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## Note

### Isotachophoresis of cyclic condensed phosphates

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Various oxoanions of phosphorus have been separated and determined by isotachophoresis<sup>1-6</sup>. In the present study, the separation and quantification of a series of cyclic condensed phosphates. P<sub>3m</sub>, P<sub>4m</sub>, P<sub>6m</sub> and P<sub>8m</sub>, were investigated by this technique.

Since sufficient difference in the potential unit (PU) values of the sample ions is required for good separation in isotachophoresis, a suitable leading electrolyte solution must be selected. The PU value represents the ratio of the potential gradient difference between the sample ion and the leading ion to that between the terminating ion and the leading ion  $P_S - P_L / P_T - P_L$ . With the leading electrolyte (0.01 M histidine hydrochloride-histidine-0.1% Triton X-100) used previously for the separation of oxoanions of phosphorus<sup>1-3</sup>, all four condensed phosphates could not be separated satisfactorily.

The addition of cations to the leading electrolyte is generally employed to vary the PU values of condensed phosphates, through complexation with the phosphate oxoanions. In this study we used cations of the alkaline-earth group.

### EXPERIMENTAL

#### Preparation of cyclic condensed phosphates

Sodium trimetaphosphate (Na<sub>3</sub>P<sub>3</sub>O<sub>9</sub> · 6H<sub>2</sub>O) was obtained by the condensation of orthophosphate: sodium dihydrogenorthophosphate was heated at 500°C for 3 h in an electric furnace, the chief product was extracted with warm water (30°C), ethanol was added stepwise with vigorous stirring, and the resulting precipitate was dried overnight in air.

Sodium tetrametaphosphate (Na<sub>4</sub>P<sub>4</sub>O<sub>12</sub> · 4H<sub>2</sub>O) was prepared by the hydrolysis of phosphorus pentoxide according to the modified method of Bell *et al.*<sup>7</sup>: phosphorus pentoxide in small amounts was added with vigorous stirring to cold water, keeping the temperature of the solution below 15°C. The resulting solution was neutralized with 30% sodium hydroxide and sodium tetrametaphosphate was precipitated by the stepwise addition of ethanol.

Sodium hexametaphosphate ( $\text{Na}_6\text{P}_6\text{O}_{18} \cdot 6\text{H}_2\text{O}$ ) was prepared by the procedure of Griffith and Buxton<sup>8</sup>: to 85% phosphoric acid was added lithium carbonate, slowly so as not to cause foaming. The slurry was placed into a platinum dish, and heated at 200°C for 1 h, then at 275°C for 5 h. The product was pulverized, extracted with water and the filtrate passed through a cation-exchange resin (acidic form). The eluate was neutralized with sodium carbonate, and product precipitated by the addition of methanol.

Sodium pentametaphosphate ( $\text{Na}_5\text{P}_5\text{O}_{24} \cdot 6\text{H}_2\text{O}$ ) was obtained by the use of Schülke's method<sup>9</sup>: to 300 ml of 0.1 *M* lead nitrate solution was slowly added 100 ml of 0.15 *M* sodium tetrametaphosphate with stirring. The lead tetrametaphosphate precipitated was heated at 150°C for 2 h, pulverized and then heated at 310°C for 0.5 h. After pulverizing again, to 300 ml of 0.1 *M* sodium sulphide solution was added the fine powder suspended in 50 ml of water, the precipitated lead sulphide was filtered off and a white precipitate was obtained by the addition of 200 ml of ethanol to the filtrate.

All the four products were recrystallized from an aqueous solution three times, giving white crystals or powders.

The other chemicals used were reagent grade.

#### *Isotachophoresis*

The apparatus, and the leading and terminating electrolytes, were the same as reported previously, except for the addition of metal chloride to the leading electrolyte.

#### RESULTS AND DISCUSSION

##### *Separation and quantification of $P_{3m}$ , $P_{4m}$ , $P_{6m}$ and $P_{8m}$*

When the four cyclic oxoanions of phosphorus,  $P_{3m}$ ,  $P_{4m}$ ,  $P_{6m}$  and  $P_{8m}$ , were analyzed with a leading electrolyte containing no metal ions,  $P_{3m}$ ,  $P_{4m}$  and  $P_{6m}$  were separated from each other but  $P_{8m}$  was not.

Fig. 1. shows the changes in PU values of the cyclic condensed phosphates due to the addition of  $\text{Ca}^{2+}$  to the leading electrolyte. The PU values increased with increasing concentration of  $\text{Ca}^{2+}$ , and the differences between the PU values of the

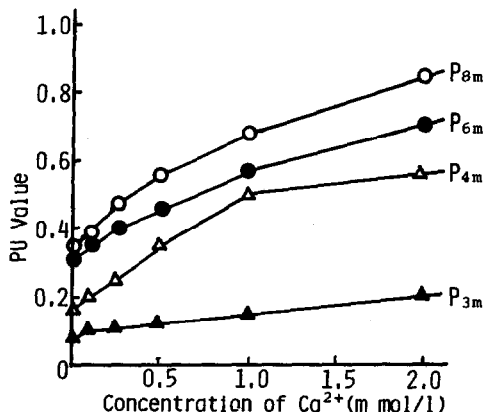


Fig. 1. Effect of addition of  $\text{Ca}^{2+}$  on the PU values of cyclic condensed phosphates.

phosphates were greater except for that between  $P_{4m}$  and  $P_{6m}$ . At low concentrations of  $Ca^{2+}$ , the difference between the PU values of  $P_{6m}$  and  $P_{8m}$  was small. On the other hand, when more than 0.5 mmol/l  $Ca^{2+}$  was added, the boundary of the zone between  $P_{6m}$  and  $P_{8m}$  was not clear. From these results, the optimum concentration of  $Ca^{2+}$  for the separation of the four cyclic condensed phosphates was 0.25 mmol/l (Fig. 2).

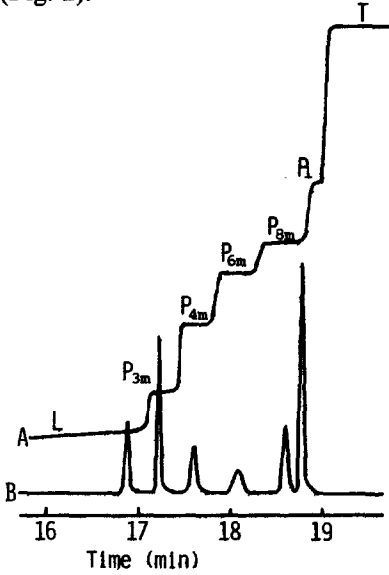


Fig. 2. Isotachopherogram obtained upon addition of 0.25 mmol/l  $Ca^{2+}$ . L = Leading ion ( $Cl^-$ ); T = terminating ion (hexanoate); A = potential gradient; B = differential potential. Chart speed: 40 mm/min.

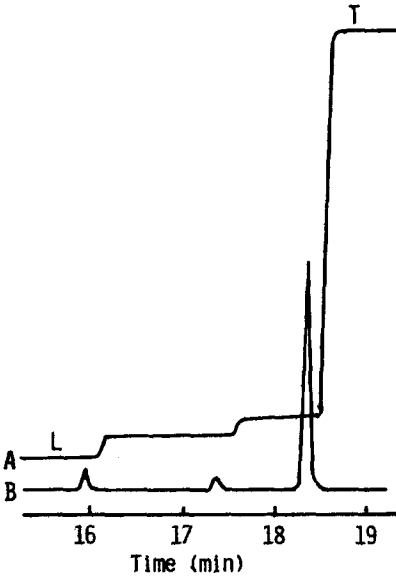


Fig. 3. Formation of a mixed zone between  $P_{3m}$  and the leading ion ( $Cl^-$ ). Sample size of  $P_{3m}$ : 20 nmol. Other details as in Fig. 2.

The calibration curves of these phosphates were linear, and the molar response, which is the value of the zone length per nmol of each phosphate, was 2.71 for  $P_{3m}$ , 2.91 for  $P_{4m}$ , 3.26 for  $P_{6m}$  and 3.64 for  $P_{8m}$ , respectively, increasing with increasing molecular weight. All the coefficients of variation, *i.e.*, the ratio of the standard deviation to the average zone length, were less than 1.5%.

The addition of other alkaline-earth metal ions,  $Mg^{2+}$ ,  $Sr^{2+}$  and  $Ba^{2+}$ , had similar effects to that of  $Ca^{2+}$ , but that of  $Cu^{2+}$ ,  $Ni^{2+}$ ,  $Fe^{3+}$  and  $Al^{3+}$  resulted in insufficient separation.

#### Determination of $P_{3m}$

When trimetaphosphate ( $P_{3m}$ ) was determined using the leading electrolyte previously described<sup>1</sup>, the injection of more than 8 nmol  $P_{3m}$  caused problems with the quantitative separation because a mixed zone with the leading ion ( $Cl^-$ ) was formed during electrophoretic migration, as shown in Fig. 3. This is due to a small difference (0.08) in the PU values between  $P_{3m}$  and  $Cl^-$ . As shown in Fig. 1, the PU value of  $P_{3m}$  increased upon addition of  $Ca^{2+}$  to the leading electrolyte, inhibiting the formation of the mixed zone, and so the range of injected sample sizes would be broadened: for example, when 1 mmol/l  $Ca^{2+}$  was added, the PU value of  $P_{3m}$  was 0.146 and  $P_{3m}$  could be determined up to 80 nmol.

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