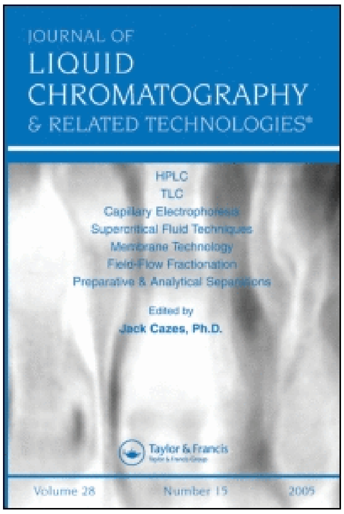

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# Separation of Cd(II), Pb(II), Zn(II), and Cu(II) by High Performance Centrifugal Partition Chromatography with Di-2-methylnonylphosphoric Acid

Min Xue[a]; Yukio Nagaosa[a]
[a] Graduate School of Engineering, University of Fukui, Fukui, Japan

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# Separation of Cd(II), Pb(II), Zn(II), and Cu(II) by High Performance Centrifugal Partition Chromatography with Di-2-methylnonylphosphoric Acid

**Min Xue and Yukio Nagaosa**
Graduate School of Engineering, University of Fukui, Fukui, Japan

**Abstract:** The separation of heavy metal ions such as Cd(II), Pb(II), Zn(II), and Cu(II) has been investigated by high performance centrifugal partition chromatography (HPCPC) employing 0.12 M di-2-methylnonylphosphoric acid (DMNPA)/heptane as a stationary phase. The liquid–liquid extraction for the above four metal ions was examined with 0.10 M tartrate, citrate, and trichloroacetate media. Based on the different half extraction values obtained, the HPCPC parameters were optimized. Among organic acid buffers as the mobile phase tested, tartrate was found to be the most suitable for separating Cu(II) and Cd(II), citrate for Pb(II) and Zn(II), and trichloroacetate for Cd(II), Pb(II), and Zn(II). A two step elution method using two kinds of mobile phases (pH different) could be well applied to the separation of the four kinds of metal ions in mixture. The HPCPC system was operated with 2,136 partition channels, at a rotation speed of 800 rpm, and at a flow rate of 1.0 $cm^3$/min.

**Keywords:** Separation, High performance centrifugal partition chromatography, Heavy metal ions, Di-2-methylnonylphosphoric acid, Organic acids

## INTRODUCTION

Toxic heavy metal ions such as Cd(II), Pb(II), and Cu(II) are well known to cause serious effects on human health when taken in from the environment. The techniques for the removal and separation of toxic metal ions from

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Address correspondence to Yukio Nagaosa, Graduate School of Engineering, University of Fukui, Bunkyo 3-9-1, Fukui 910-8507, Japan. E-mail: nagaosa@acbio2.acbio.fukui-u.ac.jp

sewage, industrial, and mining wastes have been extensively developed and used on a large scale in recent years. Among numerous methods to separate metal ions from each other, the most common are liquid–liquid extraction, ion exchange, electrolysis, membrane separation, etc.[1–4] High performance centrifugal partition chromatography (HPCPC) is considered to be one of the most useful and feasible methods for the separation of chemically resembling materials, since it has good separation efficiency and a wide range of applications. HPCPC is conducted under a constant gravity field, and is comprised of a single axis rotation together with rotor seals for delivery of solvent. In principle, this technique is based on the partition of a sample between two immiscible solvents and enables separation of any complex mixture without requiring a solid stationary phase. In the reversed-phase HPCPC system, an organic solvent is a stationary phase through which an aqueous mobile phase passes under a centrifugal force. Similar to partition liquid chromatography, HPLC and counter-current chromatography, HPCPC involves multiple extraction steps and, therefore, has the advantages of selectivity, versatility, and convenience. Both trace analysis and macro scale separations by HPCPC have been reported until now.[5–12]

Over the past two decades, many kinds of reagents have been synthesized to extract metal ions from aqueous media.[13–15] Previously, di-2-methylnonylphosphoric acid (abbreviated as DMNPA) was found to be one of the most efficient and selective extractants for the HPCPC separation of such divalent metal ions as Cu(II), Mn(II), Co(II), and Ni(II). Furthermore, we have reported successful applications of the HPCPC technique to the separation of transition and alkaline earth metal ions using bis-2-ethylhexylphosphinic acid in heptane.[16,17]

In the present paper, our attention has been focused on the mutual separation of Cd(II), Pb(II), Zn(II), as well as Cu(II) by HPCPC. First, we investigated the extraction of the above four metal ions into DMNPA/heptane to obtain the half extraction pH ($pH_{1/2}$) under the fixed experimental conditions. The $pH_{1/2}$ values were found to greatly vary with different kinds of organic acid buffers as the aqueous phases. Therefore, HPCPC separations are expected to be specific by using an organic acid as the mobile phase and DMNPA/heptane stationary phase. The isocratic elution method was used to separate two or three kinds of metal ions, and a two step elution for the separation of four metal ions. Here, it is shown that good separation could be achieved for Cd(II), Pb(II), Zn(II), and Cu(II) in mixtures by HPCPC.

## EXPERIMENTAL

### Reagents

DMNPA (above 96% in purity) was kindly supplied by Daihachi Chemical Industry Co. Ltd (Osaka, Japan) without further purification. Heptane of analytical reagent grade (Wako Pure Chemical Industry Co. Ltd, Tokyo, Japan) was used as the organic solvent. Standard solutions of metal ions were prepared by dilution of the corresponding metal standards for AAS use. Tartrate, citrate, and trichloroacetate of analytical reagent grade (Wako Pure Chemical Industry Co. Ltd) were employed as mobile phases in HPCPC. 4-(2-Pyridylazo) resorcinol (PAR) from Dojin Industry Co. Ltd (Kumamoto, Japan) was used as the post column derivatization reagent. All other chemicals were of analytical reagent grade and used without further purification.

## Apparatus

An Iwaki Model V-S KM shaker was used to shake the aqueous and organic phase. A TOA-DKK Model IM-55G Ion meter fitted with a Model GST-5721C glass electrode (TOA-DKK Electronics Co. Ltd, Tokyo, Japan) was used to measure the pH of aqueous phase after equilibration. A Senshu Science (Tokyo, Japan) high performance centrifugal partition chromatography (Model LLB) has 2,136 partition channels and 230 $cm^3$ total cell volume in the rotor. A Shimadzu Model SPD-6AV UV-VIS spectrophotometric detector was used to monitor the absorbance of the metal complex formed by post labeling with 4-(2-pyridylazo)-resorcinol (PAR) following HPCPC separations.

## Procedures

### Extraction Studies

Extraction experiments were carried out at $25 \pm 1°C$ by shaking 10.0 $cm^3$ of the aqueous and organic phases in a 50.0 $cm^3$ separation funnel using a mechanical shaker. The initial concentration of metal ion was $5.0 \times 10^{-4}$ M, and the ionic strength of the aqueous phase was kept at 0.10. After shaking for 30 min, the phases were allowed to stand for 6 hours. The pH and the metal ion concentration of aqueous phase were determined after phase separation. The concentration of metal ion in the aqueous phase was initially determined by spectrophotometry. The concentration of the metal ion in the organic phase was then calculated from the difference between the initial and final concentrations in the aqueous phase.

### HPCPC Separation of Metal Ions

A heptane solution containing 0.12 M DMNPA (50 $cm^3$) as the stationary phase was placed into the channels while the rotor was operated at 800 rpm. Under the centrifugal force, an aqueous mobile phase (0.10 M organic acid buffer) was passed through the stationary phase. When a steady flow of the mobile phase was attained, a sample solution containing $5.0 \times 10^{-4}$ M each of metal ion was injected through a sample loop (1.0 $cm^3$) and the HPCPC separation was then conducted. The absorbance of the metal complexes after derivatization with PAR was recorded at 515 nm. In the present work, the rotation speed was fixed at 800 rpm.

## RESULTS AND DISCUSSION

### Liquid–Liquid Extraction of Metal Ions

The liquid–liquid extraction of metal ions from aqueous solutions containing 0.10 M each of tartrate, citrate, and trichloroacetate buffer was investigated. Figure 1 shows the relationship between extraction percentage (E) and pH with 0.10 M trichloroacetate, which indicates that the extractability was in the order of Pb > Zn > Cd > Cu. The $pH_{1/2}$ values increased in line with this extractability order, as shown in Table 1. The extractability of metal ions also varied with organic acid buffer used as the aqueous phase. In HPCPC separation, generally, the reversed-phase mode is used in which the organic acid solution is the mobile phase. Therefore, the order of extractability coincides with that of the retention volume for the HPCPC separation of metal ions of interest. Furthermore, it has been accepted that the degree of separation for two metal ions, the so called separation factor ($\alpha_D$), which parallels the difference in $pH_{1/2}$ value, is defined as:

$$\alpha_D = D_1/D_2 \tag{1}$$

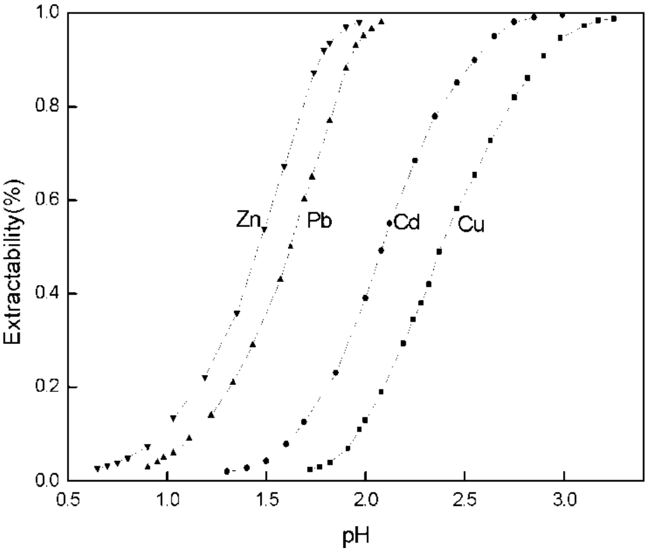

*Figure 1.* Relationship between extractability and pH. Concentration of metal ion in aqueous phase: $5.0 \times 10^{-4}$ M; Organic phase: 0.12 M DMNPA/heptane; Aqueous phase: 0.10 M trichloroacetate.

***Table 1.*** Half-extraction pH and $\Delta pH_{1/2}(pH_{1/2,2}-pH_{1/2,1})$ values for some metal ions by 0.12 M DMNPA/heptane at 25°C

| Organic acid (0.10 M) | Cu(II) | | Cd(II) | | Pb(II) | | Zn(II) |
|---|---|---|---|---|---|---|---|
| Tartrate | | | | | | | |
| $pH_{1/2}$ | 3.54 | | 2.03 | | 1.63 | | 1.56 |
| $\Delta pH_{1/2}$ | | 1.51 | | 0.40 | | 0.07 | |
| Citrate | | | | | | | |
| $pH_{1/2}$ | 2.54 | | 2.14 | | 1.75 | | 1.59 |
| $\Delta pH_{1/2}$ | | 0.40 | | 0.29 | | 0.16 | |
| | Cu(II) | | Cd(II) | | Zn(II) | | Pb(II) |
| Trichloroacetate | | | | | | | |
| $pH_{1/2}$ | 2.55 | | 2.08 | | 1.63 | | 1.53 |
| $\Delta pH_{1/2}$ | | 0.47 | | 0.45 | | 0.10 | |

As can be seen from Table 1, the $pH_{1/2}$ difference was the largest for Cu(II)-Cd(II) with 0.10 M tartrate, and for Pb(II)-Zn(II) with 0.10 M citrate, respectively. It is also seen that trichloroacetate gave relatively large differences in $pH_{1/2}$ for Cd(II)-Zn(II)-Pb(II). Based on the liquid–liquid extraction data, the HPCPC separations were attempted for the metal ions in two- or three-component mixtures.

## Principle of the HPCPC Separation

Since multiple and replicate partitions are performed in HPCPC, we can predict the possibility of separation between two metal ions from the $pH_{1/2}$ difference. Some chromatographic parameters can be related to the D value obtained by liquid–liquid extraction. The retention volume ($V_R$) is directly proportional to the D:

$$V_R = V_M + DV_S \tag{2}$$

where $V_R$ and $V_M$ are the volumes of the stationary and mobile phase, respectively.

The separation factor ($\alpha_C$) is calculated from the retention volumes of individual components 1 and 2:

$$\alpha_C = \frac{V_{R,2} - V_0}{V_{R,1} - V_0} \tag{3}$$

where $V_0$ is the retention volume of a non-retained component.

The theoretical plate number ($N_M$) is related to the retention volume and the half bandwidth ($W_{1/2}$):

$$N_M = 5.54\left(\frac{V_R}{W_{1/2}}\right)^2 \tag{4}$$

The degree of separation for two adjacent bands is defined as resolution (Rs):

$$Rs = \frac{1.18(V_{R,2} - V_{R,1})}{W_{1/2,2} + W_{1/2,1}} \tag{5}$$

Nm and Rs values are generally influenced by experimental parameters and separation efficiencies of HPCPC used.

## Separation of Cu(II) and Cd(II) with Tartrate Buffer as a Mobile Phase

The HPCPC separation of Cu(II) and Cd(II) was investigated with 0.10 M tartrate as a mobile phase (Fig. 1); interestingly, the $pH_{1/2}$ difference around 1.5 was relatively large (Table 1). Base line separation of the two metal ions could be achieved, as shown in Fig. 2. The separation factor ($\alpha_C$) and the theoretical plate number ($N_M$) for the two metal ions were 2.36 and $N_{Cu} = 1{,}066$, $N_{Cd} = 119$, respectively. It is expected that tartrate

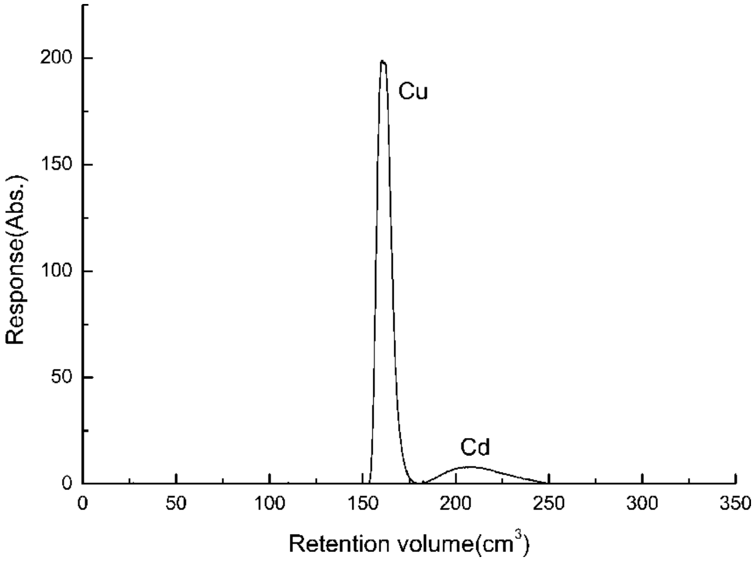

***Figure 2.*** HPCPC separation of Cu(II) and Cd(II). Partition channel: 2,136; Stationary phase: 50 cm$^3$ 0.12 M DMNPA/heptane; Mobile phase: 0.10 M tartrate, pH: 2.60; Rotation speed: 800 rpm; Flow rate: 1.0 cm$^3$/min.

buffer can be used as the mobile phase for the HPCPC separation of any other metal ions except Pb(II)-Zn(II). The $pH_{1/2}$ difference more than 0.10 was preferable to separate two metal ions in mixture by HPCPC.

### Separation of Pb(II) and Zn(II) with Citrate Buffer as a Mobile Phase

Citrate (0.10 M) was chosen as being the most optimum mobile phase for the HPCPC separation of Pb(II) and Zn(II) due to the largest difference in $pH_{1/2}$. Trichloroacetate and tartrate buffers gave overlapped elution peaks. Figure 3 shows typical HPCPC chromatograms for Pb(II)-Zn(II) at different pH values of mobile phase (pH 1.80, 1.88). The chromatographic parameters at pH 1.88 were $\alpha_C = 3.56$, $N_{Pb} = 30$, $N_{Zn} = 18$, and $Rs_{Pb/Zn} = 1.33$, respectively. No resolution of the two metal ions was obtained at pH 1.80.

### Separation of Cd(II), Pb(II), and Zn(II)

The HPCPC separation of Cd(II)-Zn(II)-Pb(II) was carried out with trichloroacetate, tartrate, and citrate buffers. As can be seen from Fig. 4, trichloroacetate buffer gave a well resolved chromatogram for the three metal ions, but tartrate and citrate buffers had overlapping peaks for Cd(II) and Pb(II) by the present HPCPC.

The effect of flow rate on HPCPC separation of the above three metal ions was studied with 0.10 M trichloroacetate (pH 2.06). The HPCPC separation efficiency is also controlled by the flow rate of mobile phase; this may be because a

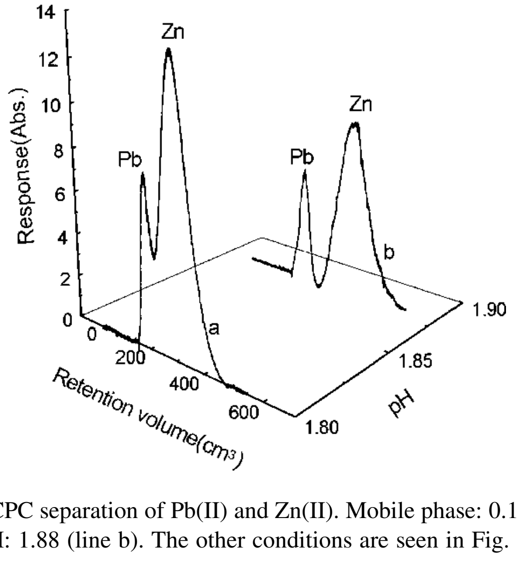

*Figure 3.* HPCPC separation of Pb(II) and Zn(II). Mobile phase: 0.10 M citrate; pH: 1.80 (line a); pH: 1.88 (line b). The other conditions are seen in Fig. 2.

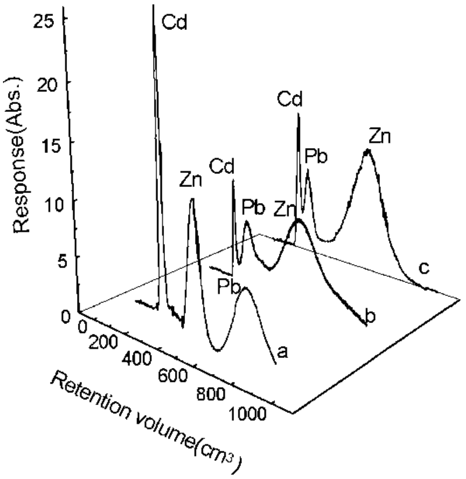

*Figure 4.* HPCPC separation of Cd(II), Zn(II), Pb(II) with different kinds of mobile phase. Flow rate: $1.0\,\mathrm{cm^3/min}$; Mobile phase: 0.10 M tartrate, pH = 2.12 (line a); Mobile phase: 0.10 M citrate, pH = 2.02 (line b); Mobile phase: 0.10 M trichloroacetate, pH = 2.06 (line c). The other conditions are seen in Fig. 2.

slow flow rate leads to a longer time of mixing the two phases on the forward and back extraction. Thus, the separation of Cd(II)-Pb(II)-Zn(II) by HPCPC was carried out at 0.5, 1.0, and $2.0\,\mathrm{cm^3/min}$ (Fig. 5). The chromatograms shown in Fig. 5(b) indicate that a flow rate of $1.0\,\mathrm{cm^3/min}$ is the most appropriate

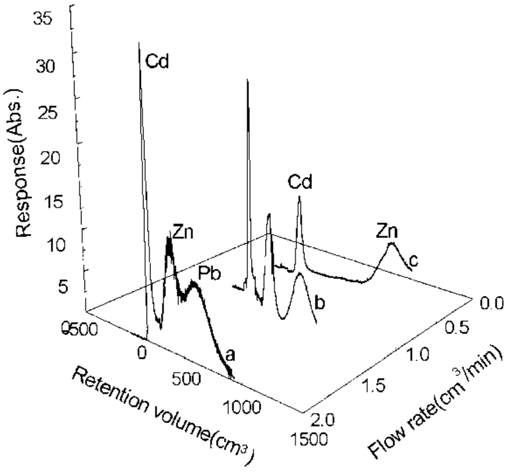

*Figure 5.* Effect of flow rate on HPCPC separation of Cd(II), Zn(II), Pb(II). Mobile phase: 0.10 M trichloroacetate, pH: 2.06; Flow rate: $1.5\,\mathrm{cm^3/min}$ (line a); $1.0\,\mathrm{cm^3/min}$ (line b); $0.5\,\mathrm{cm^3/min}$ (line c). The other conditions are seen in Fig. 2.

because of the base-lined separation for Cd(II)-Zn(II)-Pb(II). As seen from Fig. 5(c), the HPCPC separation at 0.50 $cm^3$/min flow rate took much longer to complete the elution, although good separation could also be obtained.

As Eq. (2) indicates, the retention volume of each metal ion increased with the increasing D value of mobile phase; D value also increased with pH. Therefore, the pH of the mobile phase was the most important factor governing the separation of metal ions by HPCPC. The effect of pH (0.10 M trichloroacetate mobile phase) on the separation of Cd(II)-Zn(II)-Pb(II) was investigated at pH 1.83, 1.93, 2.00, and 2.06 (Fig. 6). It can be seen from Fig. 6(d) that the above three metal ions could be base line separated well at pH 2.06. The chromatographic parameters were $\alpha_{C(Cd/Zn)} = 10.81$, $\alpha_{C(Zn/Pb)} = 2.30$, $N_{Cd} = 143$, $N_{Zn} = 136$, $N_{Pb} = 122$, $Rs_{(Cd/Zn)} = 2.46$, and $Rs_{(Zn/Pb)} = 1.59$ respectively.

## Separation of Cu(II), Cd(II), Pb(II), and Zn(II) with Trichloroacetate Buffer as a Mobile Phase

Preliminary experiments indicated that isocratic elution took considerably longer times for the separation of Cu(II)-Cd(II)-Zn(II)-Pb(II) with all the kinds of mobile phases investigated. Therefore, we selected a two step elution method for the separation of the four metal ions in which the pH of 0.10 M trichloroacetate buffer mobile phase was lowered at fixed retention volumes. The HPCPC chromatograms shown in Fig. 7 indicate that base-lined separation could be obtained for Cu(II)-Cd(II)-Zn(II) but not for

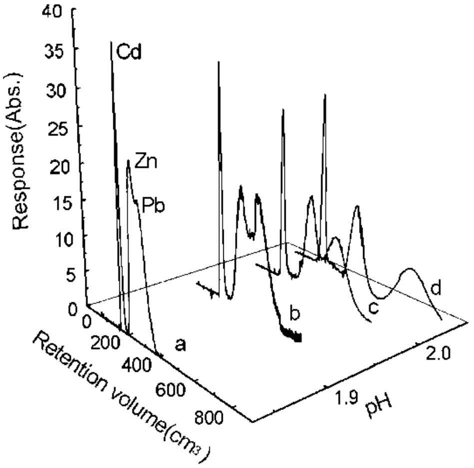

*Figure 6.* Effect of mobile phase pH on HPCPC separation of Cd(II), Zn(II), Pb(II). Mobile phase: 0.10 M trichloroacetate; Mobile phase pH: 1.83 (line a); 1.93 (line b); 2.00 (line c); 2.06 (line d). The other conditions are seen in Fig. 2.

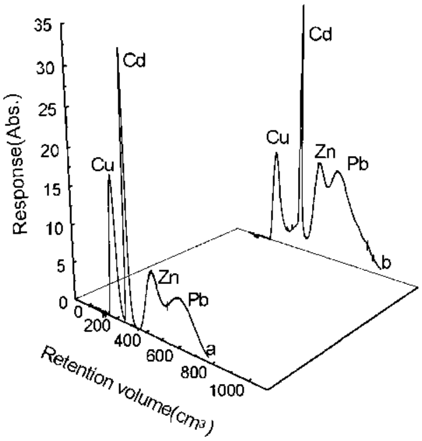

*Figure 7.* HPCPC separation of Cu(II), Cd(II), Zn(II) and Pb(II) by a two step elution method. Mobile phase: 0.10 M trichloroacetate; Flow rate: 1.0 $cm^3/min$; Mobile phase: $pH_1 = 2.65$, $pH_2 = 2.00$, changed at 190 $cm^3$ retention volume (line a); Mobile phase: $pH_1 = 2.69$, $pH_2 = 2.06$, changed at 105 $cm^3$ retention volume (line b). The other conditions are seen in Fig. 2.

Zn(II)-Pb(II). The HPCPC separation became more difficult when an elution peak became broader at a larger retention volume.

## CONCLUSIONS

The results obtained in this work show that DMNPA can be used as an effective reagent for the separation of Cu(II), Cd(II), Zn(II), and Pb(II) in organic acid media. The HPCPC separation of the heavy metal ions was investigated using DMNPA/heptane as a stationary phase. The effect of some experimental factors on separation parameters was examined in order to choose the optimum operating conditions for the present separation system. The separation of Cu(II)-Cd(II)-Pb(II)-Zn(II) was tested with 0.10 M trichloroacetate buffer mobile phases (pH 2.60 and pH 2.06) as a flow rate of 1.0 $cm^3/min$. The HPCPC separation of Cu(II)-Cd(II)-Zn(II) was found to be well resolved but Zn(II)-Pb(II) co-eluted. We also obtained the base line separation of such metal ion mixtures as Cu(II)-Cd(II), Pb(II)-Zn(II), and Cd(II)-Zn(II)-Pb(II) by the present HPCPC method.

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