



ELSEVIER

Journal of Chromatography A, 772 (1997) 339–346

JOURNAL OF
CHROMATOGRAPHY A

Influence of cationic polymers on separation selectivity in kinetic differentiation mode capillary electrophoresis of metal–4-(2-pyridylazo)resorcinolato chelates

O.V. Krokhin^{a,*}, H. Hoshino^b, O.A. Shpigun^a, T. Yotsuyanagi^b

^aChemistry Department, Lomonosov Moscow State University, 119899 Moscow, Russia

^bDepartment of Applied Chemistry, Graduate School of Engineering, Tohoku University, Aoba-ku, Sendai 980-77, Japan

Abstract

The influence of four kinds of cationic polymers on electrophoretic mobilities of V(V), Co(II), Ni(II), and Fe(II)–4-(2-pyridylazo)resorcinolato chelates was investigated in kinetic differentiation mode capillary electrophoresis (CE). It was shown that the addition of cationic polymers strongly changes selectivity of such a type of separation. Four parameters of carrier electrolyte (pH, counter-ion, polymer and acetonitrile concentrations) were changed to examine the elution order of the complexes. The behaviour of the chelates can be described in terms of ion-exchange separation. Fast separation of the four chelates with efficiency of more than 450 000 theoretical plates per meter in CE with reversed polarity power supply was easily performed.

Keywords: Buffer composition; 4-(2-Pyridylazo)resorcinol complexes; Metal chelates

1. Introduction

The behaviour of metal complexes in HPLC (CE) systems depends on their kinetic properties. Only kinetically stable chelates can be separated and detected in the absence of ligand in the eluent (carrier electrolyte solution). This separation manner—kinetic differentiation (KD) mode capillary electrophoresis—is the most attractive technique for CE analysis of metal ions. First, the sensitivity of direct spectrophotometric detection is greater than in indirect UV and non-KD modes of metal chelate detection [1]. Secondly, it is possible to vary selectivity both by precolumn complexation conditions and by carrier electrolyte compositions. Precolumn complexation permits to eliminate interferences from complex sample matrix [2]. Metal–4-(2-pyridyl-

azo)resorcinolato (PAR) chelates were separated in both the KD and non-KD mode CE. Different parameters of carrier electrolyte—sodium dodecyl sulfate (SDS) concentration [1], type of counterion [3] and pH [1,4]—were used for selectivity control.

Traditionally, the separation of metal chelates by CE has been performed with a positive polarity power supply. Nevertheless, more convenient (from the viewpoint of an increase in analysis speed) is reversed polarity, because PAR chelates exist as anions under alkaline conditions. A wide range of cationic surfactants has been used for reversing the electroosmotic flow and changing in the separation selectivity of small anionic species [5,6]. High-molecular-mass cationic polymers have also been used for this purpose. Successful separation of small organic and inorganic anions using cationic polymers for electroosmotic flow and selectivity control was demonstrated in the past decade [7–11]. Polymeric

*Corresponding author.

capillary modifiers provide stable electroosmotic flow and high separation efficiencies for inorganic anions [10]. These systems have many analogies with ion chromatographic (IC) separation of anions (the term 'ion-exchange electrokinetic chromatography' was introduced by Terabe and Isemura [7]), except one evident advantage: high solubility of cationic polymers allows separation media with a wide range of capacities to be obtained, in comparison with the complex (micelle forming) systems of cationic surfactants.

The separation of metal chelates has been made using differences in the mobility of complexes [4,12], in their affinity to SDS micelles [1,3], and in ion-association constants with short chain quaternary ammonium ions [13]. This investigation is the first attempt to use the interaction between metal complexes and cationic polymers for this purpose. The aim of this work is to examine the possibility of the metal-PAR chelate separation with reversed polarity power supply using four kinds of cationic polymers for selectivity variation. As a test mixture metal-PAR complexes of V(V), Co(II), Ni(II), and Fe(II) were chosen. The effect of pH, concentration of counterion, polymeric additive, and acetonitrile content were examined for control of the separation selectivity.

2. Experimental

2.1. Instrumentation

Two types of CE instrument were used. The first consisted of a high-voltage power supply (Matsusada, Japan) and a variable wavelength UV-Vis detector for capillary electrophoresis (Jasco-CE 970, Japan). Fused-silica capillaries (GL Science, Japan) were 60 cm long (46 cm to detector) \times 375 μ m O.D. \times 50 μ m I.D. Zones of metal-PAR complexes were detected in capillary mode (490 nm). A sample solution was introduced manually by hydrostatic injection (13 cm, 15 s). A Chromatopac-CR5A (Shimadzu, Japan) was used for electropherograms recording. The second instrument was a Bio-Focus 3000 (Bio-Rad, USA). The same type of capillary (51 cm, 46.4 cm to detector) was used with a User Assembled Cartridge Kit. Detection wavelength was

490 nm. All the pH measurements were performed with a Horiba, M-13 pH meter (Horiba, Japan).

2.2. Reagents and procedures

The polymer solutions: Polybrene, poly-(diallyldimethylammonium chloride) (PDADMACI, Aldrich, Milwaukee, WI, USA), polyethyleneimine (PEI, Wako, Japan) and poly-(N-ethyl-4-vinylpyridinium bromide) (PVPyBr, High-molecular-mass compound division of Moscow State University) were prepared by dilution (dissolving) of the initial products. All the polymers were used as received and not converted to other ionic form. Sodium sulfate, hydrogen- and dihydrogenphosphates, Na₂EDTA, and acetonitrile (all reagent grade) were used for electrolyte solution preparations. Carrier electrolytes were filtered through a 0.45 μ m membrane filter (Advantec, Tokyo, Japan) and degassed. Non-modified capillaries were purged with: water (5 min), 0.1 M NaOH (10 min), water (10 min) and a carrier electrolyte solution (30 min). Between each run the capillary was washed with the electrolyte solution (1 min). Sample solutions were prepared in the following manner: the samples of metal stock solutions were added to 1 mM PAR in a slightly alkaline solution and heated at 60°C for 15 min. Doubly distilled water (Still-N2, Iwaki, Japan) was used to prepare all the solutions.

2.3. Calculations

The electroosmotic flow (EOF) was determined from the equation $\mu_{eos} = v_{eos}/E$, where v_{eos} is a migration speed of the bulk electrolyte solution (acetone added to sample, cm/s) and E is a field strength (kV/cm). Electroosmotic mobility was arbitrary chosen as positive when EOF reversed. The electrophoretic mobilities (μ_{ep}) of analytes were calculated from the expression $\mu_{ep} = (v_{mig}/E) - \mu_{eos}$, where v_{mig} is a migration speed of an analyte. The separation efficiency (theoretical plate per meter) was calculated as: $n = 16(t_{mig}/W)^2(100/l)$, where t_{mig} , W , and l are component migration time (min), peak width on a baseline (min), and working length of capillary (cm), respectively.

3. Results and discussion

3.1. The choice of carrier electrolyte composition

The use of cationic polymers can be useful for metal–PAR chelate separation in the KD mode, because the reversed electroosmotic flow might provide a high speed of analysis. We supposed initially that the behaviour of complexes will obey the laws of ion-exchange separation. In this case, the separation selectivity will be mainly determined by an affinity of analytes to polymeric chains and a choice of a counterion (eluting anion in IC transcription) is most important. The requirements for eluting anion in IC are as follows: it must easily displace analytes on ion-exchange sites and provide a possibility for a high detection sensitivity [14]. The metal–PAR chelates exist as singly and doubly charged anions under slightly alkaline conditions [4]. Large aromatic moiety of these ions must provide a strong hydrophobic interaction with polymers. Consequently, these anions can be referred to the strongly-retained type. In IC methods for strongly retained anions determination has two different approaches. The first one uses concentrated eluents or doubly/triply charged hydrophobic anions as eluent components [15]. The second approach employs special ion-exchange resins with low affinity to hydrophobic anions [16]. Direct detection of metal–PAR chelates does not provide particular restrictions for spectral characteristics of eluting anions. Sulfate ion was chosen because of its high elution strength and absence of specific absorbance in a visible range.

Fig. 1 shows the structure of polyelectrolytes used

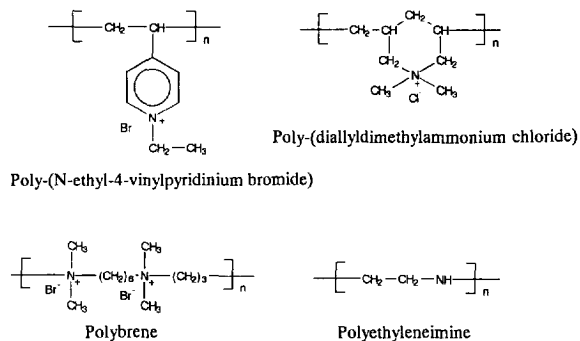


Fig. 1. The chemical structure of used polymers.

as modifiers. It was supposed that the presence of slightly retained anions (Cl^- , Br^-) in the carrier electrolyte will not influence on the selectivity of chelates separation. The test carrier electrolyte system was chosen as follows: 20 mM Na_2SO_4 , a 4 mM phosphate buffer (for pH adjustment) pH 8.0, 5.35 mM (functional group) of polymers, 0% acetonitrile, and 1 mM Na_2EDTA . The latter is usually added to carrier electrolytes (eluent) during CE (HPLC) separations of metal chelates, for the elimination of heavy metals impurities influence [17]. The possible elution orders for HL^{1-} , VO_2L^{1-} , CoL_2^{1-} , FeL_2^{2-} , NiL_2^{2-} , and reversed electroosmotic flow shown in Fig. 2A–C. Fig. 2A is a case of absence of polymer–analyte interaction (elution order determined by own chelates mobilities), or very high elution power of the carrier electrolyte; Fig. 2B shows strong polymer–chelate interaction (low elution strength). In this case, analytes will migrate to anode with the rate of the electroosmotic flow (if we suppose that the own mobility of large polymeric molecule is close to zero). The most interesting case is Fig. 2C, with

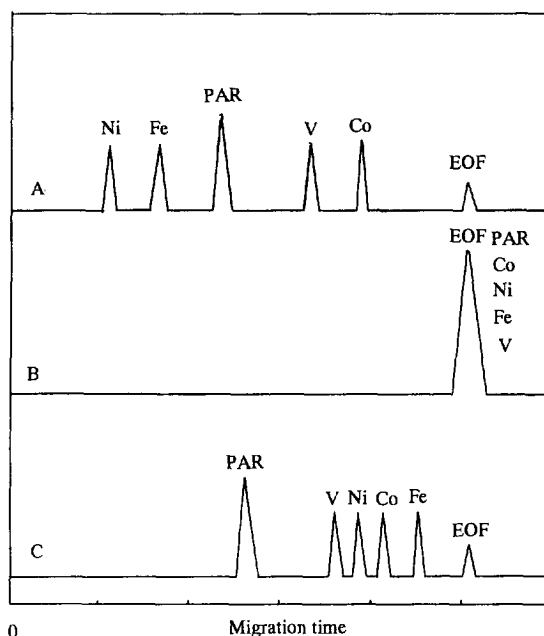


Fig. 2. Possible elution order for the systems with polymer–chelate interactions and reversed electroosmotic flow. A, without chelate–polymer interaction; B, very strong interactions; C, medium conditions.

intermediate affinities, which will allow us to manipulate separation selectivity.

In Fig. 3, the values of μ_{eos} and μ_{ep} of metal–PAR complexes for the test electrolyte composition are presented. In each case changes of own analyte mobilities (100% values were calculated for non-modified conditions, pH 8.0 [4]) were different. Thus, PEI provide the weakest decrease in μ_{ep} — only 9.5% (average magnitude for four complexes). The next follows Polybrene, 62.5%, PDADMACI, 73.4%, and PVPyBr, 82.2%. These data are in accordance with previous investigation of modified silica for IC separation of anions [16]. It was found that 2,5-Ionene (Polybrene type) has a low affinity to strongly retained on an ion-exchange resins hydrophobic anions (I^- , SCN^- , and ClO_4^-). It is clear that the differences in affinity of chelates (not only with PAR) to the polymers can be used for a choice of suitable modifiers for the definite type of separation. Thus, for system with a poor selectivity (chelates

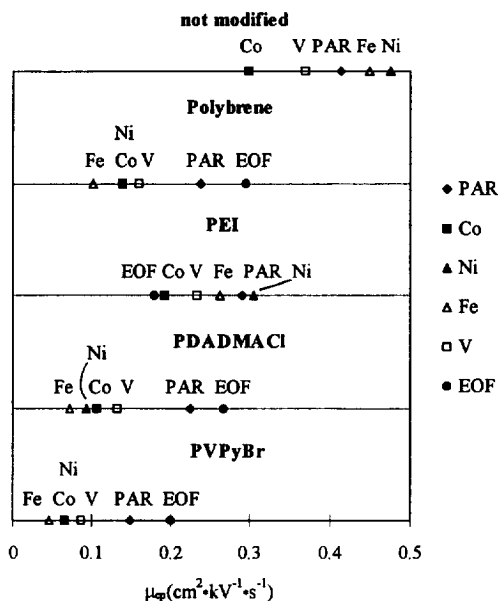


Fig. 3. Electrophoretic mobilities of metal–PAR chelates for four kinds of polymers under the same carrier electrolyte condition. Capillary: 60 cm (46 cm effective length) \times 50 μm I.D.; buffer: 20 mM Na_2SO_4 , 4 mM Sodium phosphate buffer pH 8.0, 1 mM EDTA, 5.35 mM of polymers; sampling: gravity 15 s, 13 cm; conditions: -20 kV; detection: Vis 490 nm; sample: $[\text{PAR}]_{\text{T}} = 1$ mM, $[\text{M}]_{\text{T}} = 0.01$ mM. Mobilities arbitrary chosen as positive; unmodified fused-silica conditions, phosphate–borate buffer, pH 8.0 [4].

with close mobilities) PVPyBr is preferable. And, in contrary, in the case of good selectivity, the best selection is Polybrene because it provides the highest speed of analysis. The order of an polymer–analyte as affinities increase is as follows: CoL_2^{1-} (49.1% of μ_{ep} decrease, average for four polymers) $<$ VO_2L^{1-} (51.1%) $<$ NiL_2^{2-} (63.4%) $<$ FeL_2^{2-} (67.4%). It confirms our assumption about an ion-exchange mechanism of the separation because the retention of an analyte in IC increases with their charge number.

The values of EOF decrease in the order Polybrene $>$ PDADMACI $>$ PVPyBr $>$ PEI. Cationic polymers are fixed on silica surface by electrostatic interaction and provide reversed electroosmotic flow. A change in EOF rate for different polymers cannot be explained only in terms of charge density in the polymeric chain because all the modifiers give different viscosity of the carrier electrolyte solution. But it is evident that PEI must provide the smallest EOF because it is the only weak base among all the list of modifiers.

3.2. The pH influence

As it can be seen from Table 1 [18], it is impossible to share out a single cause of analytes μ_{ep} changing under different pH conditions, because it will influence on the own mobilities and on the analytes–polymer interactions. Fig. 4 presents the pH dependences of μ_{ep} and μ_{eos} for Polybrene-containing carrier electrolyte. A change in migration times for PDADMACI and PVPyBr is similar and can be explained by the two different factors. The first one is decreasing in the analyte charge (μ_{ep}) for Fe–PAR and Ni–PAR complexes with pH decreasing ($\text{p}K_{\text{a}2}$ 7.66 and 7.1, respectively). The second is a decrease in the carrier electrolyte elution strength, since $\alpha\text{H}_2\text{PO}_4^{2-}$ (molar fraction of dihydrophosphate

Table 1
Acid dissociation constants for species in separation system [18]

| Species | $\text{p}K_{\text{a}1}$ | $\text{p}K_{\text{a}2}$ |
|--|-------------------------|-------------------------|
| PAR, H_2L | 5.43 | 12.13 |
| $[\text{Co}^{\text{III}}(\text{HL})_2]^+$ | 3.64 | 4.41 |
| $[\text{V}^{\text{V}}\text{O}_2(\text{HL})]$ | 4.40 | |
| $[\text{Fe}^{\text{II}}(\text{HL})_2]$ | 6.47 | 7.66 |
| $[\text{Ni}^{\text{II}}(\text{HL})_2]$ | 6.2 | 7.1 |
| H_3PO_4 | 2.15 | 7.2 |

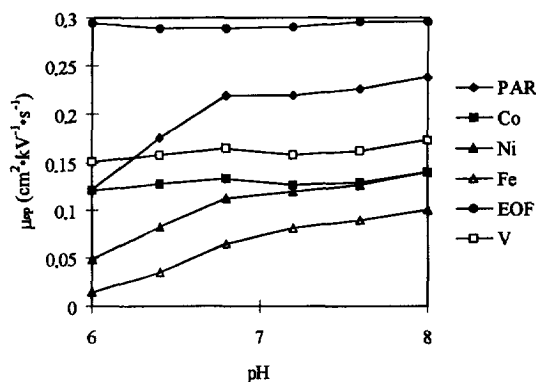


Fig. 4. The dependence of electrophoretic mobilities of metal-PAR chelates vs. pH of carrier electrolyte for Polybrene. 4 mM Sodium phosphate buffer with different pH, other conditions see Fig. 3.

anion) also depends on pH. The basic equation of IC gives the dependence of capacity factors from eluent concentration:

$$\log k' = (a/b) \log C - (a/b) \log E + \text{Constant}; \quad (1)$$

where a , b , C , and E are charges of the analyte, eluting anions, column capacity, and eluent concentration, respectively [14]. In our case, an increase in capacity factors (chelate-polymer interaction) leads to a decrease in μ_{ep} of complexes. Consequently, the second reason acts on singly and doubly charged anions in a different manner because of differences in a/b ratio. In Fig. 4, μ_{ep} of VO_2L^{1-} and CoL_2^{1-} decrease more slowly (second reason) under lowering pH, than mobilities of FeL_2^{2-} and NiL_2^{2-} (both reasons together). The μ_{eos} does not change dramatically for all the polymers in pH range of 6–8.

3.3. The influence of counterion concentration

Sulfate anion provides effective elution of metal-PAR chelates in these anion-exchange systems. Like IC eluent concentration dependence, the slope of analyte mobilities vs. the counterion concentration must depend on the charges of the analytes and eluting anion as mentioned above. Fig. 5 indicates that this model produces quite a strict description of the order of SO_4^{2-} influence: $\text{NiL}_2^{2-} (pK_{a_2} = 7.1) > \text{FeL}_2^{2-} (pK_{a_2} = 7.66) > \text{VO}_2\text{L}^{1-} > \text{CoL}_2^{1-} > \text{HL}^{1-}$. The

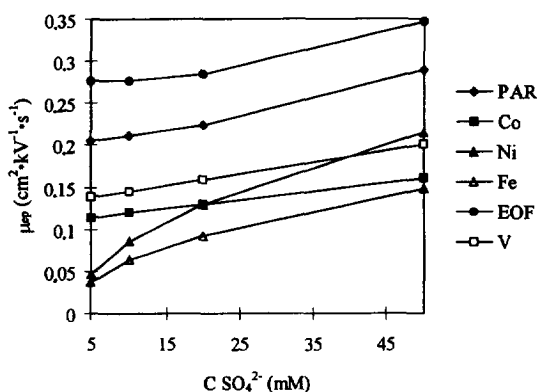


Fig. 5. The dependence of electrophoretic mobilities of metal-PAR chelates vs. SO_4^{2-} concentration in carrier electrolyte for Polybrene containing carrier electrolyte. x mM Na_2SO_4 , other conditions see Fig. 3.

character of the dependences for PDADMACI and PVPyBr is equivalent. However, in the case of PEI, a change in selectivity is minimum due to the weakest ion-exchange interaction. EOF increases with Na_2SO_4 concentration for all the polymers as we expected.

3.4. The polymer concentration influence

The most attractive feature of this system is the possibility to change capacity of separation media. Fig. 6 shows the dependences of metal-PAR chelates mobilities vs. Polybrene concentration in the carrier electrolyte. According to Eq. (1), the influence of the

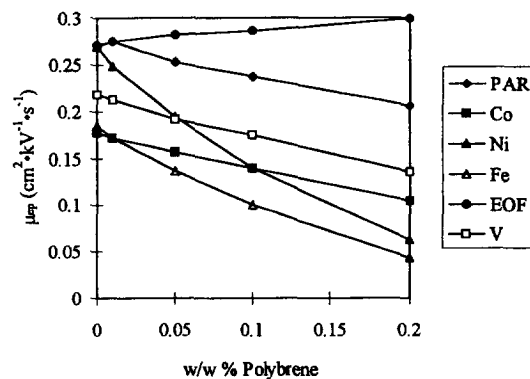


Fig. 6. The dependence of electrophoretic mobilities of metal-PAR chelates vs. Polybrene concentration in carrier electrolyte. $x\%$ (w/w) of Polybrene, other conditions see Fig. 3.

system capacity is equivalent to the eluent (counterion) concentration, but with opposite sign. Thus, the order of mobilities decreasing with the growth of capacity is: $\text{NiL}_2^{2-} > \text{FeL}_2^{2-} > \text{VO}_2\text{L}^{1-} > \text{CoL}_2^{1-} > \text{HL}^{1-}$. Only EOF has the same sign of changing.

3.5. The influence of organic modifier (acetonitrile)

The addition of acetonitrile to carrier electrolyte decreases the electroosmotic flow and, as it can be seen from Fig. 7, does not practically change the separation selectivity. But it is interesting that for polymers having relatively weak interaction (PEI, Polybrene) with the complexes, the mobilities decreased by an acetonitrile concentration increase, and inversely increase for PDADMACI and PVPyBr. Unfortunately, we do not have any information about

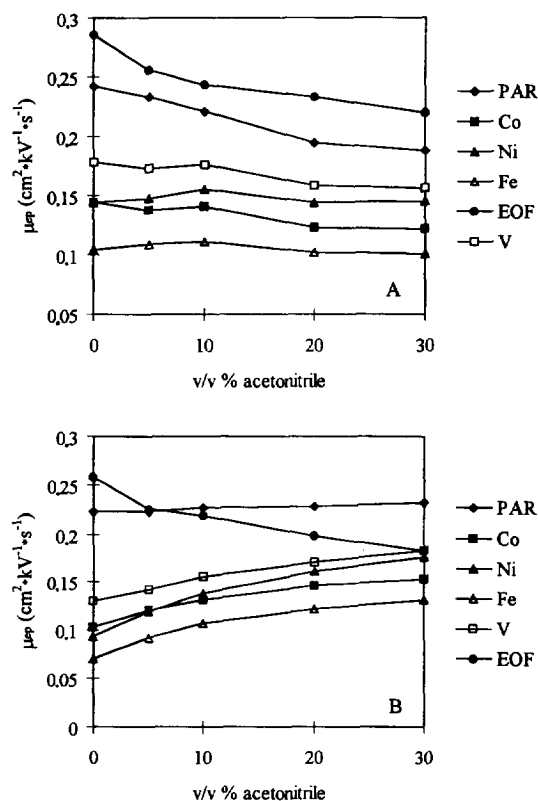


Fig. 7. The dependence of electrophoretic mobilities of metal-PAR chelates vs. acetonitrile concentration in carrier electrolyte. Acetonitrile content of $x\%$ (v/v), other conditions see Fig. 3. (A) Polybrene, (B) PDADMACI.

acetonitrile–chelate association constants; however, it is a common knowledge that the mobilities must decrease under the association conditions. This situation takes place in the case of Polybrene and PEI. PDADMACI and PVPyBr provide strong interaction (ion-exchange and hydrophobic); however acetonitrile addition disturb it so that mobilities increase. A PEI containing carrier electrolyte solution forms a double-phase system under acetonitrile concentration more than 10% (v/v).

3.6. Separation efficiency

CE separation technique easily provides a high separation efficiency. For metal-PAR chelates this value is 240 000–425 000 (55 cm effective length, free zone electrophoresis) [4] and 105 000–120 000 (60 cm effective length, micellar electrokinetic capillary chromatography with SDS addition) [1]. Probably, the appearance of additional interaction mechanisms decreases separation efficiency. Table 2 contains theoretical plate number for all the polymers studied with the test electrolyte composition. For this experiment, we used the same (6.0 nl) sample volume as in [1,4], which was determined by the continuous injection method. The other experimental sets indicated that separation efficiency slightly decreased with a polymer and acetonitrile addition and increased with the increment of SO_4^{2-} concentration. The pH conditions have practically no influence in the range of pH 6–8.

3.7. Migration times, analytical signal reproducibility

Typical electropherograms of metal-PAR chelates are shown in Fig. 8. Polybrene-containing carrier electrolyte was chosen as a test system because of the minimum separation time with the high ef-

Table 2

Separation efficiency for studied polymers (average magnitude for four metal-PAR complexes, theoretical plates) in the conditions of test carrier electrolyte composition

| | Polybrene | PDADMACI | PVPyBr | PEI |
|-----|-----------|----------|---------|---------|
| n | 195 000 | 213 000 | 215 000 | 281 000 |

Effective capillary length 46 cm, other conditions see Fig. 3

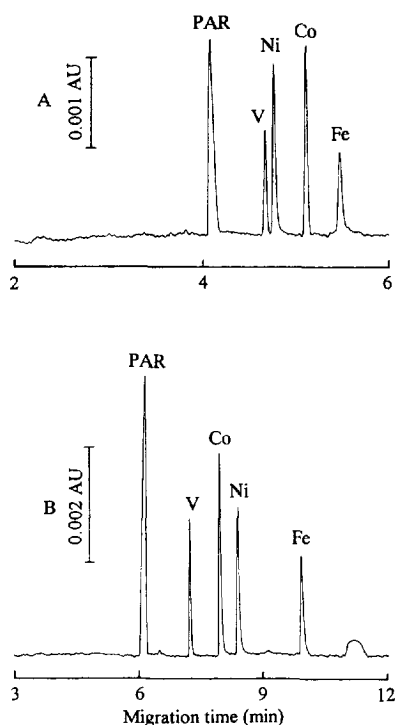


Fig. 8. The typical examples of metal-PAR chelate separations. Capillary: 50 cm (45.5 cm effective length) \times 50 μ m I.D.; buffer: 20 mM Na_2SO_4 , 4 mM sodium phosphate buffer pH 8.0, 1 mM EDTA; condition: -20 kV; detection: Vis 490 nm; sample: $[\text{PAR}]_T = 1$ mM, $[\text{M}]_T = 0.01$ mM, $[\text{V}] = 0.02$ mM. (A) Polybrene (3.2 mM), sampling: pressure 3 p.s.i.·s. (B) PVPyBr (5.35 mM), sampling pressure 10 p.s.i. s (1 p.s.i. = 6894.76 Pa).

iciency. The reproducibility data for retention time, area, and height of Co-PAR chelate peak are summarized in Table 3. Detection limit (at $S/N=3$) was found to be 0.6 μ M (6 nl injection). The plot of analytical signal (peak height) vs. Co(II) concentration indicates linearity in the range 1 to 20 μ M (5 points).

Optimization of carrier electrolyte composition

Table 3
The relative standard deviations (S_r) of retention time, area and peak height for CoPAR_2^{1-} and Polybrene-containing carrier electrolyte (S_r , $n = 10$)

| | Retention time (s) | Peak area ($\text{mV}\cdot\text{s}$) | Peak height (mV) |
|-------|--------------------|--|------------------|
| S_r | 0.042 | 0.0089 | 0.004 |

Conditions see Fig. 3

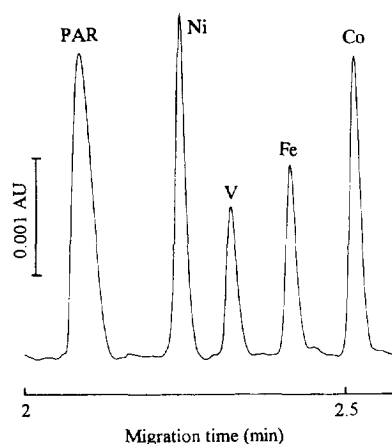


Fig. 9. Fast separation of metal-PAR chelates. Capillary: 50 cm (45.5 cm effective length) \times 50 μ m I.D.; buffer: 20 mM Na_2SO_4 , 10 mM NaClO_4 , 4 mM sodium phosphate buffer pH 8.0, 2.7 mM Polybrene; condition: -30 kV; detection: Vis 490 nm; sample: $[\text{PAR}]_T = 1$ mM, $[\text{M}]_T = 0.01$ mM, $[\text{V}] = 0.02$ mM; sampling: pressure 3 p.s.i.·s.

allows us to obtain fast (2.6 min) separation of the test chelates mixture (Fig. 9).

4. Conclusion

An ion-exchange electrokinetic chromatography mechanism was used for the separation of four kinetically stable metal-PAR chelates in KD mode CE. The use of cationic polymers provides stable reversed electroosmotic flow and makes possible the highly efficient separation of negatively charged complexes using negative polarity power supply. The affinity of the studied polymers to metal-PAR chelates decreases in the order: poly(N-ethyl-4-vinylpyridinium bromide) > poly(diallyldimethylammonium chloride) > Polybrene > polyethyleneimine. Such a system allows us to change the selectivity using a variety of the carrier electrolyte parameters (pH, counterion, polymer, and organic modifier concentration) for a definite type of separation.

Acknowledgments

The authors thank the Japan Society for the Promotion of Science for the financial support of

O.V.K. (ID No. PS95671) in his stay in Tohoku University for 16 March–12 June 1996.

References

- [1] T. Saitoh, H. Hoshino and T. Yotsuyanagi, *J. Chromatogr.*, 469 (1989) 175.
- [2] S. Motomizu, S. Nishimura, Y. Obata and H. Tanaka, *Anal. Sci.*, 7 (1991) 253.
- [3] A.R. Timerbaev, O.P. Semenova, P. Jandik and G.K. Bonn, *J. Chromatogr. A*, 671 (1995) 419.
- [4] N. Iki, H. Hoshino and T. Yotsuyanagi, *Chem. Lett.*, (1993) 701.
- [5] W.R. Jones and P. Jandik, *J. Chromatogr.*, 546 (1991) 445.
- [6] P.R. Haddad, A.H. Harakuwe and W. Buchberger, *J. Chromatogr. A*, 706 (1995) 571.
- [7] S. Terabe and T. Isemura, *Anal. Chem.*, 62 (1990), 650.
- [8] S. Terabe and T. Isemura, *J. Chromatogr.*, 515 (1990) 667.
- [9] G. Schomburg, D. Belder, M. Gilges and S. Motsch, *J. Cap. Electrophoresis*, 1 (1994) 219.
- [10] C. Stathakis and R.M. Cassidy, *Anal. Chem.*, 66 (1994), 2110.
- [11] C. Stathakis and R.M. Cassidy, *J. Chromatogr. A*, 699 (1995) 353.
- [12] F.B. Regan, M.P. Meaney and S.M. Lunte, *J. Chromatogr. B*, 675 (1994) 409.
- [13] N. Iki, H. Hoshino and T. Yotsuyanagi, *J. Chromatogr. A*, 652 (1993) 539.
- [14] D.T. Gjerde and J.S. Fritz, *Ion Chromatography*, Hüthig, Heidelberg, 1987, pp. 73, 107.
- [15] O.V. Krokhin, O.N. Obrezkov and O.A. Shpigun, *Zh. Anal. Khim.*, 48 (1993) 1180.
- [16] O.V. Krokhin, A.D. Smolenkov, N.V. Svintsova, O.N. Obrezkov and O.A. Shpigun, *J. Chromatogr. A*, 706 (1995) 93.
- [17] M. Macka, P.R. Haddad and W. Buchberger, *J. Chromatogr. A*, 706 (1995) 493.
- [18] H. Hoshino and T. Yotsuyanagi, *Talanta*, 31 (1984) 525.