# *Studies on Several Phosphates from the View-point of Analytical Chemistry. IV. A Radioactive Paper Chromatography on Several Meta- and Poly-Phosphates labelled with $P^{32}$**

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Recently the studies on several meta- and poly- phosphoric acids and their phosphates have been carried on by many investigators, but it appears that no definite method for separational analysis has been yet established. Therefore, the authors synthesized several phosphates labelled with $P^{32}$ and investigated their chromatograms at various rates using pyridine-water as solvent.

### Material and Reagents

**(1) Samples**

Samples were obtained by synthesizing sodium ortho-, pyro-, tripoly-, trimeta- and hexametaphosphates, each labelled with $P^{32}$.

As there was only a little radioactive material, it was impossible to synthesize the labelled substances to a great degree. Hence we were unable to use it as a purified sample.

*a) Disodium-hydrogen-orthophosphate* ($Na_2HPO_4$)1).—With reference to Richard C. Vogel and H. Podall's report1), the preparation was made by adding 10 ml. of "carrier-free" phosphate (by diluting 2 mc. of "carrier-free" to 50 ml. of distilled water slightly acidified by hydrochloric acid) to 1 g. of disodium-hydrogen-orthophosphate. After agitation at 110°C it was used as a dehydrated salt.

*b) Sodium-pyrophosphate* ($Na_4P_2O_7$)2).—About 1 g. of sodium-dihydrogen-orthophosphate was heated in a platinum-crucible and maintained at 210°C for twelve hours. The purity was more than 97%.

*c) Sodium-trimetaphosphate* ($Na_3P_3O_9$)2).—About 1 g. of sodium-dihydrogen-orthophosphate was heated in a platinum-crucible and maintained at 300°C for one hour and then at 610°C for three hours, lowered to 390°C slowly in that same condition, and then allowed to stand for fourteen hours. After the finely powdered substance was placed in a desiccator, this was lowered to room temperature.

*d) Sodium-tripolyphosphate* ($Na_5P_3O_{10}$)2).—Sodium-dihydrogen-orthophosphate and disodium-hydrogen-orthophosphate were mixed in the proportion of 1:2. This was heated to 800°C and maintained at this temperature for a period of eighteen hours, so it could completely melt. The temperature was then lowered gradually from 650°C to 250°C, which required about eight hours, and then cooled in a desiccator.

*e) Sodium-hexametaphosphate* ($Na_6P_6O_{18}$)2).—Monosodium-dihydrogen-orthophosphate was heated to about 900°C and maintained at this temperature for three hours in a platinum crucible and then the melted mass was cooled rapidly. Different batches of the resulting glassy product contained from 90% to 95% sodium-hexametaphosphate.

Each phosphate was dissolved in water and about 0.1 mol. solutions were prepared.

**(2) Solvents**

The solvents were prepared by mixing $n\text{-}C_4H_9OH$, and $H_2O$ and 11 N $NH_4OH$ in the ratio of 8:1:1 in volume respectively and by mixing pyridine and water in the ratios of 0:1, 1:4, 3:7, 1:2, 2:3, 1:1, 3:2, and 1:0 in volume.

**(3) The Method of Development**

The chromatograms were developed by the ascending method on Toyo Rosi No. 50 paper for six to ten hours in a chromatographic cylinder, which was kept at 25±1°C in the thermostat. The making of the cylinder was based on W. H. Longenecker3) and Otosai's apparatus4): The size of paper was 1 cm.×40 cm. and sufficiently long to provide a chromatograph path of 30 cm. On this about 0.02 micro-mol. of samples were placed with capillary on each sheet approximately 4 cm. above the surface of the solvent.

**(4) The Method for Detection**

Recently studies on application of chromatography by using the radioactive method were made by H. Muller5) and H. H. Strain6), who also devised a convenient apparatus for measuring radiation. The authors devised a simple apparatus, which was used in the test as shown in Fig. 1.

The following were used in this apparatus.

Hundred scaling G. M. Counter (Kobe Tech. Co.).

Two sheets of stainless steel (10 cm.×10 cm.×2 mm.) for slit.

A glass plate (10 cm.×30 cm.×2 mm.) upon which the papers were slid. A 30 cm. ruler.

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1) R.C. Vogel and H. Podall, *J. Am. Chem. Soc.*, **72**, 1420 (1950).

2) L.T. Jones, *Ind. Eng. Chem. Anal. Ed.*, **14**, 536 (1942).

3) W.H. Longenecker, *Anal. Chem.*, **11**, 402 (1949).

4) K. Otosai, *Kagaku no Ryoiki*, **8**, No. 8, 25 (1950).

5) R.H. Muller, *Anal. Chem.*, **23**, 208 (1951).

6) T.R. Sato, W.P. Norris and H.H. Strain, *Anal. Chem.*, **24**, 776 (1952).

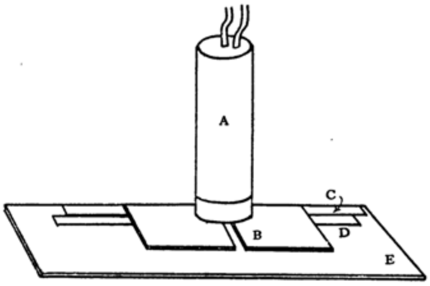

Fig. 1. Counting technique. A, counter tube holder; B, stainless steel slit; C, 30 cm. ruler; D, paper; E, glass-plate.

As shown in Fig. 1 the position of the G. M. tube is fixed. Two sheets of stainless steel were used to make a slit, the width of which was adjustable from 0.25 to 0.5 cm. These steel plates were placed over the glass plate, between which the paper could be moved at intervals of 0.25 or 0.5 cm. At each interval the count was made for one minute. The measured counting rates per minute were plotted against the developed distances.

During the measurement, there was no possibility of contamination on the surface of the slit or elsewhere. This was proved from the results of various tests.

Rf's were determined from the positions which are shown as the maximum counting rate.

Several phosphate ions came to their characteristic places regularly in the paper according to the differences of mobilities.

## Results and Discussion

a) Although the separation of several phosphate ions was examined with the solvent of $n\text{-}C_4H_9OH-H_2O-11\,N\ NH_4OH\ (8:1:1)$, the result was not successful. Rf's were approximately zero.

b) The developed states of a crude hexameta-phosphate at the various rates of pyridine-water solvent are shown in Fig. 2, in which the greatest peak should be hexametaphosphate, the main component, while others having the smaller peaks should be some impurities of the other types of phosphates.

From Fig. 2 it could easily be recognized that the most satisfactory result was obtained in the case of pyridine-water (1:1) and the result was found to be reproducible. Fig. 3 was introduced from the relation of Rf's of several phosphates ions with the various rates of pyridine-water solvent.

The results showed the following. When samples were developed only with water, all Rf's were nearly 1.0, but trimetaphosphate ions could be separated a little from other ions. Consequently, the other mutual ions except trimeta-ion could not be separated completely.

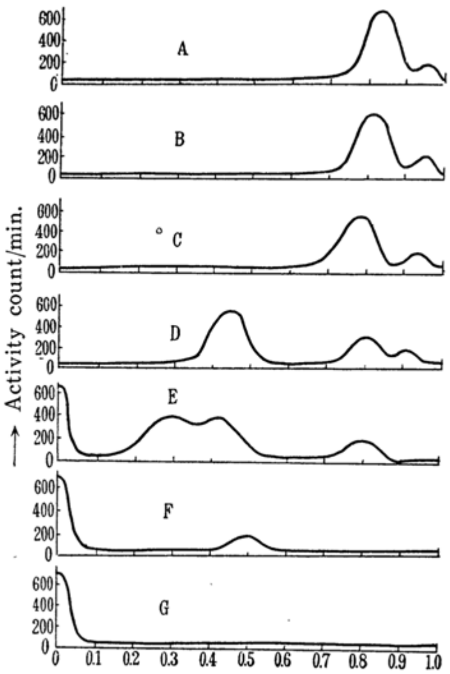

Fig. 2. Radioactive chromatograms of synthesized hexametaphosphate at various rate of pyridine-water.

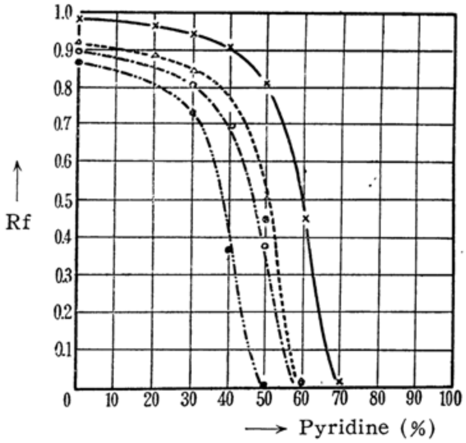

Fig. 3. Diagrams of Rf-amount of pyridine (%). △, orthophosphate ion; ⊙, pyrophosphate ion; ×, trimetaphosphate ion; ○, tripolyphosphate ion; ●, hexametaphosphate ion.

Corresponding to the increase of pyridine the mutual separation among several phosphate ions gradually became possible. Pyridine-water (1:1) appeared to be the most desirable solvent for separation.

When the rate of pyridine was increased further, Rf's of each phosphate ion decreased and approached zero. However, the decrease of trimeta ions was comparatively delayed.

A in Fig. 4 shows the peak of trimetaphosphate ion. B in Fig. 5 shows the case in which hydrolysis of hexametaphosphate did not occur. The small peak at the right hand side in the figure indicates an impurity of the hexametaphosphate. This can be quite conceivable from the results of the other investigators[7]. C in Fig. 4 shows the result of the tripolyphosphate ion having a indistinct double peak. The peak having smaller Rf-value was due to tripolyphosphate ion and the other was due to orthophosphate ion, according to the peak shown in G in Fig. 4 which was due to orthophosphate ion. A peak of similar nature can be seen in D in Fig. 4, which was obtained with the mixed sample of several phosphate ions. E in Fig. 4 shows the result of pyrophosphate ion.

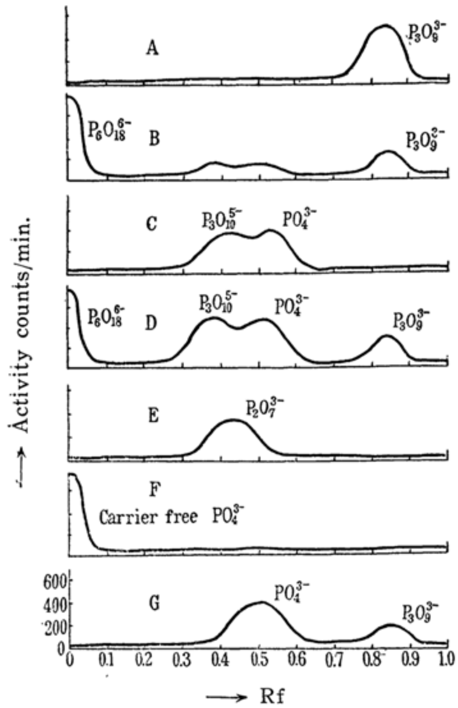

Fig. 4. Radioactive chromatogrames for several phosphates at 1-pyridine-1-water.

A. trimetaphosphate
B. hexametaphosphate C. triphosphate
D. trimeta-, ortho-, tri- and hexametaphosphate E. Pyrophosphate
F. "carrier free" orthophosphate
G. orthophosphate labelled with 'carrier free' orthophosphate.

Especially interesting cases are shown in F, G in Fig. 4. In the case of F, "carrier-free" orthophosphate was developed. The peak can be seen only at the position of its origin. In the case of G the figure was obtained with the orthophosphate ion labelled with the "carrier-free" phosphate ion just mentioned, and showed two peaks. The same result was also obtained when the trimetaphosphate was used as carrier. Judging from the results of A, B and D, the right-side peak must be due to trimetaphosphate ions and the left-side peak is large enough to be considered due to the main component; i. e. the orthophosphate ions. The peak of trimetaphosphate ion must be an impurity of the "carrier-free" orthophosphoric acid which was imported from U. S. A.

Strain[8] has already made a report on a similar fact in the case of electrochromatographic separation for calcium phosphate, but he did not clearly mention what type of ions the impurity had.

## Summary

1) To separate various meta-and poly-phosphate ions respectively, pyridine-water (1:1) was found to be the most desirable developing solvent. Especially, both trimeta- and hexameta-phosphate ions can be separated from the ortho-, pyro-, and tripoly-phosphate ions, but the latter were not completely separated. The authors plan to separate them after further study of solvents.

However, by using labelled radioactive substances, it may possible to detect the impurity in synthesized phosphate or the traces of other phosphates in the main component. If the radioactive dilution method should be applied, this method may be used in determining nonactive substances. No special care was necessary in this case concerning the atom-exchange of phosphorus between active phosphate ions and non-active[1].

2) As shown in Fig. 4 it may be possible to determine hydrolysis products by the present simple method.

3) There is a certain regular relationship between the ionic structures and their Rf's. That is to say, Rf's will decrease in proportion to the increase of the number of phosphorus atoms contained in each kind of ions with chain structure, while trimetaphosphate having a cyclic structure has greater Rf value than others with chain structure.

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[7] M. Kobayashi and M. Shinagawa, *Kagaku no Ryoiki*, 8, No. 2, 39 (1954).

[8] T.R. Sato, W.E. Kisieleski, W.P. Norris and H.H. Strain, *Anal. Chem.*, 25, 438 (1953).