Enrichment and Determination of Zinc by High-Speed Countercurrent Chromatography

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The capability of high-speed countercurrent chromatography (HSCCC) has been investigated for enrichment and determination of metal ions at trace levels. Separation of selected divalent metal ions was performed using a small coiled column. A hexane solution of 2-ethylhexylphosphonic acid mono-2-ethylhexyl ester (EHPA) was employed as the stationary phase. Loaded divalent metal ions such as Ni, Co, Cu, and Zn were chromatographically eluted in the order of increasing extractability by passing a mobile phase buffered at a desired pH. Individual metal ions showed good linearity between concentrations and chromatographic peak areas of the absorbance, as detected by postcolumn reaction with 4-(2-pyridylazo)resorcinol (PAR). Metal ions enriched into the stationary phase from a sample solution were separated into individual metal ions. The trace quantity of zinc in natural mineral water was determined by enrichment separation through an HSCCC column.

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Introduction

High-speed countercurrent chromatography (HSCCC) using a coiled column is a unique liquid-liquid distribution technique, which does not require any solid supports. HSCCC has been applied to separation of various kinds of natural and synthetic products.¹⁻³ Inorganic substances were also separated employing desirable extracting reagents as the stationary phase materials.⁴⁻⁸ For example, separation of chemically similar rare earth elements has been extensively investigated using a coiled column containing acidic organophosphorus extractants.^{4,9-11} These novel techniques have become of interest in an application to enrichment of trace metals into group and/or individual elements,^{6,8,10,11} in which experimental conditions can be designed based on the liquid-liquid partition behavior. The liquid stationary phase containing extracting reagents serves as a continuous extractor for enrichment of trace elements from a sample solution. This is free from the contamination and adsorption loss due to column packing materials encountered in other types of chromatography.

Several attempts have been made to enrich metal ions from dilute sample solutions. Kitazume *et al.* performed enrichment separation of heavy metals by HSCCC having a multilayer coiled column of a relatively large capacity (300 cm³), and then metal ions in eluted fractions were determined by plasma spectroscopy.^{5,6} Further developments in analytical applications have been expected. A small coiled column will be conveniently used for an analytical scale separation of desired species from a sample solution.^{12,13} This offers great savings in amounts of the stationary and mobile phases over the large column currently used for preparative separation.¹⁴

Chromatographic separation of individual metal ions can be performed, based on the difference in the distribution ratios, by

passing the mobile phase through the column. Each chromatographic peak will probably be accessible for analytical purposes if the quantitative relation between the concentration and detection signals is presented.¹⁰ Spectrophotometric detection is highly sensitive and seems promising for on-line recording of the absorbance of metal complexes.^{15,16} Fundamental procedures in chemical analysis, such as enrichment, separation, and determination, may be continuously performed by means of the HSCCC that employs a single coiled column. This new process makes successive tedious procedures unnecessary, and hence reduces errors and contamination.

This study was performed in order to test the feasibility of this new approach of HSCCC procedures for enrichment, separation, and determination of divalent metal ions, using a small column (10 cm³) containing an acidic organophosphorus extractant, 2-ethylhexylphosphonic acid mono-2-ethylhexyl ester (EHPA), as a support free stationary phase. The proposed approach was applied to the determination of trace zinc ions in natural mineral water.

Experimental

Reagents

2-Ethylhexylphosphonic acid mono-2-ethylhexyl ester (EHPA) was obtained from Daihachi Chemical Ind. Co., Ltd., and was diluted with hexane. 4-(2-Pyridylazo)resorcinol (PAR, Dojindo Co.) was used as a post-column reagent for the detection of divalent metal ions.¹⁶ The metal ion solutions were prepared by diluting standard solutions of metal ions for atomic absorption analysis (Wako Pure Chemical Ind., Ltd.). A certified reference solution (XSTC-480) for water analysis was obtained from SPEX Certiprep Inc. (USA). The concentrations (ppm) of components were Fe 30, Al 10, B 10, Cu 10, Zn 10, Mo 7, Cr 5, Mn 5, Pb 5, As 1, Cd 1, Ni 1, Se 1, Sb 0.2, and U 0.2 in 5% HNO₃.

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HSCCC apparatus

A desktop model of a high-speed countercurrent chromatograph apparatus (HSCCC-R1; Hitachi Tokyo Electronics Corporation) was equipped with a single column holder and a counter weight on the opposite side to balance the column weight.¹³ The coiled column consisted of three layers of 150 helical turns of a 50 m PTFE tubing (i.d. 0.5 mm). The inner volume of the column was 10 cm³. The holder undergoes a synchronous planetary motion around the vertical axis and a revolution around the central axis of the centrifuge with 10 cm orbital radius in the same direction. The ratio of rotation and revolution radii (β) is 0.5, and the maximum revolutionary speed is 1300 rounds per minute (rpm).

Chromatographic procedures

The coiled column was initially filled with a hexane solution of 0.02 M ($M = \text{mol dm}^{-3}$) (EHPA)₂, and then equilibrated with an aqueous mobile phase passing through the column revolving at 1200 rpm at 35°C. After equilibration that maintained a definite volume of the stationary phase was reached, a sample solution containing divalent metal ions was injected into the column. The pH values of the mobile phase were adjusted with 0.01 M acetic, chloroacetic, dichloroacetic, trichloroacetic acids, and 2-morpholinoethanesulfonic acid (MES) buffer solution. Chromatographic separation was performed by passing the mobile phase through the column at a rate of 0.30 cm³ min⁻¹. The eluted metal ions were monitored by spectrophotometric detection of the absorbance at 500 nm by adding 10⁻⁴ M PAR in 0.1 M NH₃-acetone to an effluent stream at a rate of 0.15 cm³ min⁻¹.

Results and Discussion

Distribution of divalent metals

Acidic organophosphorus compounds such as 2-ethylhexylphosphonic acid mono-2-ethylhexyl ester (EHPA) and bis(2,4,4-trimethylpentyl)phosphinic acid (DTMPPA) are useful extractants for a variety of metal cations.¹⁷ These organophosphorus extractants seem likely candidates for stationary phase materials in HSCCC process for the separation of divalent metal ions. The stable retention of a suitable stationary phase of 0.02 M (EHPA)₂ in hexane was attained by equilibration with the aqueous mobile phase at a flow rate of 0.30 cm³ min⁻¹. Here, the retention ratio (V_R/V_c) of the stationary phase volume (V_s) over the column volume (V_c) was 0.46 ± 0.01 .

The chromatographic behavior of divalent metal ions injected individually was tested through a coiled column containing a 0.02 M (EHPA)₂ stationary phase. Figure 1 shows an example of chromatographic peaks obtained by isocratic elution at different pH values appropriate to each metal.

The chromatographic retention volume (V_R) of a desired element is related to the distribution ratio (D_c) between the stationary and mobile phases:

$$V_R = t_R \cdot F = V_m + D_c \cdot V_s \quad (1)$$

where t_R is the retention time; F the flow rate of the mobile phase; V_m the mobile phase volume in the coil. Figure 2 shows plots of $\log D_c$ versus pH for divalent metal ions, where the D_c values were evaluated from the V_R values according to Eq. (1). These plots yielded almost straight lines with slopes of close to 2, i.e., 1.8 for Ni, 1.5 for Co, 2.1 for Mn, 2.0 for Cu, and 1.9 for Zn, in the order of distribution ratios; $\text{Zn}^{2+} > \text{Cu}^{2+}, \text{Mn}^{2+} > \text{Co}^{2+}$

Fig. 1 Chromatographic peaks for individual metal ions. Stationary phase: 0.02 M (EHPA)₂ in hexane; sample: 0.1 cm³, $[M^{2+}] = 5.0 \times 10^{-5}$ M; mobile phase: 0.01 M CH₃COOH (pH 4.00), 0.01 M (H₂Na)CH₂ClCOO and (H₂Na)CHCl₂COO (pH 2.40).

Fig. 2 Distribution ratios evaluated from chromatographic peaks. Stationary phase: 0.02 M (EHPA)₂ in hexane; sample: 0.1 cm³, $[M^{2+}] = 5.0 \times 10^{-5}$ M; mobile phase: 0.01 M buffer solution (pH 2.20 – 5.80).

$> \text{Ni}^{2+}$. This order is consistent with the decreasing extractability by EHPA such as $\text{Zn}^{2+} > \text{Cd}^{2+} > \text{Cu}^{2+} > \text{Mn}^{2+} > \text{Co}^{2+} > \text{Mg}^{2+} > \text{Ni}^{2+}$.¹⁷ Under comparable conditions, the D_c value is essentially equal to the distribution ratio between the organic and aqueous phases in liquid-liquid extraction. This corresponds to an extraction equilibrium similar to that in batch extraction:

Fig. 3 Gradient separation of metal ions. Stationary phase: 0.02 M (EHPA)₂ in hexane; sample: 0.1 cm³, [M²⁺] = 5.0 × 10⁻⁵ M; mobile phase: 0.01 M CH₃COOH and (H₂Na)CH₂ClCOO (pH 4.15), 0.01 M (H₂Na)CH₂ClCOO and (H₂Na)CHCl₂COO (pH 2.05).

where (HA)₂ denotes the dimer of EHPA; subscripts aq and org refer to the aqueous and organic phases, respectively. This equilibrium depends on the hydrogen ion concentration, and thereby chromatographic conditions are optimized for adequate separation by controlling the pH value of the aqueous mobile phase.

Chromatography with a stationary phase of (EHPA)₂-hexane

A sample solution (0.1 cm³) containing 5.0 × 10⁻⁵ M each of Ni, Co, Cu, Mn, and Zn was injected into the HSCCC column containing 0.02 M (EHPA)₂. As Fig. 3 shows, the chromatographic separation of selected divalent metal ions was achieved by supplying a mobile phase whose pH value was optimized by the technique of the gradient elution. The individual metal ions eluted in the increasing order of their extractability, and their peaks were well separated from each other, except for an appreciable overlap which was observed for the peaks of Cu and Mn. On the other hand, the separation of Cu and Mn was accomplished through the stationary phase of (DTMPPA)₂ in hexane by an alternative experiment.

Analytical approach

The capability of HSCCC method was examined for quantitative analysis of metal ions. A sample solution (0.1 cm³) containing a desired metal ion was injected by varying its concentration over a region of 0.5 × 10⁻⁴ – 2.0 × 10⁻⁴ M. Then, the peak areas were evaluated from the chromatograms obtained by isocratic elution with mobile phases of pH values of 5.56 for Ni, 3.70 for Cu, and 2.20 for Zn. Individual metals showed linear relations with reasonable correlation factors *R* > 0.999 between peak areas and metal concentrations. From these findings, the HSCCC procedures seem to be applicable to the determination of metal ions by the peak area integration.

Chromatographic separation was also performed for sample solutions containing Ni, Co, Cu, and Zn at different concentrations. Figure 4 shows an example of chromatogram obtained by gradient elution for a sample solution (0.1 cm³)

Fig. 4 Gradient separation of metal ions. Stationary phase: 0.02 M (EHPA)₂ in hexane; sample: 0.1 cm³, [M²⁺] = 7.5 × 10⁻⁵ M; mobile phase: 0.01 M CH₃COOH and (H₂Na)CH₂ClCOO (pH 4.15), 0.01 M (H₂Na)CH₂ClCOO and (H₂Na)CHCl₂COO (pH 2.05).

containing 7.5 × 10⁻⁵ M of each metal ions. Mixtures of metal ions were separated into individual metal ions, and gave good linearity with correlation factors over 0.996 between peak areas and concentrations.

Enrichment separation

Considerable enrichment of desired metal ions from a large volume of sample solutions is required for analytical purposes of detecting trace quantities of elements. The hexane solution of 0.02 M (EHPA)₂ was used as the stationary phase in a tubing coil, while the sample solution (50 cm³) containing 0.5 × 10⁻⁷ – 2.0 × 10⁻⁷ M each of Co, Cu, and Zn was injected at a flow rate of 0.60 cm³ min⁻¹. Here, the retention ratio of the stationary phase decreased to 0.34 due to a higher flow rate; adequate separation and reproducibility are nevertheless maintained. Enrichment of metal ions from a sample solution (50 cm³) corresponds to an enrichment factor of 500-fold compared to a usual sample injection (0.1 cm³). Chromatographic separation of the metal ions enriched into the stationary phase was tested by passing the mobile phase through the column at a rate of 0.30 cm³ min⁻¹.

Figure 5 illustrates the chromatogram of a mixture of Co, Cu, and Zn enriched from a 50 cm³ solution containing 10⁻⁷ M of each related metal ion. A mixture of metal ions is successively separated with good resolution by passing the mobile phase whose pH was varied from 4.2 to 2.0 by a gradient technique. Linear relations between peak areas and concentrations (0.5 × 10⁻⁷ – 2.0 × 10⁻⁷ M each metal) were established with correlation factors (*n* = 4), *i.e.*, *R* = 0.999 for Co, 0.997 for Cu, and 0.993 for Zn. The relative standard deviations were based on three repeated runs: *i.e.*, 2.8% for Co, 2.9% for Cu, and 2.0% for Zn.

Table 1 lists the detection limits and determination limits, defined as the signals corresponding to three and ten times the base line noise level, respectively. Linear relationships were also confirmed for sample solutions of different volumes. Metal

Fig. 5 Gradient separation of metal ions. Stationary phase: 0.02 M (EHPA)₂ in hexane; sample: 50 cm³, [M²⁺] = 1.0 × 10⁻⁷ M; mobile phase: 0.01 M CH₃COOH and (H₂Na)CH₂ClCOO (pH 4.15), 0.01 M (H₂Na)CH₂ClCOO and (H₂Na)CHCl₂COO (pH 2.05).

Table 1 Detection limits and determination limits

	Detection limit, ppb	Determination limit, ppb
Co	6.2	18.7
Cu	5.8	18.0
Zn	4.5	13.7

ions involved can be continuously extracted into the stationary phase of (EHPA)₂-hexane by passing a dilute sample solution adjusted at an appropriate pH through the column. These HSCCC procedures are applicable to lower concentration regions provided considerable enrichment is carried out.

Determination of zinc in mineral water

Each chromatographic peak is essentially accessible for the determination of individual elements. We tried the determination of zinc ion, which was clearly separated from others with high sensitivity. After chromatographic enrichment of zinc from diluted standard solutions (20 cm³) adjusted at pH 4.2 with 0.01 M acetate buffer, isocratic elution of zinc was carried out at pH 2.2. The peak area (A) increased linearly with zinc concentration (C), and the calibration equation with $R = 1.000$ ($n = 5$), $A = 1.89C$ (ppb), was obtained for 10–40 ppb Zn under experimental conditions.

The reference solution was prepared by diluting the certified reference solution for water analysis (XSTC-480). The concentration of zinc is regarded as 25.0 ppb after dilution with 400 times volume of 0.01 M acetate buffer solution of pH 4.2. Zinc ion was enriched from this reference solution (20 cm³) into the stationary phase, and then chromatographic separation was performed by isocratic elution. The peak for zinc is separated from others and then the zinc quantity was determined to be 25.0 from the peak area. This agreed fairly well with the expected value. The reproducibility is good as RSD ($n = 3$)

Fig. 6 Enrichment and isocratic separation of mineral water sample. Stationary phase: 0.02 M (EHPA)₂ in hexane; sample: 20 cm³, mineral water, CH₃COOH buffer solution (pH 4.20); mobile phase: 0.01 M (H₂Na)CH₂ClCOO, (H₂Na)CHCl₂COO (pH 2.20).

Table 2 Determination of zinc in natural mineral water

	Zn, ppb	RSD, %
HSCCC	23.1	1.7 ($n = 3$)
ICP-MS ^a	21.6	1.7 ($n = 3$)
ICP-AES ^b	23	1.7 ($n = 3$)

a. Mass number 64.

b. Wavelength 202.548 nm.

1.4%. Thus the feasibility of the proposed HSCCC procedures was confirmed in the analysis of the reference sample.

The determination of zinc ion in natural mineral water (Akita Pref.) was performed according to above HSCCC procedures. Zinc ion was first enriched from a sample solution (20 cm³) adjusted at pH 4.2 with acetate buffer solution. The chromatographic peak was obtained by isocratic elution with the mobile phase of pH 2.2 (Fig. 6). The concentration of zinc in natural mineral water was determined to be 23.1 ppb from the peak area. This content is very close to the value 21.6 ppb determined by ICP-MS and 23 ppb by ICP-AES, as listed in Table 2.

The present HSCCC method is thus useful in analytical applications for enrichment, separation, and determination of trace quantities of metal ions from a dilute solution containing various metal ions. Other elements yielding chromatographic peaks may also be determined, provided a desirable reagent is used as the stationary phase and a suitable mobile phase is found. The present method still requires considerably longer times for enrichment and separation; however, this HSCCC method will be improved by finding desirable mechanical points, such as a higher revolution speed, and by optimizing chromatographic conditions.

Conclusions

The feasibility of HSCCC procedures for the enrichment,

separation and determination of trace elements was studied by employing a stationary phase of (EHPA)₂ having excellent separation properties. Mixtures of divalent metal ions were chromatographically separated in the increasing order of extractability. A linear relation was obtained between the chromatographic peak areas and the concentrations of metal ions; this allowed the quantitative determination of trace metals. The mixture of selected divalent metal ions was effectively enriched into the organic stationary phase, and then successively separated into individual ions with good resolution. The proposed procedure was tested for the determination of zinc in mineral water without any complicated treatment steps.

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