

# Cations on Ion Chromatography by Phosphate-Coated Zirconia Stationary Phase Column

M. MORI<sup>1,\*</sup>, T. MASUNO<sup>1</sup>, D. KOZAKI<sup>2</sup>, N. NAKATANI<sup>3</sup>, K. TANAKA<sup>4</sup>, AND H. ITABASHI<sup>1</sup>

<sup>1</sup>Graduate School of Science and Technology, Gunma University, 1-5-1 Tenjin-cho, Kiryu, 376-8515, Japan

<sup>2</sup>Faculty of Industrial Sciences and Technology, University Malaysia Pahang, Lebuhraya Tun Razak, 26300 Gambang Kuantan, Pahang Darul Makmur, Malaysia

<sup>3</sup>Department of Environmental and Symbiotic Sciences, College of Agriculture, Food and Environmental Science, Rakuno Gakuen University, 582 Bunkyo-dai-Midorimachi, Ebetsu, Hokkaido 069-8501, Japan

<sup>4</sup>College of Engineering, Chubu University, 1200, Matsumoto-cho, Kasugai, Aichi 487-8501, Japan

\*E-mail: mori@gunma-u.ac.jp

**Summary.** Ion chromatography of inorganic cations using a phosphate-coated zirconia stationary phase (PZ) was first attempted. The retentions of cations to PZ increased by elevating the column temperature and the reproducibility of the separation could improve at the higher temperature. The PZ functioned as a cation-exchanger from changes in the retention factor of cations as a function of eluent pH. Furthermore, the Gibbs free energies of cations were estimated from enthalpy and entropy using the retention factors of cations as a function of the column temperature. The reaction was based on the endothermic reaction.

**Key Words:** ion chromatography, zirconia, phosphate, cations, temperature

## Introduction

Separations of inorganic ion in ion chromatography (IC) are affected by not only the types of functional groups in the column but also types of supports used to fix the functional groups. The supports commonly used as IC column are silica gel and organic polymers such as polystyrene-divinylbenzene, mainly. These have widely been used as the supports to fix ion-exchangers. Because silica gel constructs microparticulation and is highly porous, the silica gel-supported ion-exchange column can provide high theoretical plate numbers of analyte peaks. However, such a column is limited as to the eluent pH that can be used because silica gel dissolves in alkali solutions. In contrast, an organic polymer-supported ion-exchange column can be used over a wide ranges of eluent pH on account of its high acid/base resistance, but it causes the shrinkage or swelling by organic solvents.

Therefore, many researchers have attempted to use supports such as graphite carbon [1, 2], apatite [3], and titania [4, 5] as alternatives to silica gel or polymers. Use of a graphite carbon stationary phase, which has excellent chemical and physical resistance [6], has been considered effective because it can fix ion-exchangers such as ionized surfactants [2] and can use a buffer solution over a wide range of pH (1–14). The ion-exchangers are primarily fixed by hydrophobic interaction with the graphite carbon stationary phase, which itself shows no ligand-exchange behavior.

In this study, our research group is proceeding by the use of a zirconia stationary phase (ZrSP) column in the IC. It functions as an anion-exchanger ( $[\text{Zr-OH}_2]^+$ ) when the eluent pH is lower than the point of zero charge of zirconia ( $\text{pH}_{\text{pzc}} = 6.4$ ) and as a cation-exchanger ( $[\text{Zr-O}]^-$ ) when the eluent pH is higher than 6.4 [7]. Such properties are also found in titania, but the chemical resistance of titania is inferior to that of zirconia. Therefore, using ZrSP column, we attempted the separation of inorganic ions under acidic and basic eluents, but it could not separate each ion.

To enhance analyte selectivity, Carr et al. [7–11] and other researchers [12–14] modified various inorganic and organic species possessing ionic functional groups and applied them to liquid chromatographic columns. However, approaches to ion chromatographic columns are discussed in fewer reports.

We electrostatically fixed various types of Lewis bases, e.g., inorganic anions (fluoride, sulfate, and phosphate), aliphatic carboxylic anions (acetate, oxalate, citrate, tartrate, and malate), or aminopolycarboxylic anions (ethylenediaminetetraacetate and iminodiacetate), which are well-known to strongly interact with zirconia, onto ZrSP [15, 16]. Consequently, coating phosphate ion to the ZrSP column conducted to IC of cations. This communication describes the feature of the phosphate-coated ZrSP (PZ) column that showed functionality as a ligand for inorganic cations, in terms of the column temperature, the eluent pH, and the reproducibility to the retention behaviors of analyte cations.

## Experimental

### Apparatus

The ion chromatograph used was a Tosoh IC-2001, which consisted of a dual pump, on-line degasser, sample injector, and conductometric detector. The eluent flow rate was  $0.6 \text{ mL min}^{-1}$ , and the injection volume was  $30 \mu\text{L}$ . The column oven used a thermostat bath (Senshu Scientific Co. Ltd., Japan) to change the temperature in the range of  $40 \text{ }^\circ\text{C}$ – $100 \text{ }^\circ\text{C}$ . A backpressure coil

(2 m length, 0.5 mm i.d.) was connected before the conductivity detector in the IC-2001 to reduce the temperature of the solutions from the separation column.

### Eluent and Standard Sample

All reagents were of analytical reagent grade and were purchased from Wako (Osaka, Japan). Solutions were prepared by dissolving them in distilled and deionized water (water) to a concentration of 0.1 M, and dilutions to the appropriate concentrations were prepared by water as necessary. The eluent was nitric acid. The standard sample comprised a mixture of chloride salts (NaCl, KCl, MgCl<sub>2</sub>, CaCl<sub>2</sub>, BaCl<sub>2</sub>, MnCl<sub>2</sub>, ZnCl<sub>2</sub>, and CdCl<sub>2</sub>) in aqueous solution, each at a concentration of 1 mM.

### Column

The ZrSP column employed in this study was a ZirChrom®-PHASE (3 μm particle, 50 × 4.6 mm i.d.). After washing in order of distilled-deionized water and acetonitrile, the coating of phosphate on the ZrSP column was obtained by passing the column through a solution with 1 mM phosphoric acid at a flow rate of 0.6 mL min<sup>-1</sup> for 1 h at 80 °C. The prepared column was a phosphate-coated ZrSP (PZ) column. The cation-exchange capacities of PZ in pH 3 by breakthrough method using 1 mM nitric acid [17] were 137 μmol g<sup>-1</sup>. It was equilibrated thoroughly with eluent for 60 min before chromatographic runs.

## Results and Discussion

The retention behaviors of several cations on bare ZrSP (i.e., uncoated ZrSP) and PZ columns were observed by elution of nitric acid at column temperatures of 40 °C and 80 °C. As shown in *Fig. 1a*, in a bare ZrSP column, anion (Cl<sup>-</sup>) was detected overlapping with the elution dip after cation, indicating function as anion-exchanger. When elevating the column temperature to 80 °C, the retention time of Cl<sup>-</sup> was prolonged but the separation from elution dip could not be achieved. This could be mainly owing to the lower selectivity of bare ZrSP for anions.

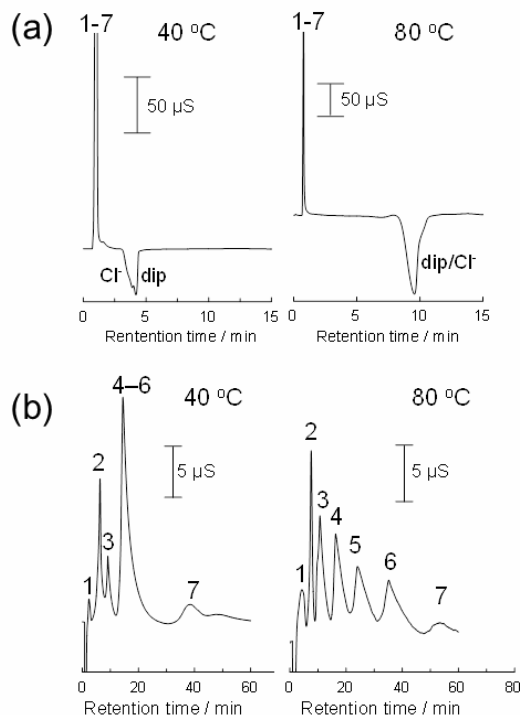


Fig. 1. Chromatograms of inorganic cations as a function of column temperature. Column: (a) bare ZrPS; and (b) PZ columns; particle size: 3  $\mu\text{m}$ ; and size: 50  $\times$  4.6 mm i.d.; eluent: 1 mM nitric acid; injected sample:  $\text{NaClO}_4$ ,  $\text{KCl}$ ,  $\text{MgSO}_4$ ,  $\text{CaCl}_2$ ,  $\text{MnCl}_2$ ,  $\text{BaCl}_2$ ,  $\text{ZnCl}_2$ , and  $\text{CdCl}_2$  (1 mM each). Column temperature: 40  $^\circ\text{C}$  and 80  $^\circ\text{C}$ . Flow rate: 0.6  $\text{mL min}^{-1}$ . Injection volume: 30  $\mu\text{L}$ . Detector: conductivity. Peaks: 1 =  $\text{Na}^+$  and  $\text{K}^+$ , 2 =  $\text{Mg}^{2+}$ , 3 =  $\text{Ca}^{2+}$ , 4 =  $\text{Ba}^{2+}$ , 5 =  $\text{Mn}^{2+}$ , 6 =  $\text{Zn}^{2+}$ , and 7 =  $\text{Cd}^{2+}$

Reversely, in a PZ column, cations were eluted after anions (Fig. 1b), indicating function as a cation-exchanger. Analyte divalent cations could separate at 80  $^\circ\text{C}$  by using the PZ column, while the monovalent cations ( $\text{Na}^+$  and  $\text{K}^+$ ) could not separate even when elevating the temperature. Also, the relative standard deviation (RSD) values in retention time and peak area of  $\text{Zn}^{2+}$  in the 20th successive runs were 11% at 40  $^\circ\text{C}$  and 2.6% at 80  $^\circ\text{C}$  in PZ column, reproducibility. The retention time of each cation at a column temperature of 100  $^\circ\text{C}$  was further enhanced compared to the column temperature of 80  $^\circ\text{C}$ , but the conductometric noise level was much enhanced (data not shown).

The retention times of cations prolonged regardless to types of the columns by elevating the column temperature. Accordingly, the reaction

was spontaneous, and this is expected to be related to the nature of zirconia itself, though origin of the effect is not well understood yet.

The  $k'$  values of the analyte cations ( $\text{Na}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{Zn}^{2+}$ ) as a function of eluent pH in PZ column at 80 °C were estimated using eq. (1):

$$k' = (t_R - t_0)/t_0 \quad (1)$$

where  $t_0$  is the retention time of  $\text{Cl}^-$  that is completely excluded from the stationary phase, and  $t_R$  is that of the analyte cations.

As shown in Fig. 2, the slopes in the approximations obtained from the relationship between logarithms of  $k'$  values of cations as a function of the eluent pH were positive. The slopes of divalent cations were near 2 and were twice larger than those of monovalent cation  $\text{Na}^+$ . The reaction between analyte cations and PZ might be based on a cation-exchange reaction.

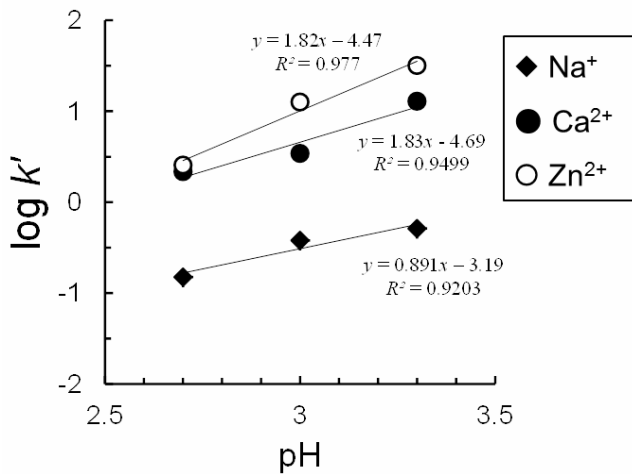


Fig. 2. Logarithms of retention factor ( $k'$ ) of analyte cations as a function of the eluent pH. Column: PZ column. Eluent: 1 mM nitric acid. Injected sample:  $\text{NaCl}$ ,  $\text{MgCl}_2$ , and  $\text{CaCl}_2$  (1 mM each). Column temperature: 80 °C. Other conditions are the same as in Fig. 1

Next, the retention factor ( $k'$ ) of the analyte cations by PZ column as a function of column temperature was estimated using eq. (1). Furthermore, the logarithms of  $k'$  values of the cations were plotted as a function of the reciprocal of column temperature. As shown in Fig. 3, the slopes in the approximations obtained from their plots had negative values. The adsorption of cations to PZ column is expected to be based on the endothermic reaction with increase of column temperature.

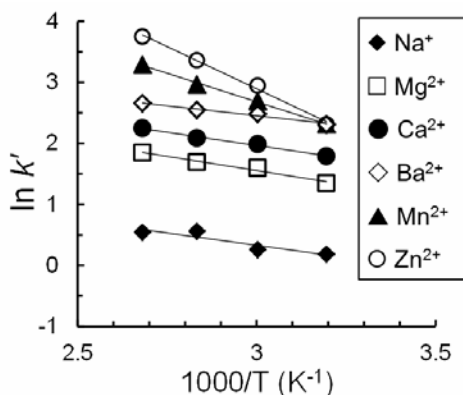


Fig. 3. Logarithms of retention factor ( $k'$ ) of analyte cations to reciprocal of column temperature. Column: PZ column. Other conditions are the same as in Fig. 1

Furthermore, van't Hoff equation [eq. (2)] was used to estimate enthalpy ( $\Delta H$ ) and entropy ( $\Delta S$ ) occurring between analyte cations and the stationary phase.

$$\ln k' = -\Delta H/RT + \Delta S/R + \ln \Phi \quad (2)$$

where  $R$  is the gas constant ( $8.31 \text{ J K}^{-1} \text{ mol}^{-1}$ ),  $T$  is absolute column temperature (K), and  $\Phi$  is ratio of volume of stationary phase to that of mobile phase. From  $\Delta H$  and  $\Delta S$ , Gibbs free energies ( $\Delta G$ ) for analyte cations were estimated using eq. (3).

$$\Delta G = \Delta H - T\Delta S \quad (3)$$

As summarized in Table I, from column temperatures of 40 °C to 100 °C, all  $\Delta G$  values were negative.

Table I. Enthalpy ( $\Delta H$ ), entropy ( $\Delta S$ ), and Gibbs free energy ( $\Delta G$ ) estimated from retention factor ( $k'$ ) of analyte cations as a function of temperature in a PS column

Analyte	$\Delta H$ (kJ mol <sup>-1</sup> )	$\Delta S$ (J mol <sup>-1</sup> K <sup>-1</sup> )	$\Delta G$ (kJ mol <sup>-1</sup> )	
			40 °C	100 °C
Na <sup>+</sup>	6.73	22.4	-0.281	-1.63
Mg <sup>2+</sup>	7.74	34.9	-3.18	-4.58
Ca <sup>2+</sup>	7.15	36.2	-4.18	-5.63
Ba <sup>2+</sup>	5.42	35.4	-5.66	-7.08
Mn <sup>2+</sup>	15.5	67.8	-5.72	-8.43
Zn <sup>2+</sup>	23.0	91.9	-5.76	-9.44

Conditions: eluent, 1 mM HNO<sub>3</sub>; column temperature, 40–100 °C. Other conditions are the same as in Fig. 1.

In conclusion, enhancement of the retention time and improvements of RSDs by elevating temperature of PZ column could be related to strong immobilization of phosphate ion to the ZrSP at the higher temperature, based on the endothermic reaction. Further investigations must be continued for selections of ligands onto the ZrSP, elution conditions, and coating method to improve peak resolutions, considering practical uses.

## Acknowledgment

This work was supported by a Grant-in-Aid for Scientific Research (23615003) from the Japan Society for the Promotion of Science.

## References

- [1] H. Nagashima and T. Okamoto, *J. Chromatogr. A*, **855**, 261 (1999)
- [2] K. Yoshikawa, M. Okamura, M. Inokuchi, and A. Sakuragawa, *Talanta*, **72**, 305 (2007)
- [3] S. Schubert and R. Freitag, *J. Chromatogr. A*, **1216**, 3831 (2009)
- [4] K. Tani and H. Kubojima, *Chromatographia*, **47**, 655 (1998)
- [5] K. Tani and Y. Suzuki, *Chromatographia*, **46**, 623 (1997)
- [6] T. Hanai, *J. Chromatogr. A*, **989**, 183 (2003)
- [7] J. Nawrocki, M. Rigney, A. McCormick, and P.W. Carr, *J. Chromatogr. A*, **657**, 229 (1993)
- [8] J. Blackwell and P.W. Carr, *J. Liq. Chromatogr.*, **14**, 2875 (1991)
- [9] J. Blackwell and P.W. Carr, *J. Chromatogr.*, **596**, 27 (1992)
- [10] A. Clausen and P.W. Carr, *Anal. Chem.*, **70**, 378 (1998)
- [11] S. Sarkar, P.W. Carr, C.V. McNeff, and A. Subramanian, *J. Chromatogr. B*, **790**, 143 (2003)
- [12] A. Mullick and M.C. Flickinger, *Biotechnol. Bioeng.*, **65**, 282 (1999)
- [13] L.-F. Yao, H.-B. He, Y.-Q. Feng, and S.-L. Da, *Talanta*, **64**, 244 (2004)
- [14] A. Subramanian and S. Sarkar, *J. Chromatogr. A*, **944**, 179 (2002)
- [15] G. Alberti, *J. Inorg. Nucl. Chem.*, **30**, 317 (1986)
- [16] IUPAC Chemical Data Series No. 22, *Stability constants of metal-ion complexes, Part B, Organic Ligands*, Pergamon Press, Oxford, UK, 1979, p. 45
- [17] Y. Ueki, T. Umemura, J. Li, T. Odake, and K. Tsunoda, *Anal. Chem.*, **76**, 7007 (2004)