

CHROM. 21 607

Note

Capillary isotachophoretic separation of phosphate, arsenate, germanate, silicate and molybdate ions using complex-forming equilibria

MASAHIKO KAN, FUMIO KOMATSU, SHUNITZ TANAKA, HITOSHI YOSHIDA and MITSUHIKO TAGA*

Department of Chemistry, Faculty of Science, Hokkaido University, Sapporo 060 (Japan)

(First received March 28th, 1989, revised manuscript received May 8th, 1989)

Capillary isotachopheresis (CITP) is an excellent technique for the separation and determination of ionic species, requiring only a short period of time and small amounts of samples.

In a previous paper¹, we have shown that CITP in combination with an enrichment technique is useful for the determination of phosphate ion at the 1 μM level. Phosphate ion was collected on a membrane filter by a facile and rapid procedure as the ion pair of molybdophosphate with bis[2-(5-chloro-2-pyridylazo)-5-diethylaminophenolato]cobalt(III). The ion pair was dissolved in N,N-dimethylformamide and the solution was injected into the CITP analyzer. In this method, however, the separation of phosphate ion from arsenate ion, which was also enriched, was not achieved.

Phosphate and arsenate ions resemble each other in chemical properties. Therefore, the determination of one of these ions is often subject to interference by the other ion in some methods, such as spectrophotometry based on the formation of a heteropoly acid with molybdate^{2,3}. The separation of phosphate and arsenate by chromatographic methods has been reported^{4,5}. However, there is no report of the separation of these ions by CITP. Such a separation is difficult because of the resemblance in chemical properties and, therefore, the similarity in effective mobilities.

Some attempts have been made to improve the separability in CITP⁶⁻⁹, of which the use of complex-forming or ion-pairing equilibria is most effective. We have also developed some migration systems using complex-forming or ion-pairing equilibria for the separation of ionic species whose effective mobilities were similar¹⁰⁻¹³ or for electrically neutral species such as catechol derivatives¹⁴.

In the present paper, the separation of phosphate and arsenate as well as of germanate, silicate and molybdate is described. The complex-forming equilibria of phosphate and arsenate ions with magnesium ion added to the leading electrolyte are effective for the separation. The difference in the effective mobilities of phosphate and arsenate ions increases with the difference in stabilities of the complexes.

EXPERIMENTAL

Apparatus

A Shimadzu Model IP-1B capillary isotachophoretic analyzer with a potential gradient detector was used. The separation was carried out in a polytetrafluoroethylene isotachophoretic tube consisting of a precapillary tube (50 mm \times 1.0 mm I.D.) and a main capillary tube (150 mm \times 0.5 mm I.D.).

Reagents

The stock solutions of phosphate, arsenate and molybdate ions were prepared by dissolving potassium dihydrogenphosphate, disodium hydrogenarsenate and ammonium molybdate in water, respectively. For the stock solutions of germanate and silicate ions, standard solutions of germanium and silicon (Wako Pure Chemical Industries, Osaka, Japan, atomic absorption spectrophotometry grade) were used. The stock solution of germanium was an aqueous solution of germanium(IV) oxide and that of silicon was a 0.02 *M* sodium carbonate aqueous solution of sodium silicate.

The leading electrolyte was prepared by diluting stock solutions of 1 *M* hydrochloric acid, 1 *M* magnesium chloride solution and 1% poly(vinyl alcohol), and the pH was adjusted by adding tris(hydroxymethyl)aminomethane (Tris). The pH of the terminating electrolyte was adjusted by adding potassium hydroxide.

The leading and terminating electrolyte systems are listed in Table I.

RESULTS AND DISCUSSION

Selection of electrolyte systems

For the migration of germanate and silicate ions which are conjugate bases of weak acids, alkaline electrolyte systems were used. As the buffering counter ions in these systems, Tris and ammonia were examined. Ammonia was not suitable because the difference in the effective mobilities of the leading and terminating ions is small. Tris is useful as a buffering counter ion up to pH *ca.* 9 and has an adequate migration velocity.

In the alkaline solutions, a zone of carbonate ion penetrated from the terminating electrolyte across the sample zones, and therefore the time required for analysis

TABLE I
LEADING AND TERMINATING ELECTROLYTE SYSTEMS

	<i>Leading electrolyte</i>	<i>Terminating electrolyte</i>
Ion	10 mM Cl ⁻ (added as HCl and MgCl ₂)	10 mM β -alaninate
Counter ion	TrisH ⁺	K ⁺ (added as KOH)
pH	8.5	10.4
Additives	0.01% poly(vinyl alcohol) 3 mM Mg ²⁺ (added as MgCl ₂) 5% Methanol	

increased. To shorten this zone barium hydroxide was added to the terminating electrolyte¹⁵ but the zone length of carbonate ion was little affected. This is explained by the fact that the major part of the carbonate ion comes from the injected sample solution containing silicate ion. The stock solution of silicate contains sodium carbonate.

Effect of pH

The effect of the pH in the leading electrolyte on the effective mobilities of the ions is shown in Fig. 1. The R_E value represents the ratio of the potential gradient of the sample ion or terminating ion to that of the leading ion. The R_E values of germanate and silicate ions decreased with increasing pH. An increase of the pH of the leading electrolyte suppresses the protonation of the silicate and germanate ions, so the effective mobilities of these ions increase. There was little variation of the R_E values of phosphate, arsenate and molybdate ions with change of pH because the degree of protonation of these ions is not so greatly influenced by the change in pH within the range mentioned here.

At pH 8, the difference in the effective mobilities of the silicate and terminating ions was small and high voltage was applied because of their low degree of ionization. At pH 9, on the other hand, arsenate and hydrogencarbonate ions had similar effective mobilities. For the separation of the ions mentioned, the leading electrolyte was adjusted to pH 8.5.

Effect of complex-forming equilibria

For the separation of phosphate and arsenate, the utility of complex-forming equilibria between these ions and magnesium ion was investigated. The effect of the concentration of magnesium ion in the leading electrolyte on the R_E values is shown in Fig. 2.

Without magnesium ion, the R_E values of arsenate and hydrogencarbonate ions were equal and that of phosphate ion was somewhat smaller. Upon addition of magnesium ion to the leading electrolyte, the R_E value of phosphate ion increased. The R_E value of arsenate also increased, but not so much as that of phosphate.

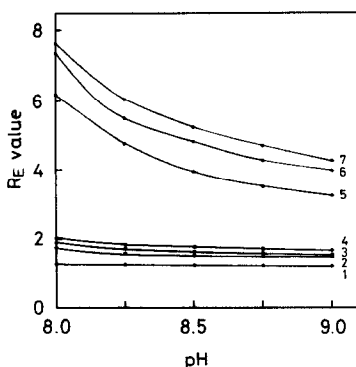


Fig. 1. Effect of the pH of the leading electrolyte on the R_E values of molybdate (1), hydrogencarbonate (2), arsenate (3), phosphate (4), germanate (5), silicate (6) and β -alanine (7) ions. Electrolyte systems as in Table I except for the pH of the leading electrolyte.

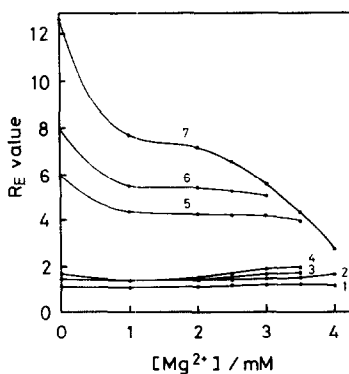


Fig. 2. Effect of the concentration of magnesium ion in the leading electrolyte on the R_E values. Curves as in Fig. 1. Electrolyte systems as in Table I except for the concentration of magnesium ion.

With increasing concentration of magnesium ion, complexes are formed between phosphate and arsenate ions with magnesium ion and the net charges of the complexes decrease. The difference in the effective mobilities of phosphate and arsenate is caused by the difference in the stabilities of the complexes.

The R_E values of germanate, silicate and molybdate ions were little influenced by the complex-forming equilibria with magnesium ion. The R_E value of the terminating ion decreased with increasing concentration of magnesium ion. The zone of silicate disappeared at 3.5 mM magnesium ion and those of phosphate, arsenate and germanate ions disappeared at 4 mM because of their effective mobilities were lower than that of the terminating ion.

Calcium ion was not suitable as a complexing agent because of the formation of a precipitate with phosphate.

Effect of organic solvents

To enhance the interaction between the above ions and magnesium ion, methanol was added to the leading electrolyte. The effect of the methanol concentration on the R_E values is shown in Fig. 3.

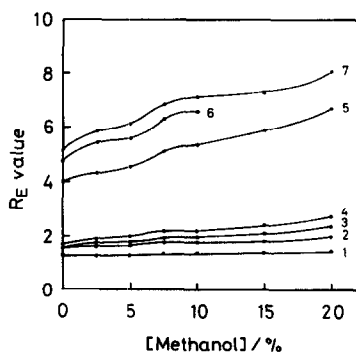


Fig. 3. Effect of the concentration of methanol in the leading electrolyte on the R_E values. Curves as in Fig. 1. Electrolyte systems as in Table I except for the concentration of methanol.

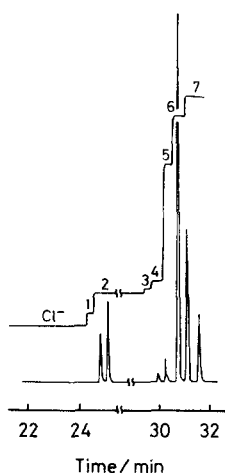


Fig. 4. Isotachopherogram: 1 = molybdate (2.5 nmol); 2 = hydrogencarbonate; 3 = arsenate (1.1 nmol); 4 = phosphate (2.5 nmol); 5 = germanate (3.6 nmol); 6 = silicate (6.4 nmol); 7 = β -alanine ions. Electrolyte systems as in Table I. Driving current: 50 μ A.

Without addition of methanol, there was no difference in the R_E values of arsenate and hydrogencarbonate ions. By the addition of only 2.5% methanol, the zone of arsenate ion was completely separated from that of hydrogencarbonate ion. With the increasing methanol concentration, the difference in the R_E values became larger. This is due to the increase in the interaction of arsenate ion with magnesium ion.

The zone of silicate disappeared at more than 15% methanol because its effective mobility is lower than that of the terminating ion.

Acetone was not suitable as an organic solvent because its boiling point is lower and migrations were often interrupted by the generation of bubbles.

Calibration graphs

An isotachopherogram obtained under the optimum conditions is shown in Fig. 4. The separation of phosphate, arsenate, germanate, silicate and molybdate ions is achieved and the boundaries of each zone are very sharp.

Calibration graphs for each ion were constructed by linear regressions and their parameters are given in Table II. Although it is known that silicate ion forms a

TABLE II
PARAMETERS OF LINEAR REGRESSIONS FOR CALIBRATION GRAPHS

<i>Ion</i>	<i>Intercept (s)</i>	<i>Slope (s nmol⁻¹)</i>	<i>Correlation coefficient</i>	<i>Range (nmol)</i>
Phosphate	0.55	10.29	0.994	0.5–2.5
Arsenate	1.21	14.14	0.994	0.5–2.5
Germanate	1.48	4.97	0.997	0.5–3.6
Silicate	1.98	3.75	0.996	2.0–12
Molybdate	1.19	5.88	0.999	2.5–21

TABLE III
RELATIVE STANDARD DEVIATIONS OF ZONE LENGTHS

<i>Ion</i>	<i>R.S.D. (%)</i>	<i>Replicate</i>	<i>Amount (nmol)</i>
Phosphate	2.0	9	2.5
Arsenate	2.6	9	1.1
Germanate	8.4	10	3.6
Silicate	3.8	10	6.4
Molybdate	3.2	10	2.5

precipitate with magnesium ion, it had no influence within the concentrations studied. Relative standard deviations of the zone lengths for each ion are listed in Table III. The linearities of calibration graphs and the reproducibilities of the zone lengths for each ion are satisfactory for the determination of these ions.

REFERENCES

- 1 M. Taga, M. Kan, F. Komatsu, S. Tanaka and H. Yoshida, *Anal. Sci.*, 5 (1989) 219.
- 2 M. Taga and M. Kan, *Bull. Chem. Soc. Jpn.*, 62 (1989) 1482.
- 3 T. Nasu and M. Kan, *Analyst (London)*, 113 (1988) 1683.
- 4 T. Takamatsu, M. Kawashima and M. Koyama, *Bunseki Kagaku*, 28 (1979) 596.
- 5 R. J. Williams, *Anal. Chem.*, 55 (1983) 851.
- 6 F. M. Everaerts, J. L. Beckers and T.P.E.M. Verheggen, *Isotachophoresis, Theory, Instrumentation and Applications*, Elsevier, Amsterdam, 1976, Chs. 16 and 17.
- 7 H. Miyazaki and K. Katoh, *Tohoku Denki Eidoh Hoh*, Kohdansha, Tokyo, 1980, Ch. 7.
- 8 P. Boček, M. Deml, P. Gebauer and V. Dolnik, *Analytical Isotachophoresis*, VCH, Weinheim, 1988, Ch. 9.
- 9 P. Boček, I. Miedziak, M. Deml and J. Janák, *J. Chromatogr.*, 137 (1977) 83.
- 10 I. Nukatsuka and H. Yoshida, *J. Chromatogr.*, 237 (1982) 506.
- 11 H. Yoshida, M. Hida and M. Taga, *J. Chromatogr.*, 325 (1985) 179.
- 12 Y. Hirama and H. Yoshida, *Nippon Kagaku Kaishi*, 1986, 943.
- 13 S. Tanaka, T. Kaneta and H. Yoshida, *J. Chromatogr.*, 447 (1988) 383.
- 14 S. Tanaka, T. Kaneta and H. Yoshida, *Anal. Sci.*, 5 (1989) 217.
- 15 P. Boček, M. Deml, P. Gebauer and V. Dolnik, *Analytical Isotachophoresis*, VCH, Weinheim, 1988, Ch. 10, p. 191.