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# Use of cationic polymers for the simultaneous determination of inorganic anions and metal-4-(2-pyridylazo)resorcinolato chelates in kinetic differentiation-mode capillary electrophoresis<sup>1</sup>

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## Abstract

Three cationic polymers were used as electroosmotic flow and selectivity modifiers for simultaneous determination of inorganic anions and metal-4-(2-pyridylazo)resorcinolato (PAR) chelates. It was shown that the ability of polymers to associate with analytes decreased in the order: poly-(*N*-ethyl-4-vinylpyridinium bromide) > poly-(diallyldimethylammonium chloride) > Polybrene.  $\text{CrO}_4^{2-}$  was chosen as a background electrolyte anion, because it enables indirect UV detection of transparent analytes at 254 nm and do not affect the detection sensitivity of chelates at 490 nm. Rapid (4 min) separation of Co(II), Ni(II), and Fe(II)-PAR chelates and of 11 inorganic anions was demonstrated. © 1997 Elsevier Science B.V.

**Keywords:** Buffer composition; Kinetic differentiation electrophoresis; Inorganic anions; Metal chelates; Pyridylazoresorcinolato chelates

## 1. Introduction

Separation with capillary electrophoresis (CE) uses the experiences of ion chromatography (IC) in problem solving: indirect UV [1], suppressed conductivity detection [2], etc. However, simultaneous determination of cations and anions by CE still remains a challenging problem. IC uses various approaches in this field of investigation. The first is simultaneous determination of anions and metal-EDTA complexes [3,4]. However CE selectivity for

separation of such types of complexes is very poor [5,6], and indirect detection is not sensitive. The second is the use of two different stationary phases [7] or sorbents with zwitterionic functional groups [8]. However, this technique is not convenient for CE because of the drastic differences in separation mechanism.

The determination of small inorganic and organic anions by CE has been intensively investigated. The common procedure includes a reverse of electroosmotic flow from cathode to anode and indirect UV detection. Aliphatic quaternary ammonium salts [9,10] and cationic polymers [11–15] were used for this purpose. These admixtures provide both electroosmotic flow and separation selectivity control.

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Cationic polymers are preferred because they allow micelle forming processes to be avoided while changing their concentration. The name 'ion-exchange electrokinetic chromatography' was introduced by Terabe et al. [11] for the mechanism of mobility differentiation with polyelectrolyte-analyte interaction.

Kinetic differentiation (KD)-mode CE is the most convenient procedure for metal-PAR chelate determination [16,17]. This technique applies carrier electrolytes without the addition of a ligand. Kinetically unstable chelates are decomposed in a capillary, whereas inert species can be separated and detected. Recently it was shown [18] that the separation of these complexes is also possible with negative power supply and cationic polyelectrolytes for electroosmotic flow and selectivity modification. This separation uses sulfate as a background electrolyte anion, and can be described in terms of ion-exchange. It is common knowledge that the ion chromatographic behaviour of  $\text{SO}_4^{2-}$  and  $\text{CrO}_4^{2-}$  is very similar with one important exception: chromate has a high specific absorbance in UV range and allows an indirect detection of light organic and inorganic species. The aim of this work is to investigate the systems for simultaneous determination of inorganic anions (indirect UV detection) and metal-PAR chelates (direct Vis) with a negative power supply and cationic polymers as selectivity modifying additives. Co(II), V(V), Ni(II), and Fe(II)-PAR chelates and 12 inorganic anions of different charge and hydrophobicity were chosen for study.

## 2. Experimental

### 2.1. Instrumentation

All instrumentation conditions were similar to that described in [18], with the exception of detector parameters. Two types of CE instruments were used. The first consisted of a high-voltage power supply (Matsusada, Japan). A variable-wavelength detector for capillary electrophoresis (JASCO-CE 970, Japan) was programmed for changing detection wavelength from 254 nm (inorganic anions) to 490 nm (metal-chelates) during the run. Fused-silica capillaries (GL

Science, Japan) had the length of 60 cm (46 cm to detector), 375  $\mu\text{m}$  O.D. and 50  $\mu\text{m}$  I.D. A sample solution was introduced manually by hydrostatic injection (13 cm, 15 s). A Chromatopac-CR5A (Shimadzu, Japan) and a Servocorder SR6211 recorder (Graphtec, Japan) were used for electrophoregram recording. The second instrument was a Bio-Focus 3000 (Bio-Rad, USA). The capillary of the same type (51 cm, 46.4  $\mu\text{m}$  to detector) was used with User Assembled Cartridge Kit. The electrophoregrams with 254 and 490 nm were recorded separately. 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), and poly-(*N*-ethyl-4-vinylpyridinium bromide) (PVPyBr, high-molecular-mass compound division of Moscow State University) were prepared by dilution (dissolving) of initial products. The structures of used polymers are shown in Fig. 1. All the polymers were converted to the chromate form as described by Stathakis et al. [6]. Only an IRA-410 anion-exchange resin was used for this procedure. Potassium chromate, sodium sulfate, hydrogen- and dihydrogenphosphate,  $\text{Na}_2\text{EDTA}$  (all reagent grade) were used for electrolyte solutions preparations. Carrier electrolytes were filtered through a 0.45  $\mu\text{m}$  membrane filter (Advantec, Toyo, Japan) and degassed. Unmodified capillaries were purged with water (5 min), 0.1 M NaOH (10 min), water (10 min) and carrier electrolyte solution (30

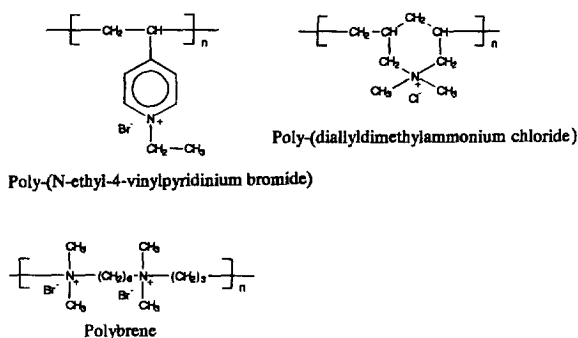


Fig. 1. Structures of the polymers used.

min). Between the runs, the capillary was washed with the electrolyte (1 min). Stock solutions of inorganic anions were prepared by dissolving sodium/potassium salts (reagent grade) in water and diluted prior injection. The sample solutions of metal–PAR complexes were prepared by adding the metal stock solution aliquots to 1 mM PAR in slightly alkaline media and heating at 60°C for 15 min. Doubly distilled water (a Still-N2, IWAKI, Japan) was used in preparing all the solutions.

### 2.3. Calculations

The electroosmotic flow (EOF) was determined from the equation  $\mu_{eos} = v_{eos}/E$ , where  $v_{eos}$  is migration speed of the bulk electrolyte (water plug gives a negative peak in indirect UV detection mode, cm/s) and  $E$  is field strength (kV/cm). Electroosmotic mobility was arbitrary chosen as positive when EOF is reversed. Electrophoretic mobilities ( $\mu_{ep}$ ) of analytes were calculated according to the equation  $\mu_{ep} = (v_{mig}/E) - \mu_{eos}$ , where  $v_{mig}$  is migration speed of analyte.

## 3. Results and discussion

There are two approaches for description of separation mechanism in similar systems. The first approach uses the terms of ion association of analyte and a polymeric molecule [11]. Nevertheless, the authors refer to the new separation technique as 'ion-exchange electrokinetic chromatography'. The second approach considers a capillary as a chromatographic column with moving anion-exchange pseudophase [18]. This system has many analogies with common IC separation of anions [19]. This allows IC terminology to be used for the qualitative description of behaviour of anions.

The eluents for IC should meet two major requirements: they must have a sufficient elution strength and provide the conditions for high detection sensitivity. However, the transfer of ion-exchange mechanism into CE separation adds extra requirements for mobility of anions of the background electrolyte (BGE). An anion with a mobility close to that of the analytes provides the highest separation efficiency. Usually, chromate ions were used for determination

of inorganic anions [9,14]. The composition of carrier electrolyte which was used previously [18], consisted of sulfate as BGE anion, phosphate buffer solution for adjusting pH, Na<sub>2</sub>EDTA for elimination of transition metal influences (which leads to the loss of peak symmetry) and polymeric admixtures. These conditions allow one to produce sharp peaks for singly and doubly charged anions of metal–PAR chelates (VO<sub>2</sub>L<sup>1-</sup>, CoL<sub>2</sub><sup>1-</sup>, FeL<sub>2</sub><sup>2-</sup>, and NiL<sub>2</sub><sup>2-</sup>). The affinity of chromate to anion-exchangers (the ability of ions to associate) is very close to that of sulfate. It allows substitution of the SO<sub>4</sub><sup>2-</sup> anion in BGE. However, the most important feature for the indirect detection technique (in IC and CE) is the elimination of all anions, which are different from UV-absorbing CrO<sub>4</sub><sup>2-</sup> because the magnitude of indirect signal depends on the substitution ratio of BGE anion for analyte. Fig. 2 shows the electropherograms with gradual elimination of phosphate, Na<sub>2</sub>EDTA and substitution of K<sub>2</sub>CrO<sub>4</sub> for Na<sub>2</sub>SO<sub>4</sub>. As it can be seen from Fig. 2, CrO<sub>4</sub><sup>2-</sup> has a higher ability to associate with polymers than SO<sub>4</sub><sup>2-</sup> because migration times of strongly retained FeL<sub>2</sub><sup>2-</sup> and NiL<sub>2</sub><sup>2-</sup> decrease significantly. It allowed us to decrease the concentration of UV-absorbing anions, which is preferable for sensitivity of indirect detection. However it is more significant that elimination of phosphate and Na<sub>2</sub>EDTA does not affect the peak shape. Separation efficiency of more than 400 000 theoretical plates per meter was obtained. This fact and closeness of CrO<sub>4</sub><sup>2-</sup> mobility to mobilities of small inorganic anions makes it possible to perform their simultaneous determination with metal–PAR chelates.

### 3.1. Effect of polyelectrolyte structure on electrophoretic mobility of analyte

It was found that different cationic polymers have different affinities to hydrophobic anions. Strongly retained anions (I<sup>-</sup>, SCN<sup>-</sup>, and ClO<sub>4</sub><sup>-</sup>) have lower capacity factors on a silica-based stationary phase modified with 2,5-Ionene (Polybrene type) in comparison with PDADMACl and PVPyBr [19]. As we expected the same picture should exist in CE separation. The relative mobilities ( $\mu_{An}/\mu_{Cl}$ ) of 12 inorganic anions and four metal–PAR chelates for

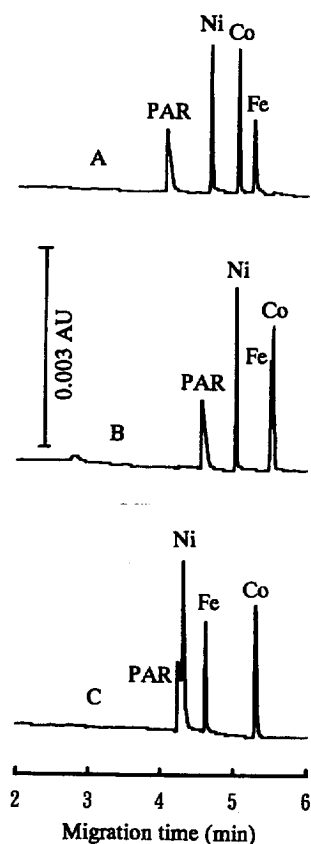


Fig. 2. Comparison of electrolyte systems with  $\text{SO}_4^{2-}/\text{CrO}_4^{2-}$  BGE anions, with/without  $\text{Na}_2\text{EDTA}$  for metal-PAR chelates separation in the presence of 5.35 mM (functional group) Polybrene. Capillary, 60 cm (46 cm effective length)  $\times$  50  $\mu\text{m}$  I.D.; buffers: A, 20 mM  $\text{Na}_2\text{SO}_4$ , 4 mM Na-phosphate buffer pH 8.0, 1 mM EDTA; B, 20 mM  $\text{Na}_2\text{SO}_4$ , pH 8.53; C, 20 mM  $\text{K}_2\text{CrO}_4$ , pH 8.86; sampling: gravity, 15 s, 13 cm; conditions:  $-20$  kV; Detection: Vis 490 nm; sample:  $[\text{PAR}]_T = 1$  mM,  $[\text{M}]_T = 0.01$  mM.

studied polymers under the same conditions of carrier electrolyte are shown in Fig. 3.

The electrophoretic mobility of analyte  $\mu_{ep}$  can be estimated from the expression:

$$\mu_{ep} = \mu_o \alpha_{A \text{ FREE}} + \mu_p \alpha_{AP}; \quad (1)$$

where  $\mu_o$  is own mobility of analyte A,  $\alpha_{A \text{ FREE}}$  is the fraction of free analyte ( $\alpha_{A \text{ FREE}} = [\text{A}]_{\text{FREE}} / [\text{A}]_{\text{TOTAL}}$ ),  $\mu_p$  is a mobility of polymeric molecule,  $\alpha_{AP}$  is the fraction of analyte associated with polymer. If we suppose for simplicity that the velocity of a polymeric molecule in an electric field is close to zero, Eq. (1) can be rewritten as:

$$\mu_{ep} = \mu_o [\text{A}]_{\text{FREE}} / [\text{A}]_{\text{TOTAL}}, \quad (2)$$

but  $[\text{A}]_{\text{TOTAL}} = [\text{A}]_{\text{FREE}} + [\text{A}]_p$ , consequently:

$$\begin{aligned} \mu_{ep} &= m_o [\text{A}]_{\text{FREE}} / ([\text{A}]_{\text{FREE}} + [\text{A}]_p) \\ &= \mu_o / (1 + [\text{A}]_p / [\text{A}]_{\text{FREE}}), \end{aligned} \quad (3)$$

where  $[\text{A}]_p / [\text{A}]_{\text{FREE}}$  is a ratio of concentrations of associates and free analyte anion. The  $[\text{A}]_p / [\text{A}]_{\text{FREE}}$  parameter is equivalent to the distribution coefficient of analyte between the stationary and mobile phase, which determines elution order of common IC separation [20]:  $\text{F}^- < \text{Acet}^- < \text{HCO}_3^- < \text{Cl}^- < \text{NO}_2^- < \text{NO}_3^- < \text{Br}^- < \text{HPO}_4^{2-} < \text{SO}_4^{2-} < \text{I}^- < \text{SCN}^- < \text{ClO}_4^-$ . The interference of this picture and electrophoretic mobilities of anions gives a description of migration order for different polymers. Thus, the largest effect was observed for hydrophobic anions  $\text{I}^-$ ,  $\text{SCN}^-$ ,  $\text{ClO}_4^-$  and metal chelates.  $\text{SCN}^-$  and  $\text{ClO}_4^-$  are not separated in the presence of Polybrene, but in the case of PDADMACl and PVPyBr good separation was observed. By the same reason, the migration order of  $\text{Cl}^-$ - $\text{Br}^-$  is reversed, the resolution of  $\text{NO}_2^-$ - $\text{NO}_3^-$  increases, and moderate changes are observed for weakly retained ( $\text{F}^-$ ,  $\text{Acet}^-$ , and  $\text{HCO}_3^-$ ) and doubly charged ( $\text{HPO}_4^{2-}$ ,  $\text{SO}_4^{2-}$ ) anions. It was not surprising that the structure of ion-exchange groups affected the elution order of anions in IC [20] and can be used for selecting a polyelectrolyte modifier for certain types of CE separation. The association ability of polymers decreased in the order: PVPyBr > PDADMACl > Polybrene.

### 3.2. Effect of BGE anion on electrophoretic mobility of analytes

A change in concentration of BGE anion affects analyte mobility through the  $[\text{A}]_p / [\text{A}]_{\text{FREE}}$  ratio in Eq. (3). An increase in chromate concentration leads to a decrease in  $[\text{A}]_p / [\text{A}]_{\text{FREE}}$  and an increase in  $\mu_{ep}$ . The same picture is observed in IC:

$$\log k' = (a/b) \log C - (a/b) \log E + \text{Constant}; \quad (4)$$

where capacity factor ( $k'$ ) can be calculated from the distribution coefficient of analyte between stationary and mobile phases;  $a$ ,  $b$ ,  $C$ , and  $E$  are the charges of analyte, eluting anions, column capacity, and eluent concentration, respectively [20]. Consequently, the

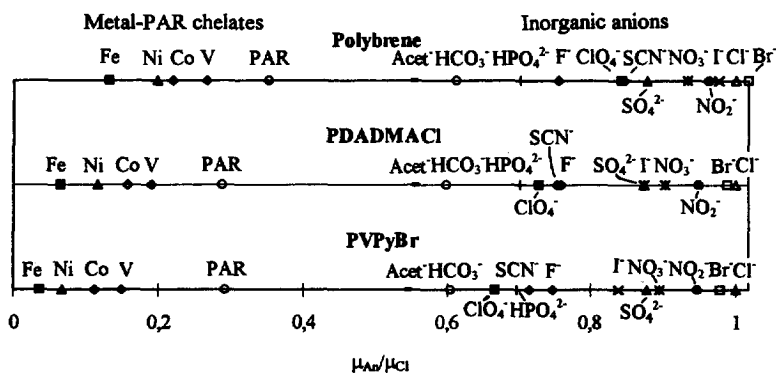


Fig. 3. Relative electrophoretic mobilities  $\mu_{An}/\mu_{Cl}$  of 12 inorganic anions and V(V), Co(II), Ni(II), Fe(II)-PAR chelates for different kind of polymers (converted to  $CrO_4^{2-}$  form). Capillary: 50 cm (46 cm effective length)  $\times$  50  $\mu$ m I.D.; buffer: 5 mM  $CrO_4^{2-}$ , pH 8.8, 5.35 mM of polymers; sampling: gravity, 15 s, 13 cm; conditions: -20 kV; detection: Vis 490 nm; sample:  $[PAR]_T=1$  mM,  $[M]_T=0.01$  mM,  $[An]_T=0.25$  mM.

slope of  $\mu_{ep}$  vs.  $CrO_4^{2-}$  concentration must be higher for doubly charged than for singly charged anions. Fig. 4 shows such a type of plot for a Polybrene-containing carrier electrolyte. As expected, the largest increases were obtained for  $HPO_4^{2-}$ ,  $SO_4^{2-}$ ,  $FeL_2^{2-}$ , and  $NiL_2^{2-}$ .

The behaviour of singly charged metal-PAR chelates can not be explained by this approach. Their

mobilities decrease on increasing chromate concentration in 3–5 mM region. In addition to ion-exchange, other types of interaction between sample solutes and polymeric chain can exist: hydrophobic interaction, absorption of ion pairs, etc. The substitution of  $NH_4^+$  for  $Na^+$  allows the authors [21] to manipulate with distribution coefficients of metal-PAR chelates, between micellar and aqueous phases

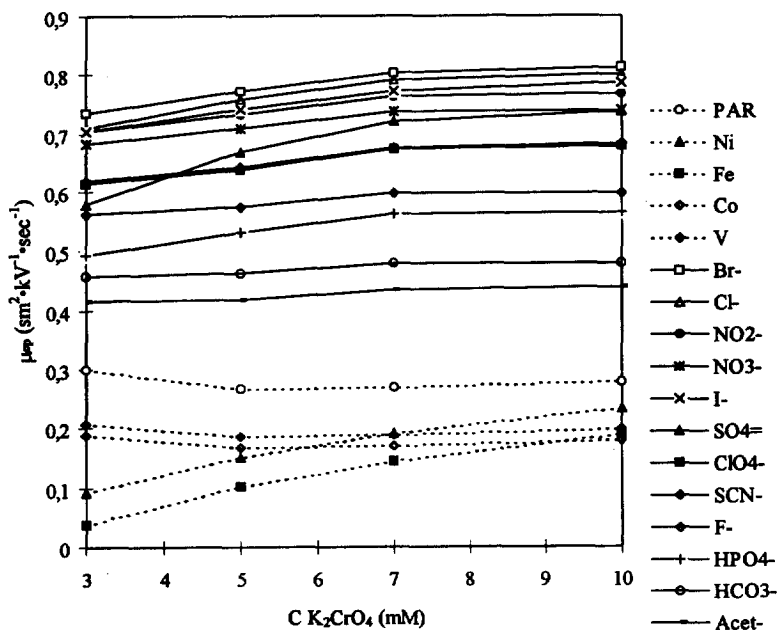


Fig. 4. Dependence of analytes electrophoretic mobility on chromate concentration in carrier electrolyte. Buffer:  $x$  mM  $CrO_4^{2-}$ , 2.67 mM of Polybrene; for other conditions see Fig. 3.

for optimization of CE separation. Probably, the sorption of ion pairs of complexes and potassium cations coexists with ion association with polymer in the case of  $\text{VO}_2\text{L}^{1-}$  and  $\text{CoL}_2^{1-}$ .

### 3.3. Effect of polyelectrolyte concentration on electrophoretic mobility of analytes

As can be seen from Eq. (4), the influence of ion-exchange capacity is equivalent to eluent concentration, though with opposite sign. Fig. 5 shows the dependence of analyte electrophoretic mobility from polymer concentration in the case of PVPyBr. The rate of decrease in mobilities increases with the charge and hydrophobicity of analyte. The use of high concentration of polymers allows  $\text{SCN}^-$  and  $\text{ClO}_4^-$  to be separated, the migration order of  $\text{Cl}^-$ – $\text{Br}^-$ ,  $\text{SO}_4^{2-}$ – $\text{I}^-$  and  $\text{NO}_3^-$ – $\text{SO}_4^{2-}$  pairs to be changed, etc. Fig. 5 illustrates the drawbacks of the simplified Eq. (3). Migration times of chelates higher than the migration time of the bulk electrolyte are observed at high polyelectrolyte concentrations. This is possibly due to the movement of polymeric molecules in the direction opposite to electroosmotic flow or inter-

action of analytes with the anion-exchange layer on the capillary wall. This process must be taken into account for correct description of retention mechanism.

### 3.4. Simultaneous determination of inorganic anions and metal–PAR chelates

As can be seen from Fig. 3, the mobility scale of a series of test anions breaks into two parts: for small inorganic anions and for metal–chelates. This allows simultaneous determination with a change of detection wavelength during analysis. Indirect detection at 254 nm was used for inorganic anions due to their weak UV-absorbing properties. Direct detection at 490 nm was used for metal–PAR complexes due to their high specific absorbance in the visible range. Fig. 6 shows an electrophoregram of a mixture of  $\text{Co(II)}$ ,  $\text{Ni(II)}$ ,  $\text{Fe(II)}$ –PAR chelates and of 11 inorganic anions. A JASCO-CE 970 detector was programmed for switching of wavelength from 254 to 490 nm at 2 min. As mentioned above, the  $\text{CrO}_4^{2-}$  anion provides a high separation efficiency (more than 400 000 theoretical plates per meter) of metal–

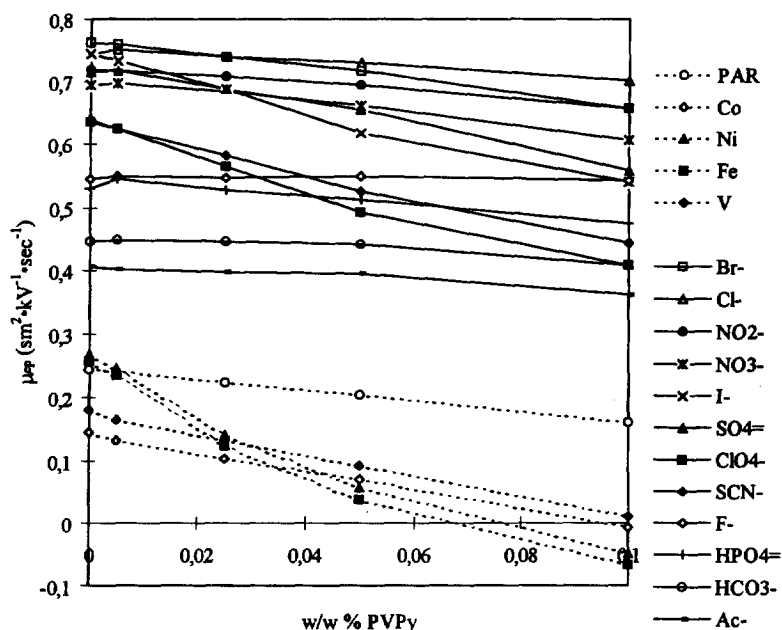


Fig. 5. Dependence of analytes electrophoretic mobility vs. polymer concentration in carrier electrolyte. Buffer: 5.5 mM  $\text{CrO}_4^{2-}$ ,  $x$  mM of PVPyBr; for other conditions see Fig. 3.

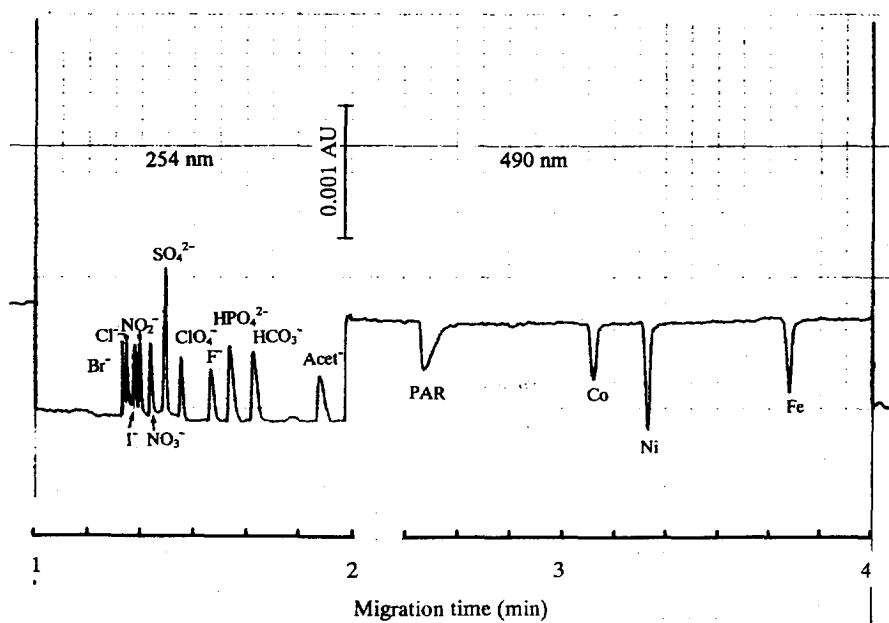


Fig. 6. Electrophoregram of simultaneous determination of 11 inorganic anions and Co(II), Ni(II), Fe(II)-PAR chelates. Capillary: 60 cm (46 cm effective length)  $\times$  50  $\mu$ m I.D.; buffer: 5 mM  $K_2CrO_4$ , pH 8.90, 0.05% (w/w) Polybrene; sampling: gravity, 15 s, 13 cm; condition: -28 kV; detection: indirect UV 254 nm changed to Vis 490 nm at 2 min; sample:  $[PAR]_T=0.6$  mM,  $[M]_T=0.006$  mM,  $[An]_T=0.25$  mM.

PAR chelates and does not deteriorate the sensitivity of their detection at 490 nm. The detection limit of  $CoL_2^{1-}$  was found to be the same as for sulfate-containing carrier electrolyte [18] (0.6  $\mu$ M/l, 6 nl injection). The values of the relative standard deviations of the migration times were 0.0051 and 0.0044, and of the peak heights -0.022 and 0.015 for  $ClO_4^-$  and  $CoPAR_2^{2-}$ , respectively. These data correspond to the characteristics of separate determination of inorganic anions [14] and metal-PAR chelates [18]. It allows the combination of two separation procedures into a single one without degradation of their analytical possibilities.

#### 4. Conclusion

The technique described provides rapid (4 min), simple simultaneous determination of inorganic anions and kinetically stable metal-PAR chelates. For the first time an ion-exchange electrokinetic chromatography mechanism was used for this purpose. The addition of cationic polymers to the background electrolyte reverses electroosmotic flow and de-

creases electrophoretic mobility of anions according to their abilities to associate with polymeric cations. Spectral characteristics of chromate as an anion of carrier electrolyte allow the combination of indirect UV detection of inorganic anions and direct Vis detection of test complexes. This approach can be useful in developing multi-component CE techniques that allow determination of analytes of different natures.

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