Retention Selectivity between 4-(2-Pyridylazo)resorcinol and Its Cobalt Chelate in the Solid Phase Extraction Systems and Its Application to the On-Line Preconcentration for Reversed Phase HPLC

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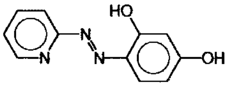
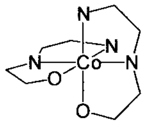
Preferential solid phase extraction of ultratrace metal chelate from the excess of its ligand has been succeeded in the case of cobalt, 4-(2-pyridylazo)resorcinol (PAR), ODS-Silica, and H_2O system, applying the reverse order of them found in reversed-phase HPLC with aqueous methanol and ODS-SiO_2. This finding makes it possible to concentrate the 1-ppt level of cobalt chelate in the presence of excess PAR from aqueous samples using kinetically selective HPLC.

The pressing needs have been claimed for ultratrace monitoring of Co at ppt levels in various samples such as natural water, human serum, and nuclear reactor coolant. The present analytical methods are unsatisfactory in terms of sensitivity, cost-effectiveness and matrix toughness. Authors have reported revesed-phase (RP-) HPLC methods for ultratrace determinations of metal ions using precolumn chelation technique with eluent containing no chelating reagent.[1,2] In these methods, the highest sensitivity of the detection system has been realized without any background signals caused by reagent stream, and we have named these methods as kinetic differentiation mode HPLC (KD-HPLC) because of the kinetic nature of chelates controlling the detection selectivity. Recently, the solid phase extraction (SPE) has been established as general method for pretreatment of real samples containing complex matrices,[3] and has also applied to preconcentration of metal chelates.[4–6] Combination of the KD-HPLC and the on-line SPE would enable construction of powerful and automated determination system for various metal ions. However, for the reliable function of the SPE systems, it is required that partition of analyte is favored than any matrices.

For example, the ligand 4-(2-pyridylazo)resorcinol (PAR, H_2L) showed stronger retention than the $[CoL_2]^-$ did in the ion-pair (IP-) RP-HPLC system where about 50 wt% aqueous methanol mobile phase containing tetrabutylammmonium bromide (TBABr) as ion pair reagent was used.[7] The order of retention of singly charged anions of the ligand and its metal chelates are $HL^- > [FeL_2]^- > [VO_2L]^- > [CoL_2]^-$. These behaviors of ‘ordinary’ HPLC system have been well explained on the basis of ion-pair liquid–liquid extraction mechanism.[7]



PAR (H_2L)



$[CoL_2]^-$

Based on these information, it seems very hard to concentrate $[CoL_2]^-$ quantitatively in RP-column from the aqueous sample solution which contain large excess amount of HL^- in precolumn chelation steps. In this paper, we presents the fact that lowering of the methanol content in the mobile phase reversed the retention selectivity and favored the partition of $[CoL_2]^-$ than HL^- in the solid phase extraction system with ODS-SiO_2. This finding makes it possible to concentrate the chelate without any trouble of competitive adsorption of excess PAR. GL Sciences ODS-2 (column: 4.6 mm i.d. × 50 mm, particle size: 5 μm) and TOSOH TSKguardgel ODS-80TM (3.2 mm i.d. × 15 mm, 5 μm) were selected as preconcentration column.

Using these short columns, break-through studies and determinations of retention behavior of the chelates were carried out. Analytical column used was TOSOH ODS-80TMCTR (4.6 mm i.d. × 10 cm, 5 μm). HPLC conditions were almost similar to those previously reported.[7] Detailed conditions were given in each figure captions.

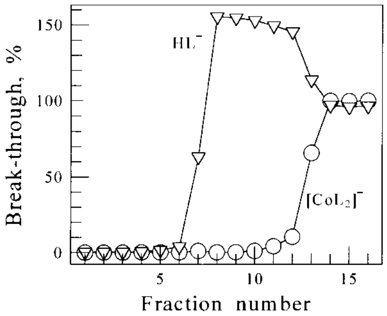


Figure 1. Break-through curves in a competitive break-through study. $[Co]_T = 1 \times 10^{-5}$ M, $[PAR]_T = 3 \times 10^{-5}$ M, pH 7 (phosphate buffer). Concentration column: TOSOH TSKguardgel ODS-80TM. One-fraction = 9 cm^3.

Figure 1 shows the break-through curves for HL^- and $[CoL_2]^-$ in the competitive conditions where the concentrations of HL^- and $[CoL_2]^-$ were almost the same. Ligand HL^- leaked at first and $[CoL_2]^-$ drove out the ligand already adsorbed on the column. Obviously, the chelate is preferably adsorbed on to the ODS packing in the SPE system. It is worth noting that in the experimental conditions in Figure 1 and Figure 2 there was no use of hydrophobic counter cations such as TBABr. In this case, concerning Co-PAR system, ODS packing shows sufficient capacity without any hydrophobic counter ions.

This selectivity results from the nature of solution from which ligand and the chelate are adsorbed; neat aqueous medium (for the preconcentration) or hydro-organic eluent (for the HPLC). Figure 2 shows retention behavior as a function of the methanol contents of mobile phase for HL^- and some PAR chelates. The selectivity between HL^- and $[CoL_2]^-$ cross over at the conditions where methanol fraction was about 20 wt%. The plots for chelates appear to be linear lines decreasing monotonously. Slopes for HL^- and 1:1 chelate $[VO_2L]^-$ were similar being smaller than those for 1:2 chelates $[CoL_2]^-$ and

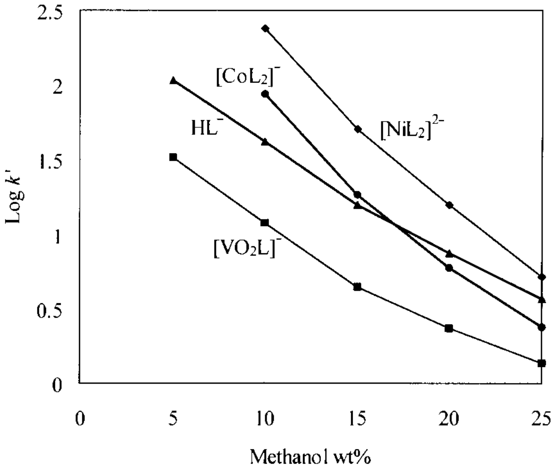


Figure 2. Capacity factor (k') vs concentration of methanol in eluent plots. Column: TOSOH TSKguardgel ODS-80TM. pH = 7 in eluent (phosphate buffer).

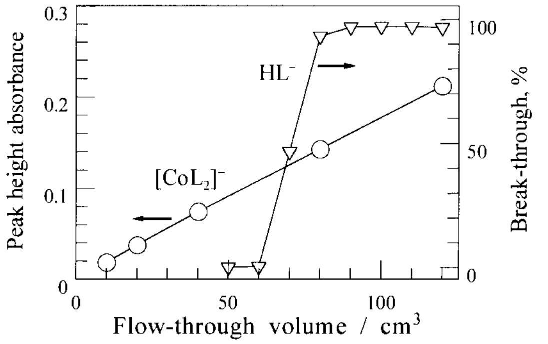


Figure 3. Peak height absorbance of [CoL$_2$]$^-$ vs column flow-through sample volume plot (○) and the break-through curve for HL$^-$ (▽). Sample: $[Co]_T = 5 \times 10^{-9}$ M, $[PAR]_T = 1 \times 10^{-5}$ M, pH 7.6 (Tris buffer). Concentration column: TOSOH TSKguardgel ODS-80TM. One-fraction was 10 cm^3 in break-through study of HL$^-$. Eluent: 50 wt% aqueous methanol containing 10 g kg^{-1} TBABr, 2×10^{-3} mol kg^{-1} Tris (pH 7.6), 1×10^{-4} M EDTA.

[NiL$_2$]$^{2-}$, which is most likely due to the differences in the molar volume.[8]

This SPE preconcentration system was connected to the KD-HPLC with column switching technique. Figure 3 shows dependence of cobalt peak heights on the sample volume loaded, and a break-through curve for the ligand. The cobalt peak height increases linearly with the flow-through volume, and does not be influenced by the break-through behavior of excess ligand, which was 2000 times higher concentration than that of cobalt. Quantitative concentrations of [CoL$_2$]$^-$ from at least more than 120 cm^3 sample solution were possible. Approximately 1000 times higher peak height than that of the direct injection of 100 mm^3 samples was obtained. This result shows that there is no problem about capacity of the concentration column. When 30 cm^3 of sample solution was applied on ODS-2 concentration column, highly sensitive and selective determination of Co was achieved with a stable base line and the good separation from added V and also from Fe and Ni as contaminants, as shown in Figure 4. The peak height was 240 times higher than that obtained by the direct injection of 100 mm^3 samples, and a linear peak height calibration graph was obtained over the concentration renges of 4×10^{-11} to 7×10^{-10} M of cobalt ion. The first-order regression line is expressed by the equation,

$$A = 1.1 \times 10^{-4} + 8.28 \times 10^{6}[Co] \qquad (r = 0.9997, n = 6)$$

and detection limit (S/N = 3) was reached down to 2×10^{-11} M (1 ppt for Co). Reproducibility in terms of r.s.d. at 1×10^{-10} M was 11.2% (n = 7).

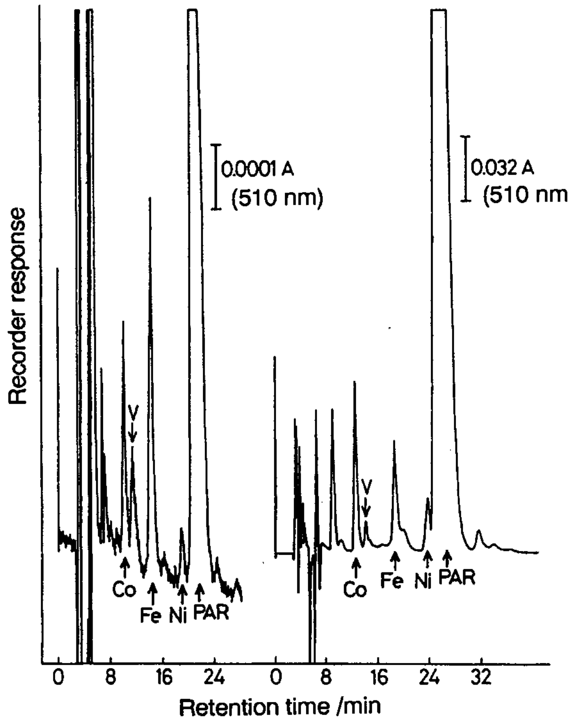


Figure 4. Typical chromatograms. Direct injection of 100 mm^3 sample solution (left), and injection after the preconcentration of 30 cm^3 sample solution by the proposed system (right). Sample: $[Co]_T = 1 \times 10^{-8}$ M, $[V]_T = 5 \times 10^{-8}$ M, $[PAR]_T = 2 \times 10^{-5}$ M. Eluent was as in Figure 3.

Controlling the composition of mobile phase, it was realized to control the retention selectivity on the SPE systems utilizing difference of molar volumes of chemical species was realized. These information will be important to design various application of SPE concentration systems.

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