High-Performance Separation and Determination of Co(III) and Ni(II) Chelates of 4-(2-Pyridylazo)resorcinol at Femtomole Levels by Capillary Electrophoresis

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Separation of 4-(2-pyridylazo)resorcinol (PAR) and its chelates of Co^{III}, Fe^{II}, Ni^{II}, and V^V was accomplished within 6 min by simple capillary electrophoresis (CE) with a spectrophotometric detection. The detection limits, defined as 3σ of base-line noise were 1.80×10^{-7} mol dm^{-3} for Co^{III} and 1.41×10^{-7} mol dm^{-3} for Ni^{II} chelates, and those on a mass basis were 1.08 fmol (63.6 fg) and 0.84 fmol (49.3 fg) in 6.0 $\times 10^{-9}$ dm^3 sample, respectively. A guideline on the ligand design for the CE system is also obtained from the pH dependence of the separation behavior of PAR chelates.

High-Performance Capillary Electrophoresis (HPCE), which is a general term of capillary electrophoresis (CE), micellar electrokinetic chromatography (MEKC), capillary gel electrophoresis, is a rapidly grown technique in modern separation sciences. Considerable advances in the applications, instrumentation, theory and optimization have appeared in the past decade.[1,2] Although many applications to the separation of biochemical or pharmaceutical matters have been achieved,[3] inorganic substances such as metal ions or complexes seem to have been scarcely treated.[4] Currently, one of the most sophisticated methodology for separation and determination of metal chelates is reversed-phase high-performance liquid chromatography (RP-HPLC) with a spectrophotometric detection,[5] using many kinds of reagents including 4-(2-pyridylazo)resorcinol (PAR, H_2L).[6,7] As compared to RP-HPLC HPCE is based on the fundamentally different separation principle and thereby has some attractive features, i.e., far higher resolution and very small sample consumption.[8] We have first utilized MEKC to separate and detect trace PAR chelates,[9] and much improved results were obtained as compared with those by HPLC. In this paper, higher efficiency of CE in the separation of PAR chelates as contrasted with MEKC and ultratrace determination of the Co^{III} and Ni^{II} chelates are demonstrated by the most basic CE system with no micellar phase.

Capillary electrophoresis equipment was the same as described elsewhere.[9] A fused silica capillary tube (700 mm long x 0.05 mm i.d.) was purchased from Scientific Glass Engineering Inc. (Australia). On-column detection at 500 nm was performed at the location of 550 mm from the positive end. The reagent, PAR received from Dojindo Lab. (Japan), was dissolved in a slightly alkaline solution (2×10^{-3} mol dm^{-3}). Standard metal ion solutions were made by dissolving metal salts of chlorides or nitrates, except for V^V and Mo^{VI}, ammonium metavanadate and ammonium molybdate, respectively, in diluted hydrochloric acid solution. Triply distilled water with Pyrex glass ware was used throughout the study. The electrophoretic buffers of pH 7 to 9.5 were composed of a mixture of NaH_2PO_4 and $Na_2B_4O_7$ solutions (each 0.01 mol dm^{-3}), and NaOH

and HCl solutions were used to adjust pH desired. The electric current was set at 20 μA resulting in the applied voltage of 21 ± 1.0 kV which was essentially unchanged in all the CE experiments. The temperature of the system was kept at 27 ± 1 °C in a safety box with an interlock system. To a sample solution containing 11 kinds of metal ions [Al^{III}, Cd^{II}, Co^{II}, Cr^{III}, Cu^{II}, Fe^{III}, Ni^{II}, Mn^{II}, Mo^{VI}, V^{V}, and Zn^{II}] were added 25 cm^3 of the PAR solution and 1 cm^3 of 1 mol dm^{-3} tris(hydroxymethyl)aminomethane – HCl buffer solution (pH 8.0). The mixture was made up to 50 cm^3 and heated at 60 °C for 15 min. After cooling the solution was injected from the positive end of the capillary tube by syphonic action (Δh = 5 cm for 50 s). The sample amount loaded was 6.0 x 10^{-9} dm^3 being determined with a continuous injection method.[10]

Typical electrophoregram for the mixture of 11 kinds of metal ions and the theoretical plate numbers under the optimum conditions are shown in Fig. 1 and Table 1. Highly resolved peaks of Co^{III}, V^{V}, Fe^{II}, and Ni^{II} chelates were obtained within 6 min. Surprisingly, the total migration time in this CE system is significantly shorter than that of the MEKC (30 min).[9] It is also noteworthy that the theoretical plate numbers obtained in the CE are two to four times greater than those in the MEKC (Table 1). This implies that in MEKC there is some contribution of micellar diffusion to the peak broadening. As a result, the CE shows superior ability to MEKC in the separation of anionic PAR chelates.

From the migration order and the pH dependence (*vide infra*), it is likely that the valence of Co^{II} and Fe^{III} (added form) is changed during the sample preparation to yield the Co^{III} and Fe^{II}–PAR chelates. No evidence of chelate formation of Al^{III}, Cr^{III}, and Mo^{VI} ions with PAR was obtained under the conditions. In addition, the peaks of the Cd^{II}, Mn^{II}, and Zn^{II} chelates are missing in Fig. 1. In a capillary, free PAR present in excess added in the complexation procedure, is separated from each metal chelates. Unless a chelate is inert, it will dissociate into metal ion and free PAR and they will be readily separated electrophoretically. Thus, the PAR chelates of Cd^{II}, Mn^{II}, and Zn^{II} seem to be quite labile on the CE time scale, while those of Co^{III}, Cu^{II}, V^{V}, Fe^{II}, and Ni^{II} are sufficiently inert to survive through a capillary tube. By contrast, when 1 x 10^{-4} mol dm^{-3} PAR was added to an electrophoretic buffer, the above three chelates also exhibited their peaks on the electrophoregram. On-column decomposition
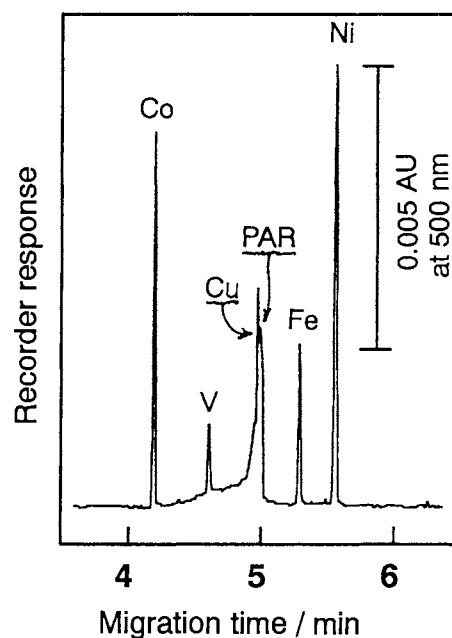

Fig. 1. CE separation of PAR chelates. pH 8.4: 20 μA (21.9 kV): Sample at pH 8.0, [PAR]$_T$ = 1 x 10^{-3} mol dm^{-3}; [M]$_T$ /(10^{-5} mol dm^{-3}), Al 2.21, Cd 2.37, Co 2.04, Cr 1.98, Cu 1.83, Fe 2.00, Mn 2.14, Mo 2.0, Ni 1.74, V 2.08, Zn 1.97.

Table 1. Theoretical plate number, N,[a] of PAR chelates

Chelate	CE[b]	MEKC[c]
Co	243000	114000
V	246000	
Fe	425000	105000
Ni	297000	120000

a) N = $5.54(t_m/W_{1/2})^2$, where t_m is migration time and $W_{1/2}$ is full peak width at half height.   b) This work, effective capillary length, l= 55 cm.   c) Ref.9, l=60 cm.

of metal chelates has been frequently observed in the separation by RP–HPLC in which a mobile phase containing no ligand is employed.[6,7] We termed this mode of HPLC separation as kinetic differentiation mode (KD–) HPLC,[11] since an analytical column behaves as if it could discriminate the kinetic stability of metal chelates to elute only inert ones. In PAR–KD–HPLC system, only Co^{III}, Cr^{III}, Fe^{II}, Ni^{II}, and V^V are detected. The similarity of the detection selectivity between the KD–HPLC and the CE may indicate that the kinetic nature of PAR chelates plays the most significant role in both systems.

During the optimization of the separation conditions, the effect of electrophoretic pH on the relative location of the peaks was investigated in terms of normalized migration time (t_m/t_0), as shown in Fig. 2, where t_0 and t_m are the migration times of solvent and sample solute, respectively. There are mainly two groups which show different pH dependence pattern. First, the t_m/t_0 values of free PAR, V^V and Co^{III} chelates simply decrease a little with increasing pH. This might be caused by the increase in the electroosmotic velocity because the larger the ζ potential of the inner wall of a capillary is at the higher pH.[12] Secondly, the values of t_m/t_0 for Fe^{II} and Cu^{II} markedly increased. It is noted that in this pH region, only these two chelates have the pK_a of 1–hydroxy groups on the resorcinol moiety (see Fig. 3 and Table 2). Thus the increment of the net anionic charge of the chelates caused by the deprotonation reasonably increases the electrophoretic mobility to the positive end. The composition of the Cu^{II} chelate seems to vary from 1:1 to 1:2 as pH is increased. At pH 7, the chelate migrates faster than $[Co^{III}L_2]^-$ does; the Cu^{II} chelate most probably has a less negative charge having the 1:1 stoichiometry, such

Fig. 2. The pH dependence of (t_m/t_0) for PAR and the chelates.

Fig.3. 1–Hydroxy group whose pK_a value is strongly dependent on the center metal ion, M.

Table 2. Acid dissociation constants for PAR and the chelates

Species [a]	pK_{a1}	pK_{a2}
PAR; H_2L	5.43	12.13 [c]
$[Co^{III}(HL)_2]^+$	3.64	4.41
$[V^VO_2HL]$	4.40	
$[Fe^{II}(HL)_2]$	6.47	7.66
$[Ni^{II}(HL)_2]$	6.2	7.1
$[Cu^{II}(HL)L]$	8.2 [b]	

a) Ref. 6. b) In 10% (v/v) aqueous acetone, from Ref. 13. c) 3–Hydroxy group.

as $[Cu^{II}L]^0$. While at pH 9.5 the chelate migrates even slower than HL^-; the 1:2 chelate, $[Cu^{II}L_2]^{2-}$ may be predominant in the CE process. This speculation is also supported by the equilibrium distribution calculation for the Cu^{II}–PAR system using the data in literature.[13] The Ni^{II} chelate behaves differently than the above mentioned species, because the pK_{a2} value of 7.1 is quite marginal in the pH range examined.

A new concept of ligand design for CE system is provided by these results shown in Fig. 2; it is necessary for a ligand to possess at least one functional group such a phenolic OH group as it can reflect the charac–
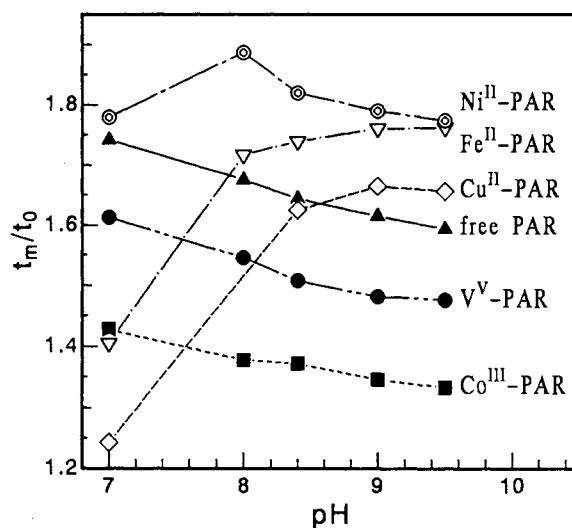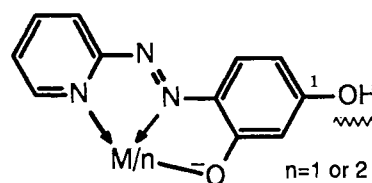
teristics of center metal ions by means of the magnitude of its acid dissociation constant.

Obviously seen from Figs. 1 and 2, an electrophoretic buffer at pH 8.4 is the most suitable for the simultaneous determination of Co^{III} and Ni^{II} ions. The concentration of each ion in the sample was reduced from 2 x 10^{-5} to 2 x 10^{-7} mol dm^{-3} and the peak height absorbance was recorded. In this concentration range the log-log calibration graphs were quite linear and expressed as,

log (peak height absorbance for Co) = 0.96_2 log ($[Co]_T$/mol dm^{-3}) + 2.31_2 r = 0.9994,

log (peak height absorbance for Ni) = 0.96_8 log ($[Ni]_T$/mol dm^{-3}) + 2.42_9 r = 0.9992.

The detection limits (D.L.), defined as 3σ of base-line noise were 1.80 x 10^{-7} mol dm^{-3} for Co and 1.41 x 10^{-7} mol dm^{-3} for Ni, respectively. The D.L.'s based on absolute amount in a 6.0 x 10^{-9} dm^3 sample were estimated as 1.08 fmol (63.6 fg) and 0.84 fmol (49.3 fg) for Co and Ni, respectively, which are of the smallest

values among the spectrophotometric methods. In case where the detection wavelength is shifted to 520 nm, the somewhat lower D.L. for Co is expected. The relative standard deviations of the peak height absorbance are summarized in Table 3, which shows the quite acceptable reproducibility.

Table 3. Relateive standard deviation of peak height absorbance [a]

	$[M]_T$ / mol dm^{-3}		
	2 x 10^{-7}	4 x 10^{-7}	2 x 10^{-6}
Co	8.5% (n=9)	8.3% (n=7)	1.3% (n=6)
Ni	15.7% (n=7)	7.0% (n=6)	1.5% (n=5)

a)$[M]_T$: total concentration of metal ion in sample solution.
The factors; f_{Co} = 1.020, f_{Ni} = 0.947. n; number of run.

In conclusion, despite that the CE system employed in this study is the simplest among those termed HPCE, this PAR-CE system shows unexpectedly high performance in the separation of the chelates and in the determination of Co^{III} and Ni^{II} ions at the fmol levels.

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