Separation of Heavy Metals by High-Speed Countercurrent Chromatography

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Separation of heavy metal elements such as Co, Cu, Fe(II), Fe(III), Mg, and Ni was performed with a high-speed CCC coil planet centrifuge equipped with a multilayer coil. The two-phase solvent systems used for the separation were composed of n-heptane containing bis(2-ethylhexyl) phosphoric acid (stationary phase) and dilute citric acid (mobile phase), where the distribution ratios of the elements can be optimized by selecting a proper acid concentration. The mobile phase was eluted through the column at a flow rate of 5.0 mL/min while the apparatus was rotated at 800 rpm. Continuous detection of the metal elements was effected by measurement of the emission intensity with direct current plasma atomic emission spectrometry (DCP-AES), where the elution curve was obtained by on-line monitoring of each emission signal. Each metal element was well resolved at high partition efficiencies ranging from 200 to 3600 theoretical plates.

INTRODUCTION

Countercurrent chromatography (CCC) is a useful method of separating many organic materials, such as biologically active substances, natural and synthetic peptides, and various plant hormones. Also it has been applied to separation of active substances, natural and synthetic peptides, and various plant hormones. It is also free from problems arising from the use of solid support, such as adsorption or irreversible binding and contamination of the sample. In addition, highly efficient chromatographic separation has been achieved using a set of three multilayer coils.

In spite of these advantages, there have been no applications of HSCCC to the separation of inorganic elements until quite recently. Radioactive calcium and strontium were separated by Zolotov et al. In previous reports we have demonstrated the efficient separation of the adjacent 14 lanthanide elements in a single run with an exponential gradient elution and also determined the trace impurities in rare earth elements using HSCCC.11

In the present paper, the capability of the single-column HSCCC centrifuge was demonstrated in the separation of several metals including divalent and trivalent irons. Under optimum conditions, excellent separation of metal elements was achieved in an isocratic elution.

EXPERIMENTAL SECTION

Apparatus. A Shimadzu countercurrent chromatograph (HSCCC-1A, prototype) was used which holds a multilayer coil separation column on the rotary frame at a distance of 10.0 cm from the central axis of the centrifuge. The column was prepared from a single piece of approximately 150-m-long, 1.60-mm-i.d. poly(tetrafluoroethylene) (PTFE) tubing by winding it directly onto the holder hub (10-cm diameter). The 0 value, an important parameter used to determine the hydrodynamic distribution of the two solvent phases in the rotating coil, ranged from 0.5 at the internal terminal to 0.6 at the external terminal. (y = r/R, where r is the distance from the column holder axis to the centrifuge.) The coil capacity is approximately 300 mL. The HSCCC can be operated at a speed of 800 rpm.

The flow diagram of the experimental assembly is illustrated in Figure 1. A Shimadzu LC-6A pump was used to pump the mobile phase; a stream splitter was used for delivering an appropriate amount of effluent to the direct current plasma (DCP) via a peristaltic pump equipped in the DCP. The DCP atomic emission spectrometry (AES) system is a SpectraMetrics Model SpectraSpan IIIB system with 20 fixed-wavelength channels for observation of the elution profile. To facilitate monitoring the

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4. Ito, Y. Countercurrent Chromatography; Marcel Dekker: New York, 1988; Chapter 3.
elution profile of the single element, an analog recorder signal from the DCP system was converted into a digital signal using a Hewlett-Packard Model 3478A digital multimeter. The digital data were stored in a Hewlett-Packard Vectra-D work station through an HP-IB bus, and the elution profile was plotted using a Hewlett-Packard 7440A graphics plotter and Advanced Graphics Software SlideWrite Plus. For simultaneous multielement measurements, the emission signal for each channel was integrated for 10 s at intervals of 20 s, and each set of integrated data was printed out. The data were entered manually into the work station and the elution profile was plotted.

Wavelengths used for the present work are listed in Table I.

Reagents. For the preparation of the two-phase solvent system, analytical reagent grade n-heptane and citric acid were purchased from Kanto Chemical Co., Inc., Tokyo, Japan, and bis(2-ethylhexyl) phosphoric acid (DEHPA) was purchased from Nacalai Tesque Inc., Kyoto, Japan. Acid treatment was not necessary for DEHPA in the present work. Samples used in the present study include Co(NO₃)₆H₂O and Ni(NO₃)₆H₂O (Kanto Chemical), FeCl₃·6H₂O and FeCl₂·6H₂O (Merck, Darmstadt, Germany), and CuSO₄·5H₂O (Kojima Kagaku Co., Ltd., Tokyo, Japan). Other metal standards were obtained as 0.1 M nitric acid solutions (1000 ppm) from Kanto Chemical.

Preparation of Two-Phase Solvent and Sample Solutions. DEHPA was dissolved in n-heptane (stationary phase). Citric acid was dissolved in deionized water (mobile phase).

Sample solutions were prepared by dissolving various nitride, chloride, and sulfate in the aqueous citric acid.

Procedure. Each separation was initiated by filling the entire DCP system with a digital signal using a Hewlett-Packard Model 3478A digital multimeter. The digital data were stored in a Hewlett-Packard Vectra-D work station through an HP-IB bus, and the elution profile was plotted using a Hewlett-Packard 7440A graphics plotter and Advanced Graphics Software SlideWrite Plus. For simultaneous multielement measurements, the emission signal for each channel was integrated for 10 s at intervals of 20 s, and each set of integrated data was printed out. The data were entered manually into the work station and the elution profile was plotted.

Wavelengths used for the present work are listed in Table I.

Table I. Wavelengths Used for Present Work

<table>
<thead>
<tr>
<th>element</th>
<th>line (nm)</th>
<th>element</th>
<th>line (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>226.5</td>
<td>Mg</td>
<td>280.2</td>
</tr>
<tr>
<td>Co</td>
<td>345.3</td>
<td>Mn</td>
<td>257.5</td>
</tr>
<tr>
<td>Cu</td>
<td>324.7</td>
<td>Ni</td>
<td>341.4</td>
</tr>
<tr>
<td>Fe</td>
<td>259.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Measurement of Distribution Ratio. The distribution ratio, KT, of each metal element was obtained using a simple test tube method

\[
K_T = \frac{(A_T - A_L)}{A_L} \tag{1}
\]

where \(A_T\) is the total concentration of the sample in the lower phase before equilibration with the upper phase; \(A_L\), the concentration of the sample in the lower phase after equilibration; \(A_T - A_L\), the concentration of the sample in the upper phase after equilibration.

The distribution ratio, KE, was also calculated from the elution curve using the following equation described previously:

\[
K_E = \frac{(R - R_d)}{(V_c - V_d)} \tag{2}
\]

Measurement of Partition Efficiency. Partition efficiencies in terms of theoretical plate numbers (N) and peak resolution (R,) were computed from the obtained chromatograph according to the following equations:

\[
N = \left(\frac{4R}{W}\right)^2 \tag{3}
\]

\[
R_s = 2(R_1 - R_d)/(W_1 + W_2) \tag{4}
\]

RESULTS AND DISCUSSION

The distribution ratios of the metal ions were measured at various sample concentration using 0.002–0.2 M DEHPA in n-heptane as the upper phase and 7–140 mM citric acid as the lower phase. The results are shown in Figure 2, and the range of \(K_T\) values observed is in the last column in Table II. Figure 2 shows the effects of sample concentration on the distribution ratios of nickel, cobalt, magnesium, and copper obtained with the test tube method using 0.2 M DEHPA in n-heptane and 7 mM citric acid. Except for the nickel ion, which elutes first, the \(K_T\) values of these metal ions slightly increase as the sample concentration is decreased. Similar results were obtained for the distribution ratio of copper, cadmium, and manganese when 50 mM citric acid was used as the mobile phase.

Metal ions eluted from the aqueous mobile phase (diluted citric acid) were detected by their emission intensity with DCP. Figure 3 shows a typical chromatogram of nickel, cobalt, magnesium, and copper obtained by eluting 7 mM citric acid at a flow rate of 5 mL/min. The partition efficiencies ranged from 280 TP (Co) to 200 TP (Cu).
while the peak resolution between the two peaks was 2.46. The great difference in the plate count for iron(II) and iron(III) probably indicates slow kinetics involved in the distribution of iron(III).

Cadmium and manganese were separated from copper using 50 mM citric acid as an eluent. Figure 4 shows a chromatogram of copper, cadmium and manganese. The partition efficiencies ranged from 630 TP (Cu) to 270 TP (Mn).

Separation of iron(II) and iron(III) was achieved at a lower DEHPA concentration in the mobile phase, where iron(II) was strongly retained in the stationary phase (Figure 5). The partition efficiency of the separation was 3700 TP for the second peak (FeCl₃), while the peak resolution between the two peaks was 2.46. The great difference in the plate count for iron(II) and iron(III) probably indicates slow kinetics involved in the distribution of iron(III).

| Table II. Distribution Ratio Values |
|-----------------------------|------------------|
| solvent system              | $K_F$ (av)  | $K_T$  |
| element (DEHPA, M)          | UP (citric acid, mM) |
| Ni                         | 0.2           | 7     | 0.02 | 0.90-0.02 |
| Co                         | 0.2           | 7     | 0.15 | 0.07-0.09 |
| Mg                         | 0.2           | 7     | 0.37 | 0.12-0.25 |
| Cu                         | 0.2           | 7     | 1.76 | 0.96-1.30 |
| Cu                         | 0.2           | 50    | 0.09 | 0.11-0.14 |
| Cd                         | 0.2           | 50    | 0.69 | 0.47-0.84 |
| Mn                         | 0.2           | 50    | 1.10 | 0.81-1.44 |
| Fe(II)                     | 0.002         | 140   | 0.04 | 0.01-0.03 |
| Fe(III)                    | 0.002         | 140   | 0.20 | 0.21-0.40 |


Figure 2. Effects of sample concentration on distribution ratio.

Table II presents the average $K_F$ computed from two or three chromatograms, while $K_T$ was obtained from the test tube method. With a few exceptions, these two distribution ratio values are in a close agreement with each other.

Although HSCCC is generally used for preparative separations, the method has the capability of performing efficient analytical separations especially when combined with a continuous detection system such as spectrophotometry or spectroscopy. By use of a sample injection loop set between the elution pump and the HSCCC, continuous repetitive measurements can be carried out as in high-performance liquid chromatography (HPLC). In the present work, the reproducibility of this method was studied using standard
solutions containing 100 ppm nickel, 200 ppm each of cobalt and magnesium, and 400 ppm copper. After the steady-state hydrodynamic equilibrium between the mobile and stationary phases was established in the column, 100 μL of the sample solution was successively injected five times at given intervals and the peak height resulting from each injection was measured. As shown in Table III, the values are quite reproducible with small relative standard deviations except for nickel, indicating that HSCCC may be used for the quantitative analysis of microgram to milligram amounts of metal elements.

The variation for detection of nickel may be related to its sharp peak and early elution. Although there may not be any difficulties when HSCCC is coupled with a DCP-AES, the detection limits for this system are influenced by that of DCP-AES. If a more sensitive detector such as ICP-MS could be coupled with HSCCC, the present system would be a highly sensitive and selective determination technique.