_2^{2-}$ was unstable after separation from an excess reagent in the column and only $\text{Co}^{\text{III}}\text{L}_2^-$ was detected.

Figure 2 shows typical chromatograms of α -PAN-4S and α -PAN-7S chelates, which respectively have the smallest and largest retention among four reagents studied. The effects of additives in the mobile phase were examined in detail for these two compounds.

Effects of buffer and TBABr

Figure 3(a) shows the capacity factor (k') of α -PAN-7S chelates plotted against the observed pH of an aqueous acetonitrile solution containing a $\text{CH}_3\text{COOH}-\text{CH}_3\text{COONa}$ buffer (10^{-3} M as a total concentration, C_B). The reading of the pH meter, which had been calibrated with aqueous standard buffers, was used without any correction for mixed solvents; for example, the observed pH of the mixture of aqueous acetate buffer (pH 5.0) and acetonitrile was 5.9 at $\text{CH}_3\text{CN}/\text{H}_2\text{O}=40/60$. The effects of the concentration of aqueous acetate buffer at pH 5.0 on the capacity factors were shown at $\text{CH}_3\text{CN}/$

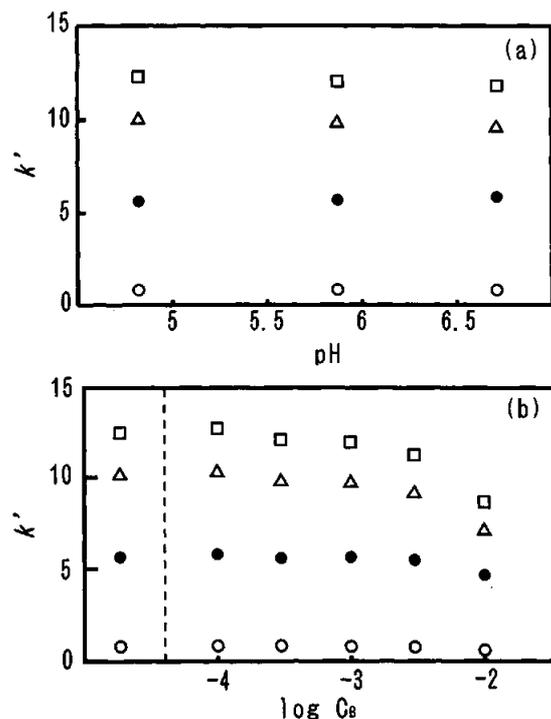


Fig. 3 Effects of pH(a) and total concentration(b) of acetate buffer on capacity factors of α -PAN-7S chelates. Mobile phase: acetonitrile-water (40:60, v/v) containing 10^{-2} M TBABr. (a) total buffer concentration: 10^{-3} M; (b) pH of buffer added: 5.0; see text about pH values. (\square) Ni; (\triangle) Fe; (\bullet) reagent; (\circ) Co.

$\text{H}_2\text{O}=40/60$ in Fig. 3(b). The symbols of the left-most position in Fig. 3(b) indicate the retention without any buffer. The same dependence on C_B and pH has been observed in the reversed-phase ion-pair HPLC retention of simple inorganic anions using phosphate or phthalate buffers and tetrabutylammonium iodide (TBAI) as an ion-pairing reagent.¹² By analogy with this extensive study, the decrease in retention with the increase in buffer concentration and with the increase in pH is ascribed to the competitive retention of acetate to anionic chelates; there are no direct effects of pH on the retention. In the subsequent studies, an aqueous buffer at pH 5.0 was added to the mobile phase (10^{-3} M).

Figure 4 shows the effects of the TBABr concentration on the retention of α -PAN-4S and α -PAN-7S chelates at $\text{CH}_3\text{CN}/\text{H}_2\text{O}=40/60$. The retention order was $\text{CoL}_2^- < \text{HL}^- < \text{FeL}_2^{2-} < \text{NiL}_2^{2-}$ at any TBABr concentration for both reagents. The retention of each species except HL^- showed convex dependence on the TBABr concentration; the increase in the lower concentration range is ascribed to the enhancement of ion-pair formation in the mobile phase and the decrease in the higher range to the competitive retention of Br^- to the anionic analytes. Such a trend was also found in the separation of simple anions; divalent ions such as SO_4^{2-} showed the maximum retention at a lower TBAI concentration compared with monovalent ions.¹² This corresponds to the behaviors

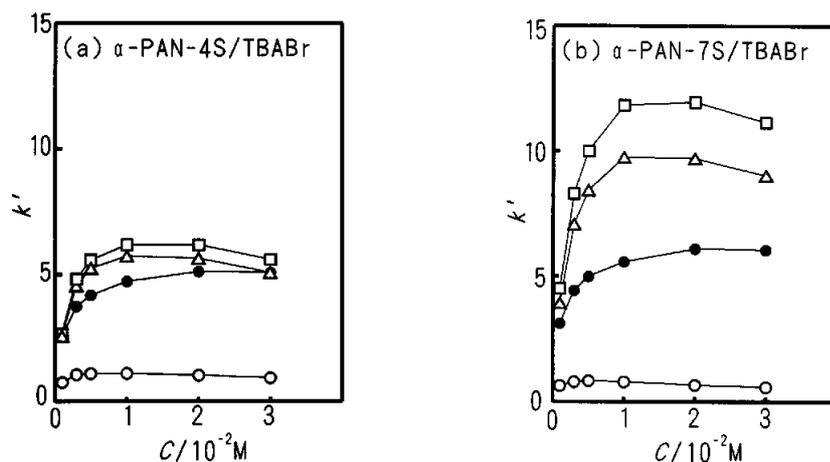


Fig. 4 Effect of TBABr concentration on capacity factors. Mobile phase: acetonitrile-water (40:60, v/v). Derivatizing reagent: α -PAN-4S (a); α -PAN-7S (b). (\square) Ni; (Δ) Fe; (\bullet) reagent; (\circ) Co.

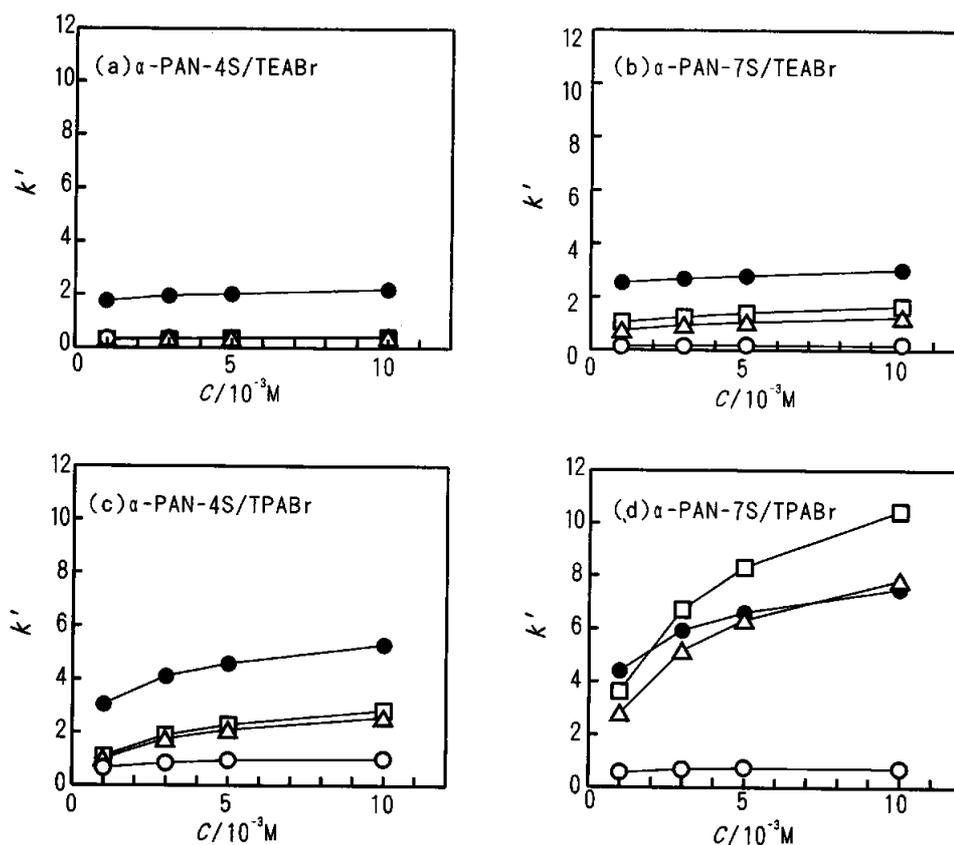


Fig. 5 Effect of TEABr (a,b) and TPABr (c,d) concentration on capacity factors. Mobile phase: acetonitrile-water (30:70, v/v). Derivatizing reagent: α -PAN-4S (a,c); α -PAN-7S (b,d). (\square) Ni; (Δ) Fe; (\bullet) reagent; (\circ) Co.

of NiL_2^{2-} , FeL_2^{2-} , and HL^- in the present study, although the maximum for HL^- was not found within the concentration range studied. In contrast, CoL_2^- , in

spite of having 1- charge, showed the maximum at the lowest TBABr concentration.

Effect of ion-pairing reagents

Using TEABr or TPABr as an ion-pairing reagent, chromatographic conditions were similarly optimized within a reasonable separation time; the acetonitrile content was decreased from 40/60 to 30/70. Figure 5 shows the effect of the concentrations of TEABr and TPABr on the retention, respectively.

The retention order was different from that using TBABr: for α -PAN-4S chelates, $\text{CoL}_2^- \approx \text{FeL}_2^{2-} \approx \text{NiL}_2^{2-} < \text{HL}^-$ with TEABr and $\text{CoL}_2^- < \text{FeL}_2^{2-} < \text{NiL}_2^{2-} < \text{HL}^-$ with TPABr; for α -PAN-7S chelates, $\text{CoL}_2^- < \text{FeL}_2^{2-} < \text{NiL}_2^{2-} < \text{HL}^-$ with TEABr or lower concentrations of TPABr. At higher concentrations of TPABr, the elution order became the same with that using TBABr: $\text{CoL}_2^- < \text{HL}^- < \text{FeL}_2^{2-} < \text{NiL}_2^{2-}$. The reversal of retention between HL^- and FeL_2^{2-} or NiL_2^{2-} was found for α -PAN-7S with the increase in TPABr concentration. The same was found for TBABr at a concentration level of 10^{-4} M and at $\text{CH}_3\text{CN}/\text{H}_2\text{O}=30/70$.

Such intersection of retention depending on the type and concentration of the ion-pairing reagents is reminiscent of the typical ion-pair extraction. In this case, distribution of doubly charged species is less favorable than that of singly charged species at lower concentrations of an ion-pairing reagent. At higher concentrations where ion-pair formation is complete in an aqueous phase, on the other hand, doubly charged species are distributed more favorably than singly charged ones. The phenomenon may also be explained by the idea that anionic chelates interact with counter cations adsorbed on the stationary phase (dynamic coating or ion-interaction; see ref. 13 as one of the most recent papers).

Comparison of chelating reagents

Performances of four chelating reagents are compared with one another at 0.01 M TBABr and $\text{CH}_3\text{CN}/\text{H}_2\text{O}=40/60$ (Fig. 6). The retention of NiL_2^{2-} and FeL_2^{2-} , which require two counter cations for ion-pair formation, is sensitive to the position of a sulfonic group. The electric charges on these metal centers are, as a rough estimate, neutralized by two phenolate oxygen atoms from two ligands. Thus NiL_2^{2-} and FeL_2^{2-} could be approximated as round molecules having two sulfonic groups. The chelates with α -PAN- x S of larger x values have sulfonic groups more distant from each other, which are more easily neutralized by two ammonium ions.

A free ligand, HL^- , is expected to have an intermolecular hydrogen bond between phenolic oxygen and one of azo nitrogen atoms¹⁴ and is appreciably retained. The CoL_2^- chelate also requires only one counter cation. In the resulting ion-pair, the negative charge on one of two sulfonic groups is neutralized by NR_4^+ but that on the other sulfonic group is located distant from the residual positive charge on cobalt. Thus, the ion-pair is not highly retained on the stationary phase, irrespective of the position of a sulfonic group.

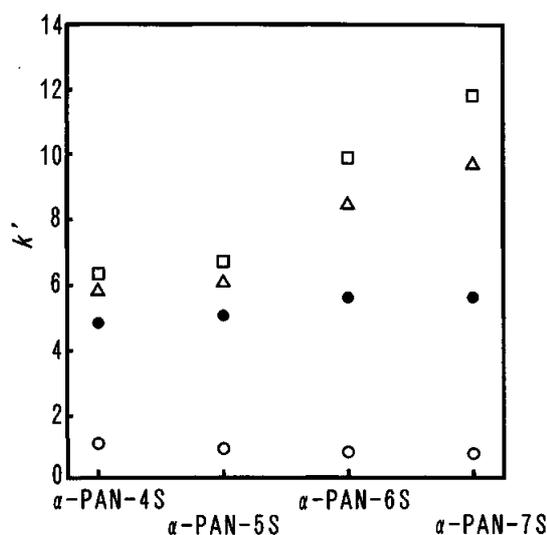


Fig. 6 Effect of chelating reagents on capacity factors. Chromatographic conditions are the same as those in Fig. 2. (□) Ni; (△) Fe; (●) reagent; (○) Co.

Further studies on the importance of charge neutralization around a metal ion are now in progress.

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