

## Spectrophotometric Determination of Calcium with Dicyclohexano-24-crown-8 and Propyl Orange by Solvent Extraction/Flow Injection Method

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Calcium in water was determined by a spectrophotometric method involving flow injection coupled with solvent extraction. An ion association complex which formed between a calcium-crown complex ion and a dye anion was extracted into an organic solvent and the absorbance of the organic phase was measured after phase separation. Six derivatives of alkylaminophenylazobenzene sulfonic acid and tetrabromophenolphthalein ethylester were examined as counter anions with dicyclohexano-24-crown-8, and a mixture of benzene and chlorobenzene was examined as an extraction solvent. A suitable method for calcium determination in water was one with Propyl Orange and a (1+1 v/v) mixture of benzene and chlorobenzene. The carrier stream was distilled water, while the reagent stream was comprised of a dye anion and lithium hydroxide. The sampling rate was 20–30 per hour. The calibration graph was linear at concentrations up to  $10^{-4}$  M using samples of 100  $\mu$ l. The detection limit corresponding to a signal-to-noise ratio of 3 was  $2 \times 10^{-7}$  M; the relative standard deviation was 0.99% for 10 injections of  $5 \times 10^{-5}$  M calcium solution. Calcium in river water was determined satisfactorily by the proposed method.

**Keywords** Calcium, Propyl Orange, dicyclohexano-24-crown-8, spectrophotometry, solvent extraction, flow-injection method

Sixty or more investigations on flow injection methods (FIA) for calcium determination have been done:<sup>1</sup> they are based on various kinds of detection methods, including spectrophotometry, atomic absorption spectrophotometry, atomic emission spectrometry and potentiometry. Of these, the methods using spectrophotometric detection with chelate reagents are simple and versatile in the determination of metal ions: phthalein complexon<sup>2,3</sup>, glyoxal-bis-(2-hydroxylaniline)<sup>4</sup>, 4-(2-pyridylazo)resorcinol<sup>5</sup>, *o*-cresol-phthalein complexon<sup>6,7</sup>, *o,o'*-dihydroxyazo compounds<sup>8</sup> and 2-(3,5-dibromo-2-pyridylazo)-5-[*N*-ethyl-*N*-(3-sulfopropyl)amino]phenol<sup>9</sup> were used. Such spectrophotometry methods are carried out in aqueous solution systems. A solvent extraction-FIA has two main advantages: (1) enhancement of sensitivity by concentration and (2) enhancement of selectivity. However, a solvent extraction-FIA for calcium determination has received little attention. In this work, the authors propose the selective and sensitive methods for calcium based on the solvent extraction of an ion association complex with a crown ether and an anionic azo dye.

### Experimental

#### Reagents

All chemicals used were of analytical-reagent grade,

except the synthesized reagents: alkylaminophenylazobenzene sulfonates (anionic azo dyes).

**Standard calcium solutions.** Dissolve accurate amounts of calcium carbonate, dried at 110°C for 1 h, in diluted hydrochloric acid, then dilute to accurate volume of stock solution. Prepare working solutions by dilution as required.

**Anionic dyes.** Tetrabromophenolphthalein ethylester (TBPE) was commercially available as a potassium salt. Anionic azo dyes (sodium salts) were synthesized as in the previous work<sup>10,11</sup>, according to the literature.<sup>12</sup> The dyes synthesized and purified as sodium salts were converted to acidic forms by acidifying the aqueous solution with hydrochloric acid. The precipitate (acidic form) was filtered, washed with water and dried in a desiccator under reduced pressure. The dyes examined in this work are shown in Fig. 1. They were dissolved in water containing lithium hydroxide.

**Crown ether.** Dicyclohexano-24-crown-8 (DC24C8) was purchased from Nippon Soda Company, Ltd. This was used by dissolving in an extracting solvent. Other crown ether compounds, 18-crown-6 (18C6), benzo-18-crown-6 (B18C6, Nippon Soda Co.Ltd.) and dicyclohexano-18-crown-6 (DC18C6, Aldrich chemical Co.) were also examined.

**Carrier stream.** Distilled water was used.

**Reagent solution stream.** This comprised an anionic

azo dye and a  $10^{-3}$  M lithium hydroxide solution. In the case of TBPE·H, a reagent stream contained only  $10^{-3}$  M lithium hydroxide solution.

**Extraction solvent stream.** A mixture of benzene and chlorobenzene was used. This contained crown ether for anionic azo dye extraction, and contained the crown ether and TBPE·H for TBPE extraction. TBPE·H organic solution was prepared as follows: an aqueous solution of potassium salt of TBPE·H, TBPE·K, was acidified with dilute hydrochloric acid and was shaken with chlorobenzene.

#### Apparatus

The absorbances were measured for anionic azo dyes at the absorption maximum of the ion association complex (410–450 nm); for TBPE·H they were measured at 620 nm, because at the absorption maximum (632 nm) the absorbance of the background was very large. Soma S-3250 visible detector with a 10-mm micro flow cell (8  $\mu$ l) was used for the absorbance measurement, and Toa Dempa FBR-251A and Hitachi

D-2000 Chromato-Integrator were used for recording the peaks and their area, respectively. Two double-plunger micro pumps (Sanuki Kogyo, DM2M-1016) were used to propel the carrier solution, the reagent solution and the extraction solvent streams. Samples were injected by a six-way injection valve with a loop (Sanuki Kogyo) into the carrier stream. The segmentor was a T-shaped connector<sup>13</sup>, in which the aqueous phase flowed straight through and the organic phase flowed at right-angles. The phase separator with porous PTFE membranes (Sumitomo Electric Industries, 0.8  $\mu$ m pore size)<sup>13</sup> was used. The efficiency of organic phase separation can be changed by changing the back pressure of the organic and aqueous phases with needle valves, which were incorporated in the aqueous waste line behind the phase separator and in the organic waste line behind the detector: 100% efficiency of organic phase separation was obtained in this work. The flow lines were made of PTFE tubing (0.5 mm i.d.). A diagram for the flow system is shown in Fig. 2.

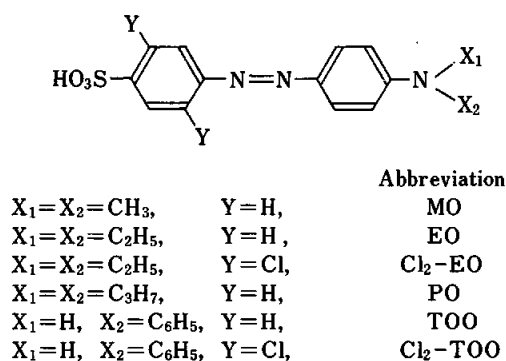


Fig. 1 Derivatives of anionic azo dyes. MO, Methyl Orange; EO, Ethyl Orange; PO, Propyl Orange; TOO, Tropaeolin OO.

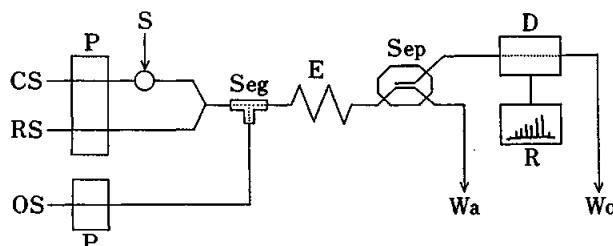


Fig. 2 Schematic flow diagram for determination of calcium. CS, distilled water; RS, Propyl Orange and LiOH solution; OS, DC24C8 (benzene+chlorobenzene=1+1) solution; P, pumps (flow rate, 0.8 ml min<sup>-1</sup>); S, sample injection (100  $\mu$ l); Seg, segmentor; E, extraction coil (0.5 mm i.d.  $\times$  2 m); Sep, separator; D, detector (spectrophotometer, 420 nm); R, recorder; Wa, aqueous phase waste; Wo, organic phase waste.

Table 1 Comparison of the sensitivities of azo dye anions for their complexes with metal-crown cations

| Dye                        | Wave-length/nm | Absorbance of $10^{-4}$ M metal ion solution<br>(absorbance ratio of metal ion to $\text{Ca}^{2+}$ ) |                 |                 |       |       |                 |                 |       |
|----------------------------|----------------|--|-----------------|-----------------|-------|-------|-----------------|-----------------|-------|
|                            |                | Li   | Na              | K               | Rb    | Cs    | Mg              | Ca              | Ba    |
| MO <sup>a</sup>            | 430            |  |                 |                 |       |       |                 |                 | 0.001 |
| EO <sup>a</sup>            | 420            |  |                 |                 |       | 0.001 |                 | 0.002           | 0.164 |
| PO <sup>a</sup>            | 420            | 0.007  | 0.008<br>(0.03) | 0.033<br>(0.12) | 0.039 | 0.057 | 0.025<br>(0.09) | 0.269<br>(1.00) | 0.542 |
| TOO <sup>a</sup>           | 410            |  |                 |                 |       |       |                 | 0.001           | 0.016 |
| $\text{Cl}_2\text{-EO}^a$  | 450            | 0.017  | 0.032<br>(0.04) | 0.122<br>(0.16) | 0.168 | 0.146 | 0.015<br>(0.02) | 0.743<br>(1.00) | 1.713 |
| $\text{Cl}_2\text{-TOO}^a$ | 430            | 0.010  | 0.018<br>(0.10) | 0.070<br>(0.37) | 0.081 | 0.088 | 0.010<br>(0.05) | 0.188<br>(1.00) | 0.408 |
| TBPE <sup>b</sup>          | 620            | 0.108  | 0.330<br>(1.21) | 1.068<br>(3.91) | 0.858 | 0.906 | 0.066<br>(0.24) | 0.273<br>(1.00) | 0.498 |

a. Concentration:  $5 \times 10^{-4}$  M. b. Concentration:  $3 \times 10^{-4}$  M.

## Results and Discussion

### Selection of the dye anion

Six anionic azo dyes shown in Fig. 1 and TBPE·H were examined with a (1+1 v/v) mixture of benzene and chlorobenzene as the extraction solvent. The results obtained are shown in Table 1, where the absorbances corresponding to the peak heights for  $10^{-4}$  M metal ions are summarized. In a series of the anionic azo dyes, the extractability of anionic azo dye ions was as follows:  $\text{MO} < \text{TOO} < \text{EO} < \text{Cl}_2\text{-TOO} < \text{PO}$ ,  $\text{Cl}_2\text{-EO}$  for alkaline earth metals and  $\text{MO}, \text{TOO} \ll \text{EO} < \text{PO} < \text{Cl}_2\text{-TOO} < \text{Cl}_2\text{-EO}$  for alkali metals. The order of the extractability of the anionic azo dyes can be predicted from the contribution values of groups to the extractability<sup>14</sup> as follows:  $\text{MO} < \text{TOO} < \text{EO} < \text{PO} < \text{Cl}_2\text{-TOO} < \text{Cl}_2\text{-EO}$ . This order is the same as the order obtained in the extractability of alkali metal ions. In alkaline earth metals, PO is a more extractable counter ion than  $\text{Cl}_2\text{-TOO}$ , this is because of the steric hindrance of a chloro group just adjacent to the sulfonic group of  $\text{Cl}_2\text{-TOO}$ . The extractability of metal ions with anionic azo dyes was found to be as follows:  $\text{Li}^+ < \text{Na}^+ < \text{K}^+ < \text{Rb}^+ < \text{Cs}^+$ ;  $\text{Mg}^{2+} < \text{Ca}^{2+} < \text{Sr}^{2+} < \text{Ba}^{2+}$ . In the TBPE extraction, potassium ion was the most extractable of five alkali metal ions, and the extractability of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Rb}^+$  and  $\text{Cs}^+$  are larger than that of  $\text{Ca}^{2+}$ , whereas in anionic azo dyes the extractability of the five alkali metal ions is smaller than that of  $\text{Ca}^{2+}$ . This is because the anionic azo dyes possessing sulfonate group probably have more interaction with alkaline earth metal ions than with alkali metal ions, just as sulfate ions react with alkaline earth metal ions such as  $\text{Ca}^{2+}$ ,  $\text{Sr}^{2+}$  and  $\text{Ba}^{2+}$  to form their precipitates.

With respect to the sensitivity for the determination of calcium ions,  $\text{Cl}_2\text{-EO}$  is the best of all. However, PO is recommended with respect to the selectivity for  $\text{Na}^+$  and  $\text{K}^+$ ; that is, the absorbance ratios of  $\text{Na}^+$  and  $\text{K}^+$  to  $\text{Ca}^{2+}$  are the smallest in PO (see Table 1). Thus, PO was selected for the calcium determination.

### Selection of the extraction solvent

Figure 3 shows the effect of a mixing ratio of benzene and chlorobenzene on the peak height. The peak height for calcium ion abruptly increases with an increase in the volume percent of chlorobenzene, whereas increases in the peak heights for alkali metal ions and magnesium ion are small.

The absorbance of the background increased with an increase in the volume percent of chlorobenzene because of the extraction of lithium ion in the reagent solution. The increase in the background absorbance results in bad base-line stability. So, in the further experiments, a (1+1 v/v) mixture of benzene and chlorobenzene was adopted.

### Selection of the crown ether compound

Crown ether compounds, 18C6, B18C6, DC18C6 and

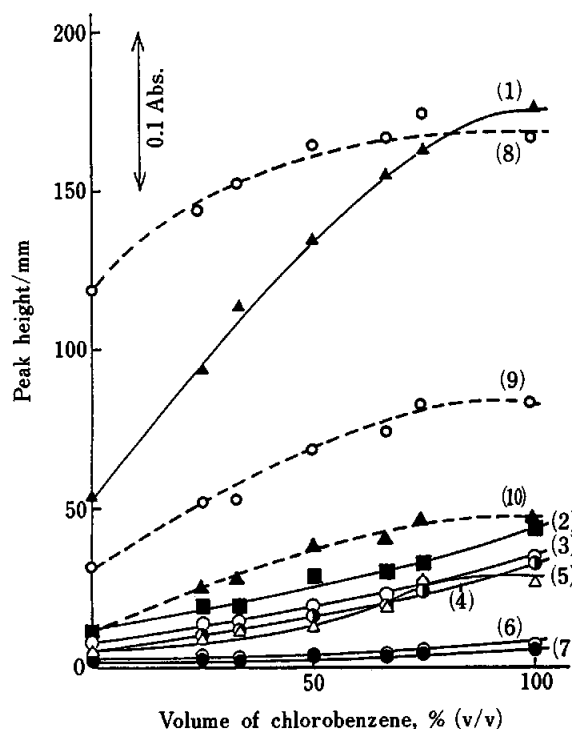


Fig. 3 The effect of mixing ratios of benzene and chlorobenzene. Metal ion: (1)–(7),  $1 \times 10^{-4}$  M; (8)–(10),  $2 \times 10^{-5}$  M. (1) and (10),  $\text{Ca}^{2+}$ ; (2),  $\text{Cs}^+$ ; (3),  $\text{Rb}^+$ ; (4),  $\text{K}^+$ ; (5),  $\text{Mg}^{2+}$ ; (6),  $\text{Na}^+$ ; (7),  $\text{Li}^+$ ; (8),  $\text{Ba}^{2+}$ ; (9),  $\text{Sr}^{2+}$ .

DC24C8 were examined using the (1+1 v/v) mixture of benzene and chlorobenzene. The ratios of peak height for  $\text{Ca}^{2+}$  to  $\text{K}^+$  were almost 1 with 18C6 and B18C6, 1.6 with DC18C6 and 8 with DC24C8, respectively. Thus DC24C8 was used in this work.

### Composition of the organic solution and the reagent solution

With a PO concentration of  $5 \times 10^{-4}$  M, the DC24C8 concentration in the organic phase was varied from  $1 \times 10^{-3}$  M to  $6 \times 10^{-3}$  M. The results obtained are shown in Fig. 4. The peak heights increased with an increase in the crown concentration, and the calibration graphs were linear up to a calcium concentration of  $1 \times 10^{-4}$  M over the examined crown concentration range. Figure 5 shows the effect of crown concentration on the peak height of  $\text{Ca}^{2+}$ , as well as on that of  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Mg}^{2+}$ . The results show that the concentration of crown considerably influences the extractability of calcium. After consideration of the sensitivity and the interference of other ions,  $2 \times 10^{-3}$  M DC24C8 solution was adopted.

With a  $2 \times 10^{-3}$  M concentration of DC24C8, the PO concentration in the reagent solution was varied from  $1 \times 10^{-4}$  M to  $1 \times 10^{-3}$  M. The results are shown in Fig. 6. The peak heights increased with an increase in the PO concentration. The calibration graphs were linear at the concentrations of PO above  $5 \times 10^{-4}$  M. After

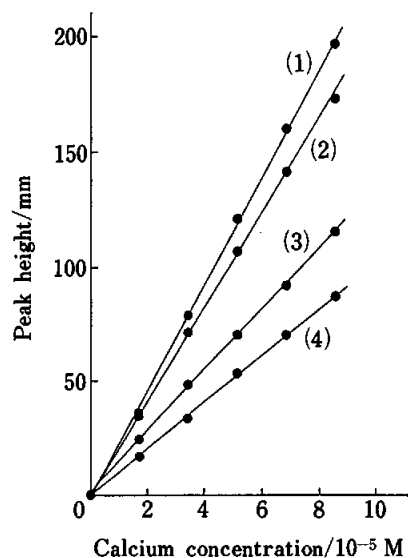


Fig. 4 The effect of DC24C8 concentration on the peak height of  $\text{Ca}^{2+}$ . PO,  $5 \times 10^{-4}$  M. DC24C8 concentration: (1),  $6 \times 10^{-3}$  M; (2),  $4 \times 10^{-3}$  M; (3),  $2 \times 10^{-3}$  M; (4),  $1 \times 10^{-3}$  M.

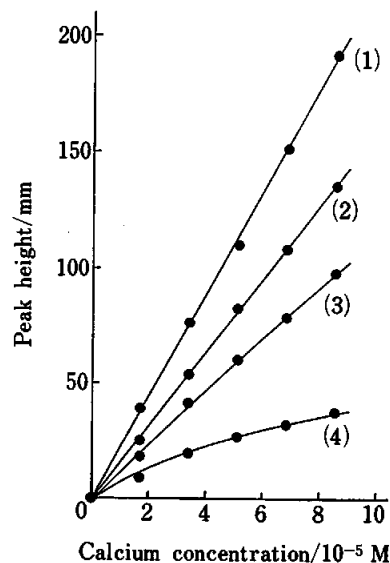


Fig. 6 The effect of PO concentration for calcium concentration. DC24C8,  $2 \times 10^{-3}$  M. PO concentration: (1),  $10 \times 10^{-4}$  M; (2),  $5 \times 10^{-4}$  M; (3),  $3 \times 10^{-4}$  M; (4),  $1 \times 10^{-4}$  M.

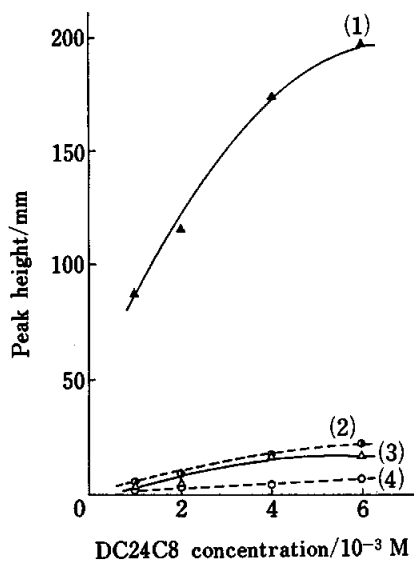


Fig. 5 The effect of DC24C8 concentration on the peak height of metal ions. Metal ion,  $1 \times 10^{-4}$  M; PO,  $5 \times 10^{-4}$  M. Metal ion: (1),  $\text{Ca}^{2+}$ ; (2),  $\text{K}^{+}$ ; (3),  $\text{Mg}^{2+}$ ; (4),  $\text{Na}^{+}$ .

considering the base-line stability, we adopted  $5 \times 10^{-4}$  M.

#### Effect of experimental variables of the flow system

The length of the PTFE extraction tubing (i.d. 0.5 mm) was varied from 0.25 to 3 m. The peak height increased with an increase in the length of the tubing up to 1 m, and then remained constant at the lengths from 1 m to 2 m. Therefore,  $2 \text{ m} \times 0.5 \text{ mm}$  i.d. tubing was chosen.

The effect of the flow rates of the three streams were

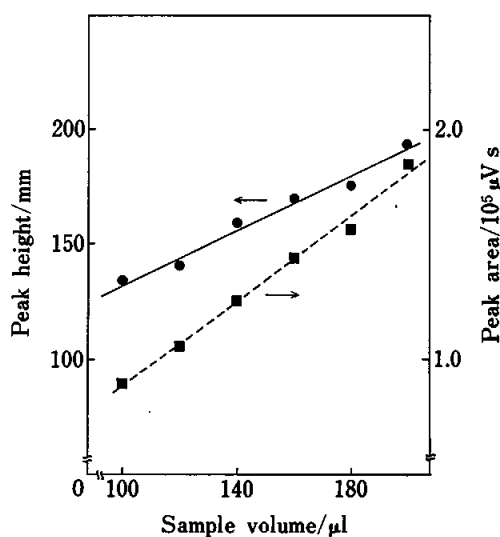


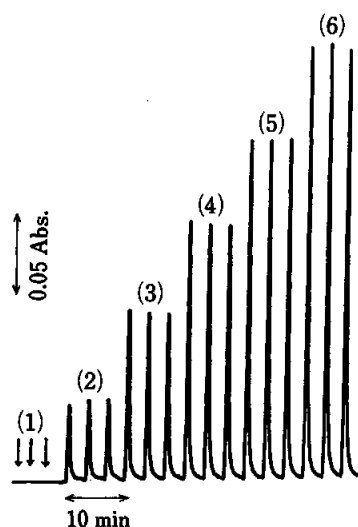
Fig. 7 The effect of sample injection volume on peak height and peak area. Calcium ion,  $1 \times 10^{-4}$  M; PO,  $5 \times 10^{-4}$  M; DC24C8,  $2 \times 10^{-3}$  M.

examined by varying the rate from 0.6 to  $1.2 \text{ ml min}^{-1}$ , the flow rate of each stream being kept identical. The peak height increased with an increase in the flow rate up to  $0.8 \text{ ml min}^{-1}$ , but then the increase in the peak height was small: a flow rate of  $0.8 \text{ ml min}^{-1}$  was adopted.

The effects of sample injection volume on the peak height and on the peak area were examined by varying the length of the loop of the 6-way injection valve (the dead volume of the valve:  $80 \mu\text{l}$ ). The results are shown in Fig. 7. The peak height and area linearly increased with increasing the sample size. The relation between

Table 2 Conditions finally adopted for the determination of calcium

|                                |  |
|--------------------------------|--|
| Carrier solution               | distilled water  |
| Reagent solution               | $5 \times 10^{-4}$ M PO, $10^{-3}$ M LiOH  |
| Organic phase                  | $2 \times 10^{-3}$ M DC24C8 in benzene-chlorobenzene (1+1 v/v)   |
| Mixing tubing                  | 30 cm $\times$ 0.5 mm i.d.   |
| Extraction tubing              | 200 cm $\times$ 0.5 mm i.d.  |
| Sample injection volume        | 100 $\mu$ l  |
| Flow rate                      | carrier stream, 0.8 ml min <sup>-1</sup><br>reagent stream, 0.8 ml min <sup>-1</sup><br>organic phase stream, 0.8 ml min <sup>-1</sup> |
| Flow-through cell (8 $\mu$ l)  | 10-mm path length  |
| Wavelength of detection        | 420 nm   |
| Linearity of calibration graph | 0 – $1 \times 10^{-4}$ M   |

Fig. 8 Typical FIA signals of calcium ion.  $\text{Ca}^{2+}$  concentration/ $10^{-5}$  M: (1), 0; (2), 1.7; (3), 3.3; (4), 5.0; (5), 6.6; (6), 8.3.

the peak area and the sample injection volume shows a straight line going through the origin, though that between the peak height and the sample injection volume does not go through the origin. This shows that peak areas can be used for the preparation of a linear calibration graph. In the determination of calcium in water, 100  $\mu$ l of sample solution were injected. The recommended conditions are summarized in Table 2.

#### Calibration graph for calcium

The calibration graph was linear up to a calcium concentration of  $1 \times 10^{-4}$  M. An example of the profile of the flow signals is shown in Fig. 8. The detection limit corresponding to a signal-to-noise ratio of 3 was  $2 \times 10^{-7}$  M of calcium. The relative standard deviations for ten injections of samples containing  $5 \times 10^{-5}$  M calcium was 0.99%.

Table 3 Effect of foreign ions on peak height of calcium

| Ion              | Added as                     | Concn./M           | Error, % |
|------------------|------------------------------|--------------------|----------|
| $\text{Na}^+$    | NaCl                         | $1 \times 10^{-4}$ | 1.4      |
|                  |                              | $5 \times 10^{-5}$ | 0.0      |
| $\text{K}^+$     | KCl                          | $1 \times 10^{-5}$ | 7.4      |
|                  |                              | $5 \times 10^{-6}$ | 1.1      |
| $\text{Mg}^{2+}$ | $\text{MgSO}_4$              | $1 \times 10^{-4}$ | 7.0      |
|                  |                              | $5 \times 10^{-5}$ | 1.4      |
| $\text{Fe}^{3+}$ | $\text{Fe}_2(\text{SO}_4)_3$ | $1 \times 10^{-5}$ | 0.8      |
|                  |                              | $5 \times 10^{-6}$ | 3.1      |
| $\text{NH}_4^+$  | $\text{NH}_4\text{Cl}$       | $1 \times 10^{-6}$ | 0.1      |
|                  |                              | $5 \times 10^{-6}$ | 7.6      |
|                  |                              | $1 \times 10^{-7}$ | 3.6      |
|                  |                              | $5 \times 10^{-7}$ | 3.4      |

$\text{Ca}^{2+}$ :  $5 \times 10^{-5}$  M.

Table 4 Determination of calcium in water samples

| Sample <sup>a</sup> | Found/ $10^{-4}$ M |                 |
|---------------------|--------------------|-----------------|
|                     | FIA                | Titration       |
| Asahi River A       | $1.90 \pm 0.03$    | $1.86 \pm 0.03$ |
| B                   | $2.11 \pm 0.03$    | $1.95 \pm 0.03$ |
| Takahashi River     | $3.93 \pm 0.03$    | $4.00 \pm 0.01$ |
| Yoshii River        | $2.56 \pm 0.00$    | $2.53 \pm 0.04$ |
| Zasu River          | $2.66 \pm 0.02$    | $2.65 \pm 0.04$ |
| Tap water           | $1.87 \pm 0.03$    | $1.93 \pm 0.02$ |

a. Sample solutions were diluted 10-fold, and 100  $\mu$ l of the diluted samples were injected.

#### Determination of calcium in river water

Interferences from foreign ions were examined; the results obtained are shown in Table 3. The amounts of ions that are generally found in river water samples tested here are, below  $3 \times 10^{-4}$  M for  $\text{Na}^+$ , below  $5 \times 10^{-5}$  M for  $\text{K}^+$ , below  $2 \times 10^{-4}$  M for  $\text{Mg}^{2+}$ , below  $10^{-5}$  M for  $\text{Fe}^{3+}$  and below  $10^{-6}$  M for  $\text{NH}_4^+$ , while the amounts of calcium ion are  $(1-4) \times 10^{-4}$  M. So the samples were diluted 10-fold. Thus, the interferences from metal ions were negligible.

Calcium in some samples of river water was determined. Table 4 shows the results obtained by the proposed FIA method and EDTA titration method. The calcium concentrations obtained by the two methods were in good agreement with each other.

In the proposed FIA method, due to the concentrations of calcium in samples, enhancement of sensitivity is less considered. When more sensitive methods are required, they can be easily achieved by using larger amounts of DC24C8 and PO and larger sample sizes. A more sensitive method also can be achieved by changing the dynamic range of detection: the detection limit can be easily lowered to  $2 \times 10^{-8}$  M under the same conditions as recommended here (Table 2). To enhance

the sensitivity of calcium determination, another anionic azo dye, Cl<sub>2</sub>-EO, can be used.

The proposed FIA system can be applied to the sensitive determination of barium and strontium ions, though it is necessary to take into account the interference from calcium ions.

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