

## Note

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### High-performance liquid chromatography using a reagent solution as a component of the mobile phase

#### I. Separation of alkaline earth metal cations with *o*-cresolphthaleine complexone

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(Received January 23rd, 1986)

Modern ion chromatography, in which inorganic ions are separated on a low capacity ion-exchange gel and detected with a conductometric detector, is of great importance in inorganic analysis<sup>1</sup>. There have been few applications to metal analysis because of its low selectivity and sensitivity. In this field, ion-exchange chromatography with post-column derivatization in which a colour-forming agent is added to the column eluent allows sensitive determination of metal cations<sup>2–4</sup>. However, the analytical precision is greatly influenced by the pump delivery system and the conditions are unstable.

Zenki<sup>1</sup> has already reported the separation of alkaline earth metal cations using a colour-forming agent as a component of the mobile phase. Though the method has the advantage of low baseline noise and simple apparatus, it is restricted to those ions which form a coloured complex and suitable separation is not always achieved.

In this paper we describe a modified system which after formation of a complex between the alkaline earth cation and a colour-forming agent in acidic solution, requires only the addition of an alkaline buffer solution for a sensitive determination. Though there have been many reports on the separation of metal cations with sulphonic acid-type cation-exchange gel columns<sup>6,7</sup>, there have been few reports on the use of carboxylic acid-type gel columns for this purpose. Thus we investigated the separation of alkaline earth metal cations on a chemically bonded carboxylic acid-type silica gel using a colour-forming agent as a component of the mobile phase. Four cations, magnesium, calcium, strontium and barium, were separated within 25 min. Isocratic and gradient elution are described.

#### EXPERIMENTAL

##### *Reagent*

*o*-Cresolphthaleine complexone (PC) was obtained from Dojin-kagaku Kenkyusho and used without further purification. All other chemicals and solvents were analytical-reagent grade from commercial sources.

### Apparatus

Chromatography was carried out with a multi-function pump CCPM (two heads, one: for the eluent and the other for the buffer; Toyo Soda), a Rheodyne 7125 injection valve and a separation column (Glycopack 5  $\mu\text{m}$ , 150 mm  $\times$  6 mm I.D., Toyo Soda). The flow-rate of the mobile phase was 1 ml/min. The eluent from the column was mixed with 0.48 *M* ammonium hydroxide solution with the use of the CCPM. The flow-rate of the alkaline solution was 1 ml/min. The absorbance of the mixed solution was monitored at 575 nm with an UV-VIS spectrophotometric detector UV-8000 (Toyo Soda). The cation-exchange capacity of Glycopack, which is a  $-\text{CH}_2\text{COOH}$  type gel, is about 0.3 mequiv./mg.

### RESULTS AND DISCUSSION

#### Isocratic elution

Parameters such as the concentration of colour-forming agent and salt, pH and column temperature were varied to optimize the elution conditions on a chemically bonded carboxylic acid-type ion-exchange silica gel.

It is well known that the salt concentration greatly affects the elution of cations in sulphonic acid-type ion-exchange chromatography<sup>8</sup>. In the present case of the carboxylic acid-type gel it moderately affected the elution. Fig. 1 shows the effect of potassium. This result suggests that at weakly acidic pH only a part of the carboxylic acid on the gel ionizes and the cation-exchange capacity is moderately altered by the change in salt concentration. When much sodium salt (0.5 *M* sodium chloride) was

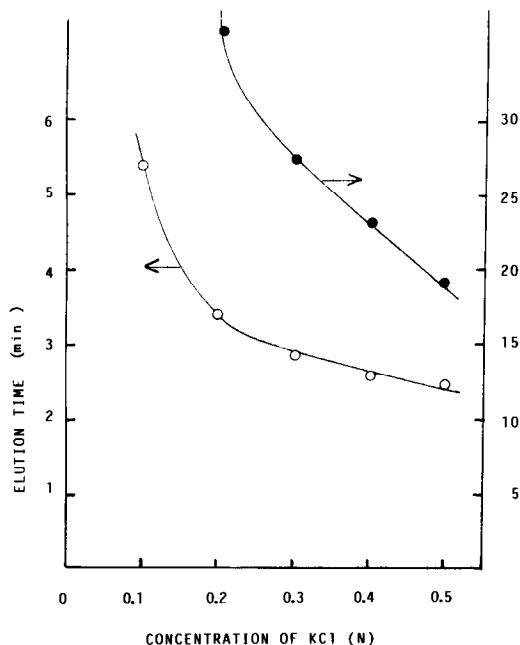


Fig. 1. Effect of potassium chloride on the elution time at pH 3.9 and 1 mM PC. Column temperature: 40°C. ○—○, Magnesium; ●—●, calcium.

injected into the mobile phase of this system, a larger peak was obtained. We concluded that potassium interacted with PC more weakly than sodium. As high salt concentrations resulted in an increase in the baseline and in baseline noise, 0.25 *M* potassium chloride was selected.

Fig. 2 shows the effect of the reagent concentration on the elution time. The lack of an effect is due to the fact that at neutral pH, PC does not act a chelating agent towards the metal ions, in contrast to the carboxylic acid of the gel. That is, in this case, PC in the mobile phase only plays an analytical rôle. As a high concentration of PC brought about a high baseline and also a high detection limit, 1 *mM* PC was selected.

Fig. 3 shows the effect of pH on the elution time. The marked effect arises because the chelating capacity of the carboxylic acid of the gel varies greatly in the weakly acidic pH range<sup>9</sup>. When the pH is increased, both the cation-exchange capacity and the elution time increased. Although magnesium was eluted at pH 4, calcium was not. So a pH of 3.9 was selected. The pH was adjusted with acetic acid and sodium acetate and the concentration of 0.02 *M* was selected because of easy alkalization by post-column addition of ammonium hydroxide.

Fig. 4 shows the effect of column temperature on the elution time. Though the elution time increased with increasing column temperature in the case of a sulphonic acid-type cation-exchange gel<sup>8</sup>, in this case the opposite result was observed. Upon increasing the column temperature, the chelating capacity of the gel may be reduced and the elution accelerated; it also resulted in a decrease in column pressure and also in high baseline noise, so 40°C was selected.

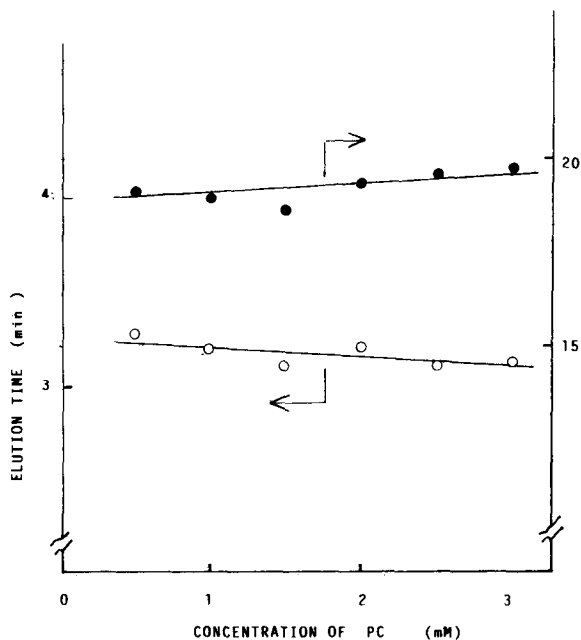


Fig. 2. Effect of reagent concentration on the elution time at 0.25 *M* potassium chloride and pH 3.9. Other details as in Fig. 1.

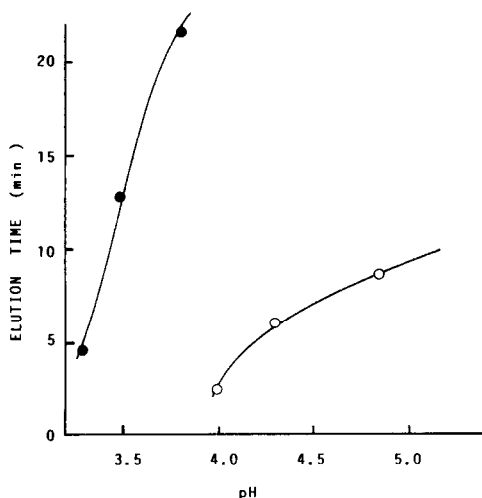


Fig. 3. Effect of pH on the elution time at 0.25 M potassium chloride and 1 mM PC. Other details as in Fig. 1.

#### Gradient elution

Sometimes alkaline earth metal cations coexist in environmental samples, especially magnesium and calcium. Under isocratic elution conditions, the magnesium was eluted rapidly and the calcium was retained. So a salt gradient was employed. Fig. 5 shows a separation of these ions within 15 min.

When a salt gradient is adopted, this method, in which the reagent is a component of the mobile phase, is preferred to the usual post-column addition of a

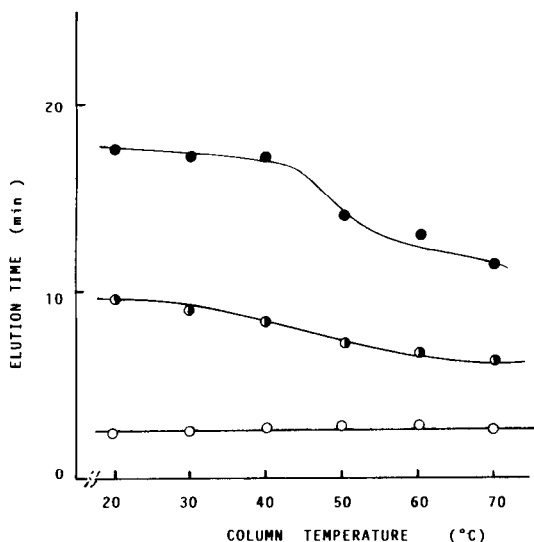


Fig. 4. Effect of column temperature on the elution time. Mobile phase: 0.25 M potassium chloride, pH 3.9, 1 mM PC. ○—○, Magnesium; ●—●, calcium; ◐—◐, strontium.

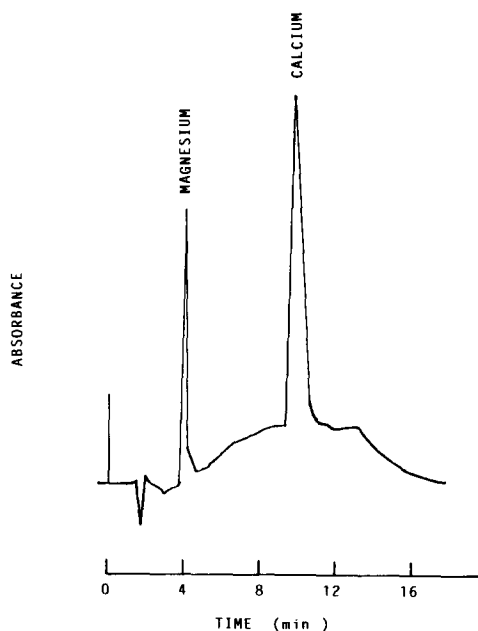


Fig. 5. Chromatogram of a 5 ng/ml standard solution of magnesium and 10 ng/ml of calcium. Mobile phase as in Table I.

reagent. That is, when we use much salt as a component of mobile phase, a reagent interacts with much salt only after the column in the usual post-column system and it sometimes causes the high baseline noise, but in this system the reagent has already interacted with the salt before the column and the high baseline noise can be eliminated.

Table I shows the reproducibility of the separation. We would emphasize that the gradient elution with a high quality pump-delivery system and a modified post-column procedure that has the advantages of good baseline in using much salt as a component of mobile phase enables an high analytical precision.

The separation of the four alkaline earth metal cations  $Mg^{2+}$ ,  $Ca^{2+}$ ,  $Sr^{2+}$  and

TABLE I  
REPRODUCIBILITY OF THE GRADIENT ELUTION

Mobile phase: 100% A to 100% B in 3 min, isocratic for 7 min, then 100% B to 100% A in 1 min; (A), 0.1 M potassium chloride, pH 3.9, 1 mM PC, 1 ml/min; (B), 0.4 M potassium chloride, pH 3.9, 1 mM PC, 1 ml/min. Injection volume: 20  $\mu$ l containing magnesium (5 ng/ml) and calcium (10 ng/ml). Column temperature: 40°C.

Element	Parameter	Average	R.S.D. (%) ( $n = 5$ )
Mg	Time (min)	4.16	3.08
	Height (cm)	5.73	1.40
Ca	Time (min)	9.75	0.00
	Height (cm)	8.13	2.15

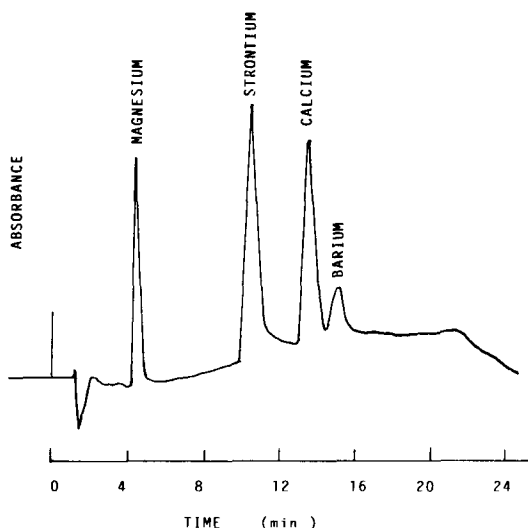


Fig. 6. Chromatogram of 5 ng/ml standard solution of magnesium, 10 ng/ml of calcium, 50 ng/ml strontium and 50 ng/ml of barium. Mobile phase: 100% A to 100% B in 15 min, isocratic for 5 min, then 100% B to 100% A in 5 min; A, B as in Table I. Column temperature: 40°C.

Ba<sup>2+</sup> is important in environmental analysis and Fig. 6 shows a separation of these ions within 25 min using this gradient procedure. It is well known that in the case of sulphonic acid-type cation-exchange chromatography the four are eluted in the order Mg, Ca, Sr, Ba. As Blasius and Brozio<sup>10</sup> had already pointed out, the order is reversed on chelating resins (pyridine-2,6-dicarboxylic acid exchanger) where calcium more strongly bound than strontium.

## CONCLUSION

A new method of ion-exchange chromatography with post-column derivatization, in which only buffer solution is added to the column eluent, was proposed for the separation of alkaline earth metal cations on a chemically bonded carboxylic acid-type gel. As this procedure does not involve post-column derivatization, the analytical precision is higher. The optimum concentrations of reagent and salt, pH and column temperature were evaluated. Good separation of these ions was obtained by gradient elution.

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