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## ION-EXCLUSION CHROMATOGRAPHY OF CONDENSED PHOSPHATES ON A CATION-EXCHANGE RESIN

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### SUMMARY

The ion-exclusion chromatography of orthophosphate ( $\text{PO}_4^{3-}$ ) and several condensed phosphates ( $\text{P}_2\text{O}_7^{4-}$ ,  $\text{P}_3\text{O}_{10}^{5-}$  and  $\text{P}_3\text{O}_9^{3-}$ ) was investigated using a cation-exchange resin column in the  $\text{H}^+$  form. The phosphates eluting from the column were monitored as the corresponding acids with flow coulometric and conductometric detectors.

The retention volumes of the phosphates increased with increase in the concentration of organic solvents (acetone and dioxane) in the eluent, and the increases were greater in water-dioxane than in water-acetone. A good separation of the phosphates from each other could be achieved by gradient elution with 80% dioxane-water.

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### INTRODUCTION

The liquid chromatographic separation of condensed phosphates has been exclusively performed by ion-exchange chromatography<sup>1-3</sup> and gel chromatography based on the steric exclusion or molecular sieve effect<sup>4-6</sup>. For ion-exchange chromatography, anion-exchange resins such as Dowex-1 and Amberlite IRA-400 have usually been used, and the phosphates have been chromatographed by stepwise or gradient elution with eluents of various pH. For gel chromatography, cross-linked dextran gels such as Sephadex gels have been used, and the phosphates have been chromatographed by isocratic elution with potassium chloride or sodium chloride solutions at various pH. The condensed phosphates separated by these chromatographic methods have been monitored by spectrophotometry such as the molybdenum blue or yellow methods after digestion with various acids.

In a previous paper<sup>7</sup>, we have reported that in ion-exclusion chromatography (IEC), various acids can be separated gel chromatographically by elution with water on a cation-exchange resin in the  $\text{H}^+$  form. The retention volumes of the acids depended on the first dissociation constants ( $\text{p}K_1$ ), and the distribution coefficients ( $K_d$ ) were as follows: 0 for strong acids such as HI, HCl,  $\text{HNO}_3$  and  $\text{H}_2\text{SO}_4$ ; 0-1 for weak acids such as  $\text{H}_2\text{PO}_4$ ,  $\text{H}_2\text{SO}_3$ , HF and  $\text{CH}_3\text{COOH}$ ; and 1 for very weak acids

such as  $\text{H}_2\text{CO}_3$ ,  $\text{H}_3\text{BO}_3$ ,  $\text{HCN}$  and phenol. The good separation of  $\text{PO}_4^{3-}$  from  $\text{Cl}^-$  by isocratic elution with water was not accomplished owing to the similar  $K_d$  values, whereas it was possible by isocratic elution with water-acetone, because the retention volume or capacity factor of  $\text{PO}_4^{3-}$  increased with increase in acetone concentration in the eluent and that of  $\text{Cl}^-$  did not<sup>8</sup>. Further, a mixture of  $\text{PO}_4^{3-}$ ,  $\text{HPO}_4^{2-}$  and  $\text{H}_2\text{PO}_4^-$  has also been separated successfully in the same manner<sup>9</sup>. The mechanism of the separation of these phosphates is based on the increasing effect of their  $\text{p}K_1$  values caused by increasing acetone concentration in the eluent. Therefore, it was recognized that the dielectric constant ( $D$ ) of the eluent is an important factor in the IEC separation of phosphates<sup>8</sup>.

The acidities of condensed phosphates, which are very strong acids, as reported by Beukenkamp *et al.*<sup>10</sup>, decrease in the order  $\text{P}_3\text{O}_9^{3-} > \text{P}_3\text{O}_{10}^{5-} > \text{P}_2\text{O}_7^{4-} > \text{PO}_4^{3-}$ . Therefore, it is expected that these phosphates can be separated ion-exclusively by elution with a mixture of water and an organic solvent with a very low  $D$ . In this work, mixtures of water with acetone ( $D = 21.7$ ) or dioxane ( $D = 2.1$ ) were used as the eluent for the IEC separation of phosphates.

This paper discusses the chromatographic conditions for the separation of mixtures of phosphates by isocratic and gradient elution and the response characteristics of each phosphate using flow coulometric and conductometric detectors. The method was applied to the separation of phosphates in a household detergent.

## EXPERIMENTAL

A Spectra-Physics 3500B liquid chromatograph equipped with a Hitachi 630 flow coulometric detector (FCD) and a Yanagimoto C-202 conductometric detector (COND) was used for isocratic and gradient elution with water-organic solvent (acetone and dioxane) mixtures. In the FCD, the following electrochemical reaction was used:  $p$ -benzoquinone +  $2\text{H}^+$  +  $2e^- \rightarrow$  hydroquinone<sup>7,11</sup>. A glass-jacketed column ( $540 \times 9$  mm I.D.) packed with a Hitachi 2613 ( $\text{H}^+$ ) strongly acidic cation-exchange resin (particle size  $18 \mu\text{m}$ , degree of cross-linking 8%) was used. The column was thermostated at  $50^\circ$  and the flow-rate of eluent was 1 ml/min. A 0.1-ml sample was introduced into the column with a loop injector.

Chromatograms were recorded with a National VP-6541A two-pen strip-chart recorder. The peak areas and retention times of the chromatograms were recorded with a Auto Lab System I computing integrator and a Takeda-riken TR-2213 digital integrator.

The phosphates studied were  $\text{PO}_4^{3-}$ ,  $\text{P}_2\text{O}_7^{4-}$ ,  $\text{P}_3\text{O}_{10}^{5-}$  and  $\text{P}_3\text{O}_9^{3-}$ . All stock solutions of the phosphates (0.1  $M$ ) were prepared by dissolving the reagent-grade sodium or potassium salts in deionized, distilled water. Organic solvents and the other chemicals were of reagent grade.

## RESULTS AND DISCUSSION

### *Chromatogram of phosphates by elution with water*

Fig. 1 shows the chromatograms of a mixture of phosphates obtained by isocratic elution with water alone. As expected from the acidities of phosphates, the retention volumes of phosphates except  $\text{PO}_4^{3-}$  were almost identical and corres-

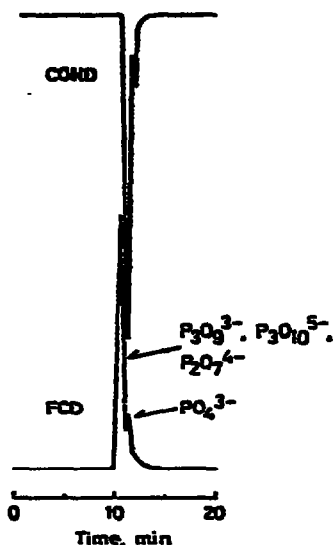


Fig. 1. Chromatograms of mixture of phosphates obtained by elution with water alone. Concentration of phosphates,  $10^{-3} M$ . FCD, 500 mV full scale; COND,  $10^3 \mu S \cdot cm^{-1}$  full scale.

ponded to the column void volume. That of  $PO_4^{3-}$  was slightly higher than those of the other phosphates. These results indicate that  $P_2O_7^{4-}$ ,  $P_3O_{10}^{5-}$  and  $P_3O_9^{3-}$  are completely ion-excluded from the cation-exchange resin phase, because the  $H^+$  of both these phosphates and the resin dissociate completely in the water.  $PO_4^{3-}$  was incompletely ion-excluded from the resin phase, because  $PO_4^{3-}$  dissociates incompletely ( $pK_1 = 2.1$ ). The IEC separation of the phosphates must be accomplished by suppressing the dissociation. Therefore, the separation of the phosphates by elution with water-organic solvents was examined and is discussed in the next section.

#### *Effect of organic solvents on retention volumes of phosphates*

In order to separate a mixture of phosphates, the effects of acetone and dioxane on the retention volumes of each phosphate were studied in the concentration range from 0 to 80% (v/v) in water.

Figs. 2 and 3 show the effects of acetone and dioxane, respectively on the retention volumes of each phosphate. It can be seen that the retention volumes of each phosphate increased with increase in concentration of acetone and dioxane, the increase being greater in the latter system; the order of elution followed the order of decreasing acidities mentioned above. These results indicated that the  $pK_1$  value for each phosphate increased with the decrease in  $D$  resulting from the increase in concentration of acetone or dioxane in the eluent, because the  $pK_1$  values are dependent on the  $D$  value of the eluent. Therefore, of increase in  $pK_1$  values for the phosphates is greater in water-dioxane than in water-acetone.

In addition to the above discussion, the partition effect between the resin phase and the mobile phase should be also considered as an important factor in IEC by elution with water-organic solvent mixtures. When water is used as the eluent, the partition effect may be negligible because the eluent composition in the resin phase

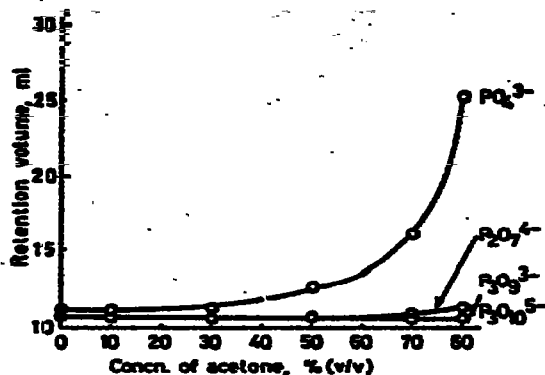


Fig. 2. Effect of acetone on retention volumes of phosphates.

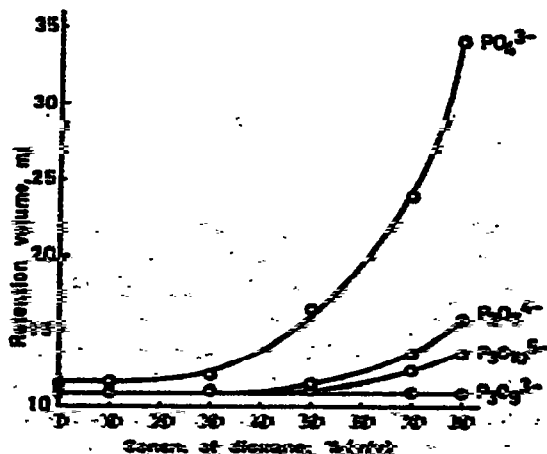


Fig. 3. Effect of dioxane on retention volumes of phosphates.

is the same as that in the mobile phase. Thus, IEC by elution with water can be considered as gel chromatography based on the ion-exclusion effect<sup>7</sup>. However, when water-organic solvent mixtures are used as the eluent, the eluent composition in the resin phase may differ considerably from that in the mobile phase because the sulphonic group in the resin becomes hydrated and the resin network is hydrophobic. Therefore, the partition effect can not be neglected because the eluent composition in the resin phase is water-rich and that in the mobile phase is solvent-rich<sup>12,13</sup>. Hence, in the IEC separation of phosphates with water-organic solvent mixtures, the partition effect and the ion-exclusion effect are very important factors.

When 80% acetone-water was used as the eluent,  $PO_4^{3-}$  and  $P_2O_7^{4-}$  were separated from the mixture of phosphates. However,  $P_3O_9^{3-}$  and  $P_3O_{10}^{5-}$  were not successfully separated, as shown in Fig. 4. When 80% dioxane-water was used as the eluent, all of the phosphates were successfully separated, as shown in Fig. 5. A shrinkage of the ion-exchange resin bed by about 4% was then observed. However, the column could be used repeatedly without re-packing and reproducible chromatograms were obtained throughout the work.



Fig. 4. Chromatograms of mixture of phosphates obtained by isocratic elution with 80% acetone-water. FCD, 200 mV full scale; COND,  $50 \mu\text{S}\cdot\text{cm}^{-1}$  full scale.

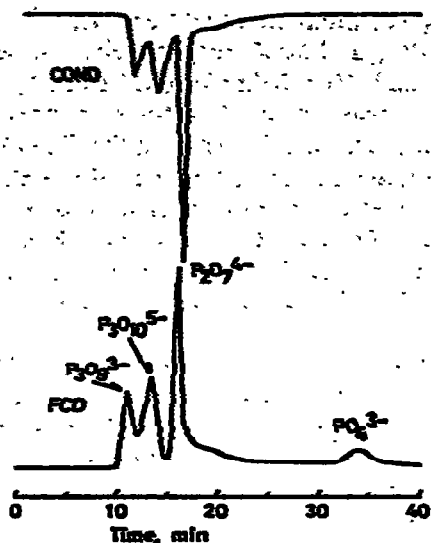


Fig. 5. Chromatograms of mixture of phosphates obtained by isocratic elution with 80% dioxane-water. FCD, 200 mV full scale; COND,  $50 \mu\text{S}\cdot\text{cm}^{-1}$  full scale.

From the above results, water-dioxane was selected as the most suitable eluent for subsequent experiments on the IEC separation of phosphates.

#### *Effect of dioxane concentration on detector response towards phosphates*

The detector responses (peak areas) of each phosphate with the FCD and COND were studied using 0-80% dioxane-water mixtures.

Fig. 6 shows the results obtained for  $\text{PO}_4^{3-}$  and  $\text{P}_3\text{O}_{10}^{3-}$ . With the COND, the peak areas of  $\text{PO}_4^{3-}$  and  $\text{P}_3\text{O}_{10}^{3-}$  decreased markedly with increasing concentration of dioxane, and the peak of  $\text{PO}_4^{3-}$  was not observed with 80% dioxane-water. The behavior of  $\text{P}_2\text{O}_4^{4-}$  and  $\text{P}_3\text{O}_{10}^{3-}$  was similar to that of  $\text{P}_3\text{O}_{10}^{3-}$ . With the FCD, the

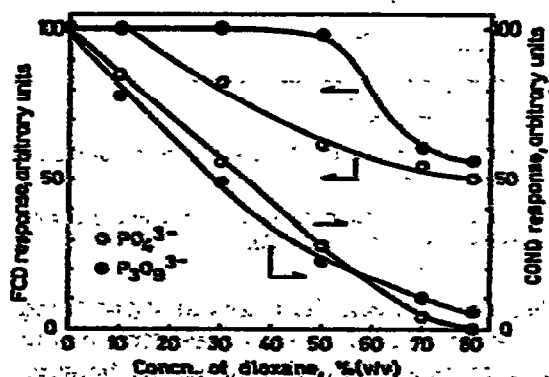


Fig. 6. Effect of dioxane on detector responses of phosphates.

peak areas of  $\text{PO}_4^{3-}$  and  $\text{P}_3\text{O}_9^{3-}$  varied little with concentrations of dioxane up to 10% and 50%, respectively, but decreased by 40–50% when 30% dioxane-water was used. The behaviour of  $\text{P}_2\text{O}_7^{4-}$  and  $\text{P}_3\text{O}_{10}^{5-}$  was similar to that of  $\text{P}_3\text{O}_9^{3-}$ . The decrease in the detector responses of the phosphates obtained on elution with water-dioxane was lower with the FCD than the COND. This indicates that, in spite of the increase in the  $\text{pK}$  values of the phosphates owing to the decreasing  $D$  value of eluent, the electrochemical reaction of  $\text{H}^+$  ions from the phosphates and  $p$ -benzoquinone in the FCD proceeds considerably, even with 80% dioxane-water. From these results, it was concluded that the FCD is a more powerful detector than the COND for the IEC of phosphates by elution with water-dioxane.

#### *Separation of phosphates by gradient elution with water-dioxane*

Gradient elution with water-dioxane was used to separate effectively a mixture of the phosphates. Fig. 7 shows the chromatograms of the mixture of phosphates obtained with the FCD and COND. The gradient, with a delay time of 10 min, was linear with a continuously decreasing concentration of dioxane from 80% to 0% during 10 min. Although the chromatographic running time of the phosphates on isocratic elution with 80% dioxane-water was about 40 min, as shown in Fig. 5, gradient elution was effected within 30 min.

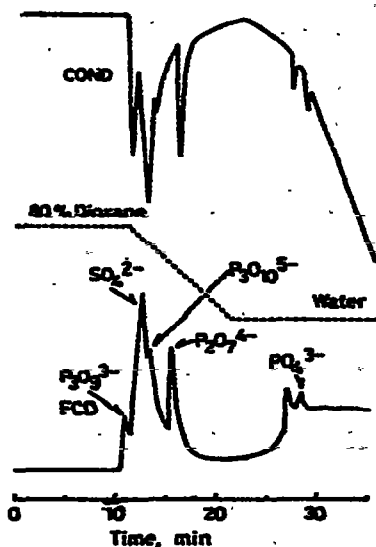
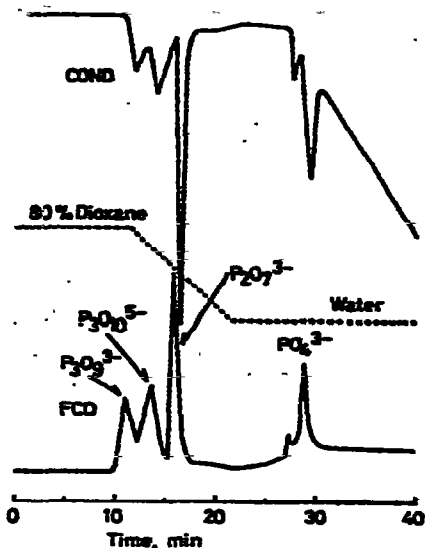


Fig. 7. Chromatograms of mixture of phosphates obtained by gradient elution with 80% dioxane-water. FCD, 200 mV full scale; COND,  $50 \mu\text{S}\cdot\text{cm}^{-1}$  full scale.

Fig. 8. Chromatograms of phosphates in household detergent obtained by gradient elution with 80% dioxane-water. FCD, 100 mV full scale; COND,  $30 \mu\text{S}\cdot\text{cm}^{-1}$  full scale.

In Fig. 7, an unknown peak was observed between the peaks of  $\text{P}_2\text{O}_7^{4-}$  and  $\text{PO}_4^{3-}$  in addition to the peaks of individual phosphates. This peak was attributed to an impurity in  $\text{P}_3\text{O}_9^{3-}$ , and may be estimated to be an acid with an acidity between those of  $\text{P}_2\text{O}_7^{4-}$  and  $\text{PO}_4^{3-}$ .

As can be seen from Fig. 7, the characteristics of gradient elution with 80% dioxane-water are a high detector response and a high column efficiency for  $\text{PO}_4^{3-}$  in comparison with isocratic elution. Background drift due to the variation of eluent composition in gradient elution was observed with both the FCD and COND. The drift was smaller with the FCD than the COND.

From the above results, it was concluded that gradient elution using the FCD is suitable for the IEC of phosphates.

#### *Separation of phosphates in household detergent*

The separation of phosphates in household detergent was carried out by gradient elution with 80% dioxane-water. Fig. 8 shows the chromatograms of the detergent (0.1% aqueous solution) obtained with the FCD and COND. Although  $\text{P}_3\text{O}_{10}^{5-}$  was not adequately separated from the  $\text{SO}_4^{2-}$  which is present in the detergent,  $\text{P}_3\text{O}_9^{3-}$ ,  $\text{P}_2\text{O}_7^{4-}$  and  $\text{PO}_4^{3-}$  could be successfully separated from  $\text{SO}_4^{2-}$ . The retention times of each phosphate agreed well with those of the phosphates in an authentic mixture.

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