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SEPARATION OF 4-(2-PYRIDYLAZO)RESORCINOLATO METAL CHELATES BY MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY

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SUMMARY

The first application of micellar electrokinetic capillary chromatography to the separation of metal chelate compounds is demonstrated. The resolution of the peaks of 4-(2-pyridylazo)resorcinolato (par) chelates is excellent on a 60 cm × 0.05 mm I.D. silica capillary filled with a 0.02 mol dm^{-3} sodium dodecylsulphate micellar eluent at an applied voltage of 16.5 kV (driving current 12 μA). The theoretical plate number for the chelates reaches to more than $1 \cdot 10^5$ per 60 cm. The elution behaviour is discussed in terms of electrokinetic and micellar partition characteristics of the metal–par chelates. The chromatographic system is highly promising for the ultratrace determination of metals at the femtomole level in a very small sample injection volume (*ca.* 6 nl).

INTRODUCTION

Recently, micellar electrokinetic capillary chromatography (MECC) has been developed[1,2] and as found applications in the determination of phenols,[1,2] amino acids,[3,4] B-6 vitamers[5] and oligonucleotides[6] in very small samples. The separation principle and the theoretical basis have been given by Terabe *et al.*[2] and Sepaniak and co-workers[7,8]. When a high voltage is applied across a capillary, an aqueous solution is forced to flow toward the negative end by electroosmosis, while anionic micelles, *e.g.*, of sodium dodecylsulphate, in the solution are considerably retarded by the electrophoretic migration, thus acting as a dynamic stationary phase of colloidal dimensions. As this chromatography has very high efficiency, multi-element simultaneous detection was thought to be achievable. Of practical importance is the minimization of the contamination from metal contacts encountered in ordinary high-performance liquid chromatography (HPLC) (pump, column and tubing), because this capillary chromatography can be performed out of contact with metals except inert platinum electrodes. In addition, as the sample volume necessary for

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injection is very small (a few nanolitres)[2], the system is suitable for monitoring metal ions in clinical testing or quality control of electronics materials, where only limited amounts of samples are available.

In this paper, the MECC of metal chelates of the well known chromogenic reagent 4-(2-pyridylazo)resorcinol (par, H_2L) is described and the separation behaviour is discussed.

EXPERIMENTAL

Apparatus and reagents

A Horiba M-5 pH meter and a Hitachi Model 124 double-beam recording spectrophotometer were used. The chromtographic system consisted of a Shimadzu Isotachophoresis IP-2A constant-current supply with platinum electrodes and a JASCO Uvidec 100-IV visible spectrophotometric detector. A fused-silica glass capillary (850 mm × 0.05 mm I.D.) was obtained from Scientific Glass Engineering. On-column absorbance detection was employed; the width of the slit made of aluminium foil was matched to the inner diameter of the capillary, and the height of the slit was 1 mm. This slit was attached 150 mm from the negative end of the tube. The chromatographic data were processed with a Shimadzu Chromatopak C-R3A LC data processor.

Sodium dodecylsulphate (SDS) was obtained from Wako and par from Dojindo Labs. was dissolved in a slightly alkaline solution. All other reagents were of analytical-reagent grade.

Procedures

The sample solutions were prepared by mixing the appropriate solutions of metal ions with the par solution, followed by additions of SDS and buffer solutions to give the same bulk composition as that of the eluent to be used. A stock chromium (III)–par chelate solution was prepared as reported previously[9].

All procedures in the MECC were carried out in a similar manner to those reported by Terabe and co-workers[1,2]. The capillary tube was filled with a buffered SDS eluent and each end was immersed in the same eluent solution. A small amount of par ($1 \cdot 10^{-4}$ mol dm^{-3}) was added to the eluent to prevent the dissociation of relatively unstable chelates such as those of Zn(II), Cd(II) and Cu(II) during the separation. The applied voltage and the driving current are given in the captions of the figures. The sample solution was injected into the capillary by syphonic action. The sample solution was introduced 3 mm from the positive end (*ca.* 6 nl). The temperature of the system was kept at 25 ± 1°C in a safety box with an interlock system.

RESULTS AND DISCUSSION

A typical chromatogram of Co(III), Cr(III). Fe(III) and Ni(II) chelates and the retention data are shown in Fig. 1 and Table I, respectively. The concentration of each metal ion was $1 \cdot 10^{-5}$ mol dm^{-3}. As can be seen from Fig. 1, very sharp peaks are produced within an acceptable total elution time. The theoretical plate numbers of the chelates were in the range 105 000–120 000 per 60 cm. The surprisingly high efficiency of the MECC was also confirmed for the chelate compounds. The elution order of the

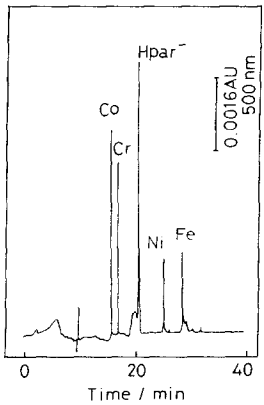

Fig. 1. MECC separation of metal–par chelates. Eluent: [SDS] = 0.02 mol dm^{-3}, [NaH_2PO_4] = 0.05 mol dm^{-3}, [$Na_2B_4O_7$] = 0.0125 mol dm^{-3}, [par] = $1.0 \cdot 10^{-4}$ mol dm^{-3}. Applied voltage: 16.5 kV (25 μA). Complexation conditions; [par] = $1.0 \cdot 10^{-3}$ mol dm^{-3}; [metal ion]/(10^{-5} mol dm^{-3}), Co(II) 0.995, Cr(III) 1.23, Fe(III) 1.00, Ni(II) 1.01; [triethanolamine] = 0.025 mol dm^{-3} (pH = 8.8 with HCl), heated at 98°C for 30 min.

chelates is $[CoL_2]^-$, $[CrL_2]^-$, $[NiL_2]^{2-}$ and $[FeL_2]^-$ under the conditions used. It should be noted that the complete separation of Co(III) and Cr(III) chelates is readily achieved, whereas this was a serious problem in ion-pair HPLC on an ODS stationary phase[9]. Other cations that form unstable par chelates, such as Mn(II)[10] and alkaline earth metal ions, gave no peaks under the conditions employed.

The retention of the chelates varies with the eluent pH, as shown in Fig. 2, owing to the acid dissociation equilibria of the par chelates (1-hydroxy groups of par ligands):

$$[M^{n+}(HL)_2]^{(n-2)+} \overset{pK_{a_1}}{\rightleftharpoons} [M^{n+}(HL)L]^{(n-3)+} \overset{pK_{a_2}}{\rightleftharpoons} [M^{n+}L_2]^{(n-4)+}$$

Under the pH conditions tested, the Co(III) and Cr(III) chelates are fully deprotonated species, $[ML_2]^-$ [the pK_{a_2} values are 4.41 and 4.35 for the Co(III)[11] and Cr(III)[9] chelates, respectively]. The acid dissociation equilibria of the Ni(II) chelate (pK_{a_1} = 6.2 and pK_{a_2} = 7.1)[12] obviously cause a decrease in the k' value in the pH range 5–8. The

TABLE I

CAPACITY FACTORS (k') AND THEORETICAL PLATE NUMBERS (N) OF par (HL^- FORM) AND THE METAL CHELATES ($[ML_2]^{n-}$)[a]

Solute	*k'*	*N (per 60 cm)*
$[Co(III)L_2]^-$	0.79	114000
$[Cr(III)L_2]^-$	0.99	108000
HL^-	1.61	65000
$[Ni(II)L_2]^{2-}$	2.54	120000
$[Fe(III)L_2]^-$ [b]	3.38	105000

[a] Chromatographic conditions as in Fig. 1.
[b] Injected form. Possibly reduced to $[Fe(II)L_2]^{2-}$.

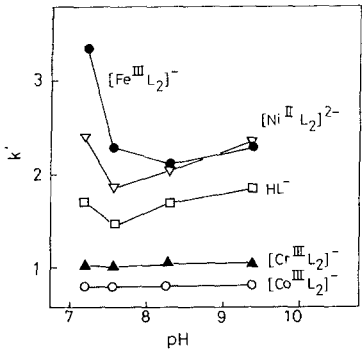

Fig. 2. Capacity factor *vs.* eluent pH plots for par and the chelates. The eluent pH was adjusted by mixing appropriate phosphate and borate buffer solutions to give a constant ionic strength. Other conditions as in Fig. 1. Temperature: 25 ± 1°C.

possibility of reduction of the Fe(III)–par chelates to the Fe(II) form, $[FeL_2]^{2-}$, during the electrokinetic separation was suggested as the elution time of the iron chelate is very close to those of the other divalent metal chelates (Ni, Cu and Zn) (see Fig. 3 and Table I). In this work, slightly alkaline conditions (pH = 9.2, 0.05 mol dm^{-3} sodium acetate, 0.01 mol dm^{-3} disodium tetraborate), where the 1-hydroxy groups of the chelates are fully dissociated,[12] were employed for the quantitative elution studies.

Several chromatograms of metal–par chelates at different concentrations of SDS (0, 0.05, 0.10 and 0.15 mol dm^{-3}) in the eluent are shown in Fig. 3. Under these conditions, the peaks of the Co(III), Fe(III), Ni(II), Zn(II) and Cd(II) chelates are detected. As shown in Fig. 3, the SDS micellar concentration in the eluent significantly influences the order of elution of the chelates. With no added SDS, *i.e.*, running the system in the electrophoresis mode, the resolution of the chelates is unsatisfactory. These observations clearly indicate that a partition process of the chelates between the bulk aqueous eluent and the micellar pseudo-stationary phase is vital for the separation. It is interesting that the separation of the anions can be accomplished very effectively using the SDS micelles of the same charge type. This may indicate that

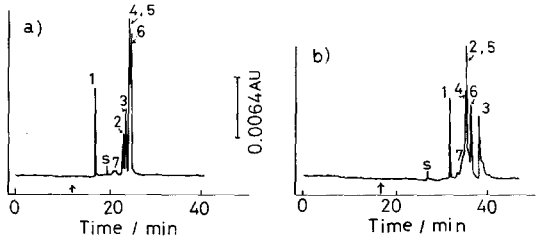

Fig. 3. Chromatograms of metal–par chelates at various concentrations of SDS present in the eluent. Peaks: (1) Co(III); (2) Cu(II); (3) Cd(II); (4) Ni(II); (5) Fe(III); (6) Zn(II); (7) HL^-; (s) system peak; arrow indiates solvent front position. Eluent: $[NaOOCCH_3]$ = 0.05 mol dm^{-3}, $[Na_2B_4O_7]$ = 0.01 mol dm^{-3}, [par] = $1 \cdot 10^{-4}$ mol dm^{-3}; [SDS], (a) none at 14.5 kV (25 μA), (b) 0.05 mol dm^{-3} at 12.6 kV (25 μA), (c) 0.10 mol dm^{-3} at 12.2 kV (30 μA), (d) 0.15 mol dm^{-3} at 13.0 kV (40 μA). Temperature: 25 ± 1°C. Complexation conditions: [par] = $1 \cdot 10^{-3}$ mol dm^{-3}, [metal ion] = $(3.6\text{–}4.4) \cdot 10^{-5}$ mol dm^{-3}, $[NaOOCCH_3]$ = 0.05 mol dm^{-3}, $[Na_2B_4O_7]$ = 0.01 mol dm^{-3}; pH = 9.2.

a relatively weak interaction of the solutes with the SDS micellar pseudo-phase is operative in the delicate MECC resolution. Presumably, the tight binding of solutes to the micelles via strong hydrophobic or electrostatic interactions, although it provides a greater retention, is unfavourable for the mutual resolution. The results in Fig. 3 suggest that in addition to the chromatographic partitioning into the pseudo-stationary phase, the concomitant electrophoretic retardation of the anions possibly plays a subtle role in the peak resolution.

The capacity factors (k'), a measure of the partition of the solutes into the micellar phase, are derived from the retention data as follows[2]: for neutral species,

$$k' = \frac{t_R - t_0}{t_0(1 - t_R/t_{mc})} = \frac{(1/v_R - 1/v_0)v_0}{(1 - v_{mc}/v_R)} \quad (1)$$

where t_0, t_R and t_{mc} are the elution times of the solvent, the solute and the micelle-bound reference solute (orange OT dye was employed), respectively, and v_0, v_R and v_{mc} are the electroosmotic flow velocity of the bulk eluent and the elution velocity of the solute and of the micelles, respectively. For anionic species, the electrophoretic velocity, v_{ep}, must be considered. Using the apparent elution velocity, v_{app}, eqn. 1 is rewritten as

$$k' = \frac{[1/(v_{app} - v_{ep}) - 1/v_0]v_0}{1 - v_{mc}/(v_{app} - v_{ep})} \quad (2)$$

As the values of v_{ep} are dependent on the potential gradient, the approximate values are estimated from the calibration graphs of applied voltage *versus* v_{ep} in the absence of SDS.

The k' values can be related to the aqueous–SDS micellar partition constant (K_d) via

$$k' = K_d V(C - \text{cmc}) \quad (3)$$

where V is the partial molar volume of the SDS micelle (0.26 $dm^3\ mol^{-1}$) [13], C is the total concentrations of SDS and cmc is the critical micellar concentration of SDS (= 0.003 mol dm^{-3}) under the conditions used[14]. The values for k' and K_d of $[CoL_2]^-$, $[FeL_2]^-$, $[NiL_2]^{2-}$ and HL^- at eluent SDS concentrations of 0.10 and 0.15 mol dm^{-3} are shown in Table II. The K_d values in Table II for each compound at the two SDS concentrations are considered to be substantially equal because of some errors involved in the v_{ep} values. In addition, a rise in temperature due to Joule heating possibly affects the results unless the voltage–current conditions are identical.

The line-shaped peaks shown in Fig. 1 provide a remarkably increased sensitivity on a mass basis. The noise level of the detection system is about $2 \cdot 10^{-4}$ absorbance at 500 nm. From the peak height of the Cr–par chelates in Fig. 1, the concentration that gives a signal three times the noise level is calculated to be $2.3 \cdot 10^{-7}$ mol dm^{-3}. This molar concentration corresponds to an absolute amount of $1.4 \cdot 10^{-15}$ mol ($7.2 \cdot 10^{-14}$ g Cr) in a 6-nl injection. This absolute detection limit (calculated) is about 700 times lowers than that obtained in ion-pair HPLC (1.0 pmol in a 100-μl injection)[9].

TABLE II
CAPACITY FACTORS (k') AND PARTITION CONSTANTS (K_d) OF METAL–par CHELATES[a]

Solute	*[SDS] (mol dm^{-3})*			
	0.10 (30 μA, 12.2 kV)		*0.15 (40 μA, 13.0 kV)*	
	k'	*Log* K_d	k'	*Log* K_d
$[Co(III)L_2]^-$	0.555	1.34	0.919	1.38
$[Fe(III)L_2]^-$[b]	0.088	0.55	0.183	0.68
$[Ni(II)L_2]^{2-}$	0.072	0.45	0.150	0.59
HL^-	0.190	0.88	0.361	0.98

[a] Other chromatographic conditions as in Fig. 3.
[b] Injected form. Possibly reduced to $[Fe(II)L_2]^{2-}$.

TABLE III
ELUTION TIMES (min) OF par AND THE CHELATE SPECIES IN MECC[a]

Species	*[SDS] (mol dm^{-3})*			
	0 (14.5 kV, 25 μA)	*0.05 (12.6 kV, 25 μA)*	*0.10 (12.2 kV, 30 μA)*	*0.15 (13.0 kV, 40 μA)*
$[Co(III)L_2]^-$	17.2	32.3	44.0	57.7
$[Fe(III)L_2]^-$[b]	24.5	35.7	39.9	40.8
$[Ni(II)L_2]^{2-}$	24.7	35.1	39.5	39.5
$[Cu(II)L_2]^{2-}$	23.7	35.7	42.0	43.5
$[Zn(II)L_2]^{2-}$	25.0	36.5	41.4	42.8
$[Cd(II)L_2]^{2-}$	24.4	39.4	47.8	52.0
HL^-	21.9	34.2	42.6	45.3
Orange OT		111.3	169.0	216.6

[a] Other conditions as in Fig. 3.
[b] Injected form. Possibly reduced to $[Fe(II)L_2]^{2-}$.

MECC seems a fairly promising approach for monitoring metal ions *in vivo* and *in vitro* and in sophisticated industrial analyses where the absolutely small amounts rather than the low concentrations are to be determined. Investigations are in progress on the application of MECC using chelating reagents such as azo dyes, hydrazones and porphyrins.

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