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Note

Anion-exchange chromatographic separation of linear phosphates with eluent containing a chelating agent

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In recent work¹, we observed that an appreciable amount of diphosphate was hydrolyzed to orthophosphate during anion-exchange chromatography, and that this hydrolytic degradation could be prevented by adding EDTA to the eluent.

The effects of metal ions on the hydrolysis of linear phosphates and phosphate esters have been reported by many investigators², and experimental observations³ show that the presence of ions of multivalent metals promotes the hydrolysis of P-O-P linkages at pH values above 4. In contrast, anions (except hydroxide) have little effect on the kinetics of hydrolysis. These phenomena can be explained by complex formation between the metal ions and phosphate, but the contribution of metal ion catalysis and the nature of the catalytic effect have not been completely clarified because of participation by protons and hydroxide ions in the hydrolysis of P-O-P linkages.

The degradation of diphosphate in an anion-exchange column seems to be attributable to the catalytic action of multivalent ions, *e.g.*, Fe³⁺, in the elution system. Although potassium chloride or sodium chloride solution is generally used as eluent in the anion-exchange chromatographic separation of phosphorus oxo-anions, contamination by traces of heavy metals is inevitable, and it is difficult completely to eliminate such metals from the reagents.

In this work, we masked the heavy-metal impurities by adding the sodium salt of citric acid or EDTA to the eluent; this prevented hydrolysis of P-O-P linkages, EDTA being found to be a more effective masking agent. Good separation of linear phosphates was achieved by anion-exchange chromatography in a sodium chloride-EDTA system.

EXPERIMENTAL

The ion-exchange column (70 cm × 13 mm) was packed with Bio-Rad AG 1-X8 (Cl⁻) anion-exchange resin (100-200 mesh). The sample solutions comprised the sodium salts of ortho- and di-phosphates and glassy sodium polyphosphate with an average chain length of 4.5. Exponential gradient elution was employed; a solution of 0.32 *M* sodium chloride was kept in the reservoir and 750 ml of 0.12 *M* sodium chloride was kept in a mixing bottle at the start. The eluents were 2.5 *mM* in trisodium citrate or 5 *mM* in EDTA (ratio of tetrasodium salt to disodium salt = 2 : 1). The

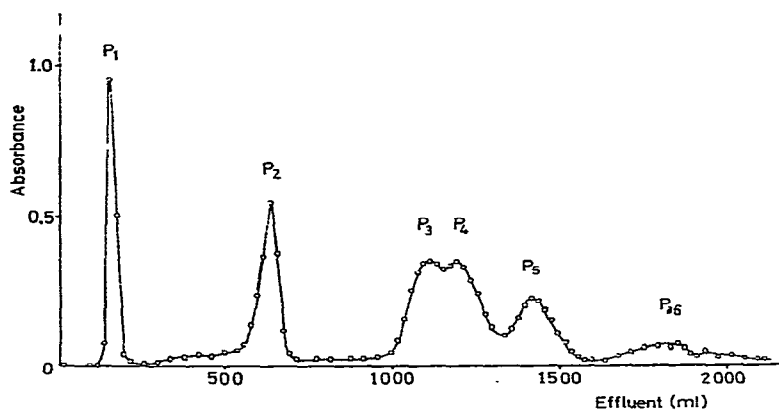


Fig. 1. Chromatogram of linear phosphates in the sodium chloride-citrate system.

pH of the eluent was about 7 for each system. The effluent was collected in 10-ml fractions, and the phosphate content of each fraction was determined colorimetrically with a molybdenum(V)-molybdenum(VI) reagent.

RESULTS AND DISCUSSION

Fig. 1 is a chromatogram of linear phosphates in the sodium chloride-citrate system. Ortho- and di-phosphates were separated from each other and from the higher linear phosphates, (e.g., triphosphate and tetraphosphate) which were eluted later, but peak resolution of the higher linear phosphates was unsatisfactory. A chromatogram of linear phosphates in the sodium chloride-EDTA system is shown in Fig. 2. Comparison of the two chromatograms shows that the EDTA-containing system is preferable for the separation of linear phosphates (from orthophosphate to hepta- or octa-phosphate) and for suppression of the hydrolysis of P-O-P linkages during chromatography. Development of the blue molybdophosphate colour between the

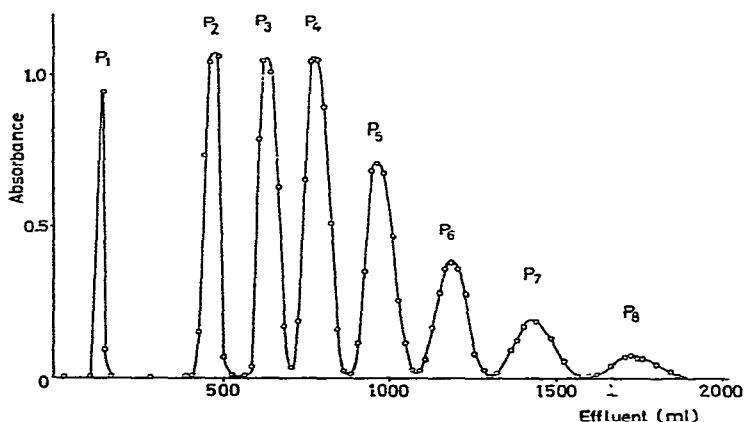


Fig. 2. Chromatogram of linear phosphates in the sodium chloride-EDTA system.

peaks for ortho- and di-phosphate in the sodium chloride-citrate system was appreciable although it was less noticeable than in the absence of any masking agent¹. These facts can be interpreted by the formation of chelate compounds between the heavy-metal ions and the masking agents in the eluents. EDTA anions are a more powerful chelating agent than is citrate, and hydrolysis of P-O-P linkages is considered to be completely suppressed in the sodium chloride-EDTA system. The amount of masking agent that can be added to the eluent is restricted because each phosphate species is eluted in a smaller volume of effluent in the presence of the masking agent than that in the absence of it, and this can cause overlapping of peaks.

REFERENCES

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