

## Separation and Sensitive Determination of Metal Ions by Capillary Zone Electrophoresis with 2-(5-Nitro-2-pyridylazo)-5-(*N*-propyl-*N*-sulfopropylamino)phenol

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Capillary zone electrophoresis (CZE) was first introduced as a powerful and important technique for the separation of charged substances by Mikkers *et al.*<sup>1</sup> Since then, one of the most important breakthroughs was achieved by Terabe *et al.*; they made it possible to separate non-charged organic compounds by using micellar electrokinetic chromatography (MEKC).<sup>2</sup> Saitoh (T) *et al.*<sup>3-4</sup> as well as Saitoh (K) *et al.*<sup>5</sup> separated non-charged metal chelates by MEKC. Besides, there have been several studies on the separation of metal ions as their complex ions.<sup>6-11</sup> These studies have mainly been aimed at utilizing the high separation ability of CZE. However, CZE seems to be very useful for the simultaneous determination of metal ions, if the sensitivity and reproducibility could be sufficiently improved for the quantitative analysis of metals. Motomizu *et al.* have reported on methods for determining alkaline earth metal ions with EDTA<sup>12</sup> and CyDTA (1,2-cyclohexanediamine-*N,N,N',N'*-tetraacetic acid)<sup>13</sup> by CZE.

In this work, the authors studied the separation and determination of metal ions based on the formation of chelate anions with Nitro-PAPS [2-(5-nitro-2-pyridylazo)-5-(*N*-propyl-*N*-sulfopropylamino)phenol] by CZE.

### Experimental

#### Apparatus, reagents and procedure

The CZE system used was the same as that in a previous study.<sup>12</sup> Separations were performed in an untreated fused-silica capillary (50  $\mu\text{m}$  i.d., 375  $\mu\text{m}$  o.d.) obtained from GL Science. The capillary, which was mounted in a temperature-controlled room (35°C), was 72 cm in total length and 50 cm in separation length from the anode to the detector.

A chelating agent (Nitro-PAPS) was purchased from Dojin Laboratories. Pairs of either  $\text{KH}_2\text{PO}_4$  and

$\text{Na}_2\text{HPO}_4$  or  $\text{CH}_3\text{COOH}$  and  $\text{CH}_3\text{COONa}$  were used for preparing buffer solutions. All of the reagents used were of analytical reagent grade, and were dissolved in water purified with both a deionizing-distilling apparatus and a Milli Q Lab (Millipore Ltd.).

Carrier solutions containing buffer components and Nitro-PAPS were used. Sample solutions (about  $9 \times 10^{-9} \text{ dm}^3$ ) containing metal ions and Nitro-PAPS were injected using a vacuum injection system.

### Results and Discussion

#### Detection of metal chelates

Most metal chelates of Nitro-PAPS exhibited absorption maxima at wavelengths from 520 to 600 nm, where the molar absorptivities were about  $10^5 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ . In this work the detection was carried out at 560 nm, where most of the metal chelates showed a relatively large absorption.

#### Effect of the pH of the carrier solution

The velocity of electroosmotic flow ( $U$ ) and electrophoretic mobility ( $\mu_{\text{eo}}$ ;  $\mu_{\text{eo}} = U/E$ , where  $E$  is the potential field strength) are almost constant in the pH region above 7, and gradually become slow in the pH region below 7. This indicates that most of the silanol is deprotonated in the pH region above 7.

Figure 1 shows the relationship between the  $\mu_{\text{ep}}$  values of metal ions and the pH. It can be seen that there are only slight differences among the electrophoretic mobilities of divalent metal ions at pH 9.3, where most of the divalent metal ions exist as divalent chelate anions,  $\text{MR}_2^-$  ( $\text{M}^{2+}$  indicates the metal ion and  $\text{R}^{2-}$  Nitro-PAPS). As for  $\text{Co}^{2+}$ , the  $\mu_{\text{ep}}$  value is about half as large as those of divalent metal ions. This indicates that the oxidation state of cobalt in the chelate is trivalent and that the chelate exists as  $\text{CoR}_2^-$ . Since the stability of such chelates as  $\text{Fe}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Co}^{2+}$  ( $\text{Co}^{3+}$ ) were very high,

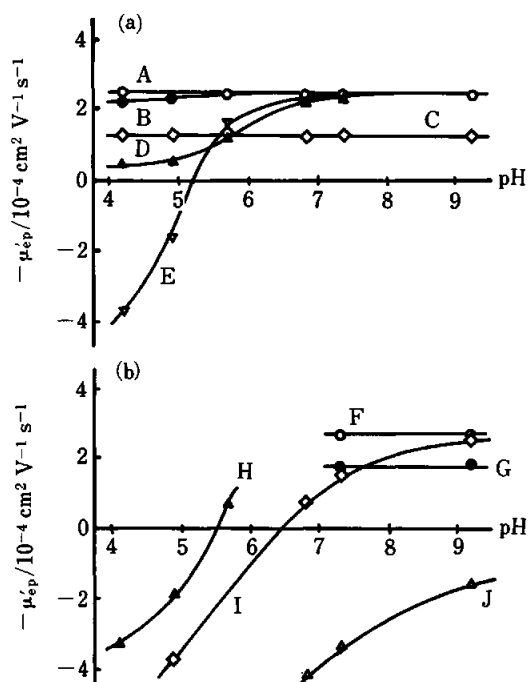


Fig. 1 Effect of the pH on the electrophoretic mobilities of metal ions. Carrier,  $1.2 \times 10^{-4}$  M Nitro-PAPS + 24 mM buffer solution; sample,  $1 \times 10^{-5}$  M metal ion +  $4 \times 10^{-5}$  M Nitro-PAPS; temperature,  $35^\circ\text{C}$ ; voltage, 30 kV; sampling time, 3 s ( $9 \times 10^{-9}$  dm<sup>3</sup>). Buffer solutions were prepared by mixing  $\text{Na}_2\text{HPO}_4$  and  $\text{KH}_2\text{PO}_4$  or acetic acid and sodium acetate. A,  $\text{Fe}^{2+}$ ; B,  $\text{Ni}^{2+}$ ; C,  $\text{Co}^{2+}$ ; D,  $\text{Cu}^{2+}$ ; E,  $\text{Zn}^{2+}$ ; F,  $\text{Hg}^{2+}$ ; G,  $\text{V}^{\text{IV}}$ ; H,  $\text{Pb}^{2+}$ ; I,  $\text{Cd}^{2+}$ ; J,  $\text{Ag}^+$ .

there was little change in the mobilities over wide pH ranges. On the other hand, since the stabilities of such chelates as  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Cd}^{2+}$  were not high, their chelates gradually dissociated with a decrease in the pH. Also, their apparent electrophoretic mobilities became small and their peaks became short with a decrease in the pH. In the pH region below 6, the signs of  $\mu'_{\text{ep}}$  for  $\text{Zn}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Cd}^{2+}$  and  $\text{Pb}^{2+}$  became opposite to that in the pH region above 6; their peaks appeared in front of the injected sample zone or the peak of a neutral sample component.

From Fig. 1 it can be seen that the optimal pH for the best separation of metal ions is about 4. Figure 2 shows electropherogram for eight metal ions. It is worth noting that the transition metals in the third period are well separated under this condition.

#### Effect of the concentration of Nitro-PAPS in the carrier solution

Figure 3 shows the effect of the concentration of Nitro-PAPS in the carrier solutions on the apparent electrophoretic mobilities of the metal ions. Metal ions, whose chelates are very stable, showed identical mobilities over a wide range of Nitro-PAPS concentrations. In the case of metal ions, whose chelates are not very stable, the apparent mobilities gradually reached constant values;

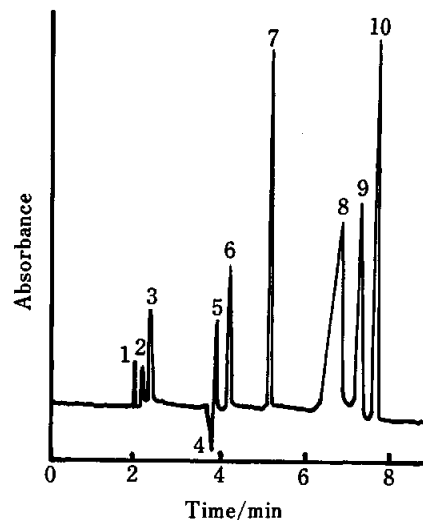


Fig. 2 Electropherogram for 8 metal ions at pH 4.1. Carrier,  $1.2 \times 10^{-4}$  M Nitro-PAPS + 24 mM acetate buffer; sample, metal ions +  $4 \times 10^{-5}$  M Nitro-PAPS. Other conditions are the same as in Fig. 1. (1)  $1 \times 10^{-4}$  M  $\text{Cd}^{2+}$ , (2)  $1 \times 10^{-5}$  M  $\text{Zn}^{2+}$ , (3)  $5 \times 10^{-5}$  M  $\text{Pb}^{2+}$ , (4) water dip, (5)  $1 \times 10^{-5}$  M  $\text{V}^{\text{IV}}$ , (6)  $5 \times 10^{-6}$  M  $\text{Cu}^{2+}$ , (7)  $5 \times 10^{-6}$  M  $\text{Co}^{2+}$ , (8) Nitro-PAPS, (9)  $5 \times 10^{-6}$  M  $\text{Ni}^{2+}$ , (10)  $5 \times 10^{-6}$  M  $\text{Fe}^{2+}$ .

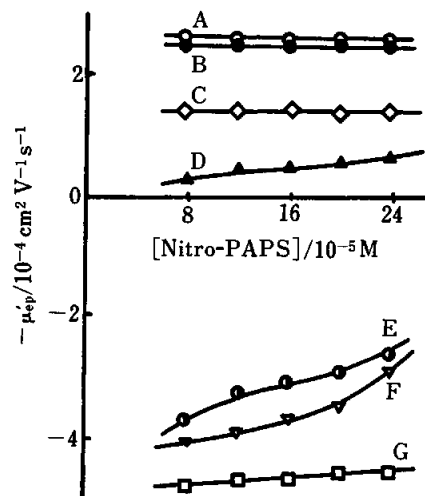


Fig. 3 Effect of the concentration of the Nitro-PAPS in the carrier solution. Carrier, Nitro-PAPS + 24 mM acetate buffer (pH 4.1); sample,  $5 \times 10^{-6}$  M metal ion +  $2 \times 10^{-5}$  M Nitro-PAPS. Other conditions are the same as in Fig. 1. A,  $\text{Fe}^{2+}$ ; B,  $\text{Ni}^{2+}$ ; C,  $\text{Co}^{2+}$ ; D,  $\text{Cu}^{2+}$ ; E,  $\text{Pb}^{2+}$ ; F,  $\text{Zn}^{2+}$ ; G,  $\text{Cd}^{2+}$ .

also, their peak heights and areas became larger and finally reached constant values along with an increase in the concentration of Nitro-PAPS. The concentration of Nitro-PAPS in the carrier solution was determined to be  $1.2 \times 10^{-4}$  M for good separation.

#### Calibration graphs and application

The calibration graphs for the eight metal ions examined in Fig. 2 were linear for concentrations at the

$10^{-6}$  M level. The detection limits corresponding to a signal-to-noise ratio of 3 were, for example,  $1 \times 10^{-7}$  M for  $\text{Fe}^{2+}$  and  $5 \times 10^{-7}$  M for  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$ , when about  $9 \times 10^{-9}$  dm<sup>3</sup> of the sample solutions was injected.

The proposed method with Nitro-PAPS was applied to the determination of small amounts of metal impurities in nickel salts. According to the electropherogram obtained at a pH of 4.1, only cobalt was found as an impurity. In the determination of cobalt in nickel salts, the concentration of Nitro-PAPS in the carrier was lowered to  $2 \times 10^{-5}$  M, and the pH of the carrier was adjusted to 7.3, due to an improvement in the sensitivity and a shortening of the measurement time. By using this carrier, the calibration graph for Co was linear over the ranges from  $5 \times 10^{-7}$  M to  $1 \times 10^{-5}$  M using both the peak heights and areas. The analytical results for the cobalt contents (%) in  $\text{NiSO}_4 \cdot 7\text{H}_2\text{O}$  (extra pure reagent: e),  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$  (e) and  $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  (e) are 0.26, 0.23 and 0.26, respectively. These cobalt contents are nearly equal to those obtained by the solvent extraction spectrophotometric method with nitroso-DMAP.<sup>14</sup> The relative standard deviation of the cobalt content was about 1%.

In conclusion, the separation and detection sensitivity of metal ions were much improved by the proposed CZE method with Nitro-PAPS, and nine kinds of transition metal ions and  $\text{Pb}^{2+}$  could be determined simultaneously within 8 min.

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