Rapid Determination of Ortho- and Polyphosphates in Soft Drinks

YASUHIDE TONOYAI and MASAIRO IWADA
National Institute of Hygienic Sciences, Osaka Branch, 1-1-43, Hoenzaka, Higashi-ku, Osaka, Japan

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ABSTRACT
Ortho-, poly (pyro- and higher)- and meta-phosphates can be separated from each other with two dimensional thin layer chromatography, the detection level being 1 µg as P₂O₅. For determination of ortho- and poly (including polymeta-)phosphates, an ion-exchange column chromatography was used. A single concentration elution with 0.2 M potassium chloride was effective for separating of orthophosphates from polyphosphates, and the residual polyphosphates could be recovered with 6 N hydrochloric acid. This system was used for a survey of polyphosphates in 61 samples of soft drinks sold in Osaka. Results showed that the total phosphate contents within the range of 0 - 0.420 g of P₂O₅/kg. Only three samples contained polyphosphates. The highest being 0.261 g of polyphosphate/kg in a soft drink containing 20% mango juice.

In Japan, phosphoric acid as well as its ammonium, potassium, sodium and calcium salts are permitted as orthophosphate food additives with no limitations or restrictions on their specification use being attached except calcium phosphates that are allowed only for nutrient use with a maximum limit of 10,000 ppm as calcium. So far as condensed phosphates are concerned, both sodium and potassium salts of pyrophosphate, polyphosphate and metaphosphate are designated as food additives. It is almost impossible to discriminate added orthophosphate from inorganic phosphates originally present in fruit juice used as raw material of soft drinks. Addition of polyphosphate to soft drinks is effective for chelating metals, prevention of discoloring, antioxidation, emulsification, dispersion of turbid substances and peptization. Though it had been considered that excess intake of polyphosphate results in lack of calcium and anemia, Lang (4) proved that it had no physiological effect. Since polyphosphates are not contained in natural resources, there arose the necessity of establishing a method for separative determination of ortho- and poly-phosphates in soft drinks. It was previously reported by one of the authors (7) that mono-, di-, tri-, tetra-, penta- and hexa-phosphates can be separated by use of ion-exchange chromatography. In this report, it was attempted to establish a simplified method of discriminating polyphosphates from orthophosphates, being accompanied with favorable results.

MATERIALS AND METHODS

Reagents and apparatus
1. Orthophosphate standard solution: Dissolve 1.917 g of KH₂PO₄ in water to make 1,000 ml. Dilute 10-ml portion of the solution to 1,000 ml with water. Each ml of the solution contains 0.01 mg of phosphorus as P₂O₅.
2. Sodium pyrophosphate (guaranteed) and sodium tripolyphosphate were supplied from Katayama Chemical Ind. Co., Ltd. Tetra and more highly condensed phosphates were prepared by heat-condensation of a mixture (5 : 4) of sodium dihydrogen phosphate (NaH₂PO₄•2H₂O) and sodium hydrogen phosphate (NaH₂PO₄•12H₂O) being followed by separative purification with ion-exchange column chromatography (5). Sodium metaphosphate (Na₂P₂O₇) was supplied by Katayama Chemical Ind. Co., Ltd., while sodium trimeta- and tetrameta-phosphates by Polyphos Chemicals Ltd.
3. Colour development solutions. Ammonium molybdate test solution: Dissolve 1 g of (NH₄)₆Mo₇O₂₄•4H₂O in a mixture of 5 ml of 70% perchloric acid and 1 ml of hydrochloric acid and make to 100 ml with water. Stannous chloride test solution: Dissolve 1 g of SnCl₂•H₂O in 10% hydrochloric acid to make 100 ml.
4. Preparation of ion-exchange column: A glass column of Ø 1.2 x 60 cm in length was packed with 40 g of Dowex 1 X 4 (100 - 200 mesh) by wetting method.
5. Eluants for ion-exchange column chromatography. Ammonium chloride buffer solution: Mix 0.01 N ammonia and 0.01 M ammonium chloride to obtain the final pH of 9.3. Potassium chloride buffer solution (0.20 - 0.35 M): Dissolve 15.0 - 27.5 g of KCl in-ammonia. ammonium chloride buffer solution to make 1,000 ml.
6. Plate for thin layer chromatography (TLC): Prepare plate by coating microcrystalline cellulose (Avicel SP) on glass plate at the thickness of 250 µm being followed by drying for 30 min at 80 C.
7. Developing solvents for TLC: Two dimensional TLC was carried out by use of two kinds of developing solvents, i.e. a mixture of 0.5% pyridine in acetone and 20% trichloroacetic acid (3 : 1) as the first and a mixture of isobutanol, ethanol, water and ammonia water (20 : 30 : 39 : 1) as the second developing solvent, respectively.
8. Atomic absorption spectrophotometer: AA-I, Mark II type of Nippon Jarrell Ash Co., Ltd. was used.
9. All the other reagents used were of analytical grade.

Qualitative analysis of phosphates with TLC
After removal of solid matter in soft drink sample by filtration with No. 5C (Toyo Roshi Co., Ltd.), develop the filtered solution together with phosphate standards, then detect the developed phosphates on the
plate by successive spraying of ammonium molybdate test solution, heating for 5 min at 80-90°C and spraying again with stannous chloride test solution. Phosphates will give blue spots.

**Quantitative analysis of total phosphates with atomic absorption spectrometry**

Pour 2.0 g of sample into a 300-ml digestion flask, add 50 ml of nitric acid and heat the mixture for 10 min. Then add 5 ml of 70% perchloric acid and continue heating until the digested solution becomes colorless and white fumes appear (volume is about 5 ml). Cool the solution to room temperature, then dilute with water to 50 ml. Take a portion (usually 10 ml) of this solution to a separatory funnel of 100 ml, then add 10 ml of 5 N perchloric acid, 4 ml of 5% ammonium molybdate solution and 20 ml of n-butyl acetate, in turn. Shake vigorously and, after separation into two layers, determine the molybdenum content of the phosphorus-ammonium molybdate complex in the upper layer by atomic absorption spectrometry, expressing the results as $P_2O_5$ content in each sample.

**Separative determination of phosphates with ion-exchange column chromatography**

Decolorize 20 g of soft drink with a small amount of activated carbon, filter and adjust the pH to 9.3 with 0.1 N ammonia, then put the whole solution on the top of the column. The flow rate should not exceed 1 ml/min. After the solution has nearly run empty, wash the column with 100 ml of ammonia-ammonium chloride buffer solution, and when this has run off add 200 ml of potassium chloride solution to elute orthophosphates. After nearly 200 ml of the effluent has been collected, charge 100 ml of 6 N hydrochloric acid on top of the column to elute polyphosphates. Determine phosphorus content of the last two fractions, using the procedure shown for quantitative analysis of total phosphates.

**RESULTS AND DISCUSSION**

**Qualitative analysis of phosphates with thin layer chromatography**

When various phosphates were developed according to previous our paper (2), separation of ortho-, pyro- and tripolyphosphate were good, but separation of triopoly(P₃O₈), trimeta(M₃) and tetrapoly(P₄) phosphate with tetrameta(M₄) phosphate were not enough and discrimination of pentapoly(P₅) and hexapoly(P₆) phosphate were no good.

Therefore, these problems were solved by secondary developing method of TLC plate. Paper chromatography of phosphates with a secondary developing system was reported (3) but the TLC method, which is superior in speed and separation, was not yet studied. Thin layer chromatogram of various phosphates is as shown in Fig. 1. As a result of using cellulose powder as adsorbent and various composition of solvent as developer, it became evident that a combination of isobutanol-ethanol-water-ammonia water (20 : 50 : 39 : 1) gave a good result; i.e. separation of various phosphates, especially trimeta(M₃) and tetrameta(M₄) phosphate having a ring-shaped structure was very good. The detection limit of phosphate with this method is 1 µg as $P_2O_5$.

**Comparison of total determination of phosphates by atomic absorption spectrophotometry and colorimetry**

The atomic absorption (A.A) method was compared with the Molybdenum blue (M.B.) method to determine total phosphates. In respect to sensitivity of determination, the A.A. method is about 10 times more sensitive than the M.B. method. In respect to stability of solution, the former is stable for 150 min but the latter sometimes increased gradually in absorbance. Therefore, Standard Methods of Analyses for Hygienic Chemists (1980) (6) uptakes 10 min after color development in the M.B. method. In respect to reproducibility of determination, the former (c.v. 7.6% in 1 µg/ml) is almost the same as the latter (c.v. 5.2% in 10 µg/ml). In respect to influence of coexistent substances, arsenic, silicon, antimony, etc. being complexed with ammonium molybdate, the influence is positively upon absorbance in M.B method (1), but the influence is minimal in the A.A method because the complex of phosphate is selectively extractable into the n-butyl acetate layer (2).

**Simplification of separative determination of phosphates by ion exchange column chromatography**

So far as inorganic phosphorus compounds are concerned, the orthophosphate content in fruit juice varies by the nature of the soil, quantity of phophatic manure used and other environmental conditions, which makes it difficult to discriminate artificial orthophosphate used as food additive from natural orthophosphate constituent derived from fruit juice used as raw material.

Contrary to this, pyro- and higher poly-phosphates are hardly present as natural constituents while their addition is known to be effective for thickening, dispersion of colloidal substances, prevention of discoloration, and so on.

Gradient elution of method I reported in previous paper (7) gave good separation of each other (as shown in Fig. 2), but it took considerable time to complete elution.
DETERMINATION OF PHOSPHATES

Figure 2. Elution pattern of ortho- and polyphosphates by gradient concentration column chromatography.
Resin: Dowex 1 × 4 (100 - 200 mesh)
Eluant: 0.20-0.35 M KCl in Ammonium ammonium chloride buffer solution (pH 9.3)
P₁, P₂, P₃, P₄ or P₅ represents mono-, di-, tri-, tetra- or penta-phosphate, respectively.
Each 1 mg of sodium salt was used.

Figure 3. Effect of potassium chloride on the elution pattern of various phosphates by anion exchange column chromatography.
Resin: Dowex 1 × 4 (100-200 mesh); Column: 1.2 × 60 cm
Buffer: 0.01 M Ammonia ammonium chloride buffer solution (pH 9.3)
Sample: 1,000 μg as P₂O₅

Figure 4. Elution patterns of orthophosphate in sample by simplified anion exchange column chromatography.

Accordingly, it was attempted to determine ortho- and poly-phosphates separately with the aid of ion-exchange column chromatography.

Influences of potassium chloride concentration on elution of phosphate are shown in Fig. 3. Isocratic elution of 0.30 M KCl in buffer is shown in method II. Elution of phosphate was very speedy but separation between orthophosphate and pyrophosphate was not sufficient. Isocratic elution of 0.20 M KCl in buffer is shown in method III. Elution of orthophosphate was speedy and separation with pyrophosphate was good, but the position of eluted triphosphate was very late.

Therefore, orthophosphate is eluted with 0.20 M KCl in buffer (200 ml) and 99.2% of orthophosphate was recovered. For eluting of polyphosphates, an addition of hydrochloric acid was effective since even sodium metaphosphate (NaPO₄)n, known to be least elutive, could be recovered with 50 ml of 6 N hydrochloric acid, recovery at 1 mg level as P₂O₅ being 91.3%.

To study the effect of coexisting substances in soft drinks, such as sugar, souring agent, flavorings, etc., the elution pattern of orthophosphate was determined with commercial sample A and B. The results are compared with the standard in Fig. 4. The eluted position of orthophosphate in samples A and B tended to be faster or later than that of the standard, but both phosphates were eluted with 200 ml of KCl in buffer with no migration of condensed phosphates. When trimeta- and tetrametaphosphate having ring-shaped structure were also added to the samples, these polyphosphates were satisfactorily eluted with 6 M hydrochloric acid (100 ml), recovery being 94.8% and 92.5%, respectively.

A comparison of A-simplified method (proposed method) with B-systematic method (original method) (7) is shown in Fig. 5. In the B-method, by the gradient elution system, polyphosphates were well separated from each other, but it took considerable time to complete the elution which makes it difficult to be utilized as a routine method. Contrary to this, in A-method, by using a single concentration of eluant, orthophosphate and polyphosphates were simply and speedily separated and the reproducibility of data was very high.

Qualitative and quantitative analysis of phosphates in commercial sample

An interlaboratory study of phosphate determination was carried out by use of mango drink, the results being summarized in Table 1. Recoveries of ortho- and polyphosphates (mean values) were 96.5 - 104.2 and 96.0 - 106.9%, respectively. Each value showed very good agreement. The difference between total phosphate and ortho-plus polyphosphates is attributed to phospholipids, nucleotides and other phosphorus compounds contained in their precipitates (8).

Finally, a survey was carried out on the actual condition of phosphate distribution in soft drinks sold in Osaka. From the survey of 61 samples, their phosphate contents were revealed to be within the range of 0 - 0.420 g/kg, expressed as P₂O₅ less than 0.100, 37 samples; 0.100 - 0.199, 7 samples; 0.200 - 0.299, 10 samples; 0.300 - 0.399, 4 samples; 0.400 - 0.499, 3 samples).

Only three samples were judged to contain poly-
TABLE 1. Results of an interlaboratory study of phosphates in mango soft drink containing 20% Juice.

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Orthophosphate P2O5 (g/kg)</th>
<th>Percent of mean value</th>
<th>Polyphosphate P2O5 (g/kg)</th>
<th>Percent of mean value</th>
<th>Total phosphate P2O5 (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.135</td>
<td>104.2</td>
<td>0.215</td>
<td>102.1</td>
<td>0.420</td>
</tr>
<tr>
<td>2</td>
<td>0.128</td>
<td>98.8</td>
<td>0.225</td>
<td>106.9</td>
<td>0.434</td>
</tr>
<tr>
<td>3</td>
<td>0.130</td>
<td>100.4</td>
<td>0.202</td>
<td>96.0</td>
<td>0.424</td>
</tr>
<tr>
<td>4</td>
<td>0.125</td>
<td>96.5</td>
<td>0.200</td>
<td>102.1</td>
<td>0.425</td>
</tr>
<tr>
<td>Mean value</td>
<td>0.1295</td>
<td></td>
<td>0.2105</td>
<td></td>
<td>0.425</td>
</tr>
</tbody>
</table>

\(^a\)Average of three trials.
\(^b\)The difference between total phosphate and ortho- plus polyphosphates are attributed to phospholipids, nucleotides and other phosphorus compounds contained in the precipitates.

TABLE 2. Distribution of ortho- and poly-phosphates in several soft drinks.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Orthophosphate P2O5 (g/kg)</th>
<th>Polyphosphate P2O5 (g/kg)</th>
<th>Total phosphate P2O5 (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (coffee)</td>
<td>0.115</td>
<td>0.047</td>
<td>0.186</td>
</tr>
<tr>
<td>B (guava)</td>
<td>0.030</td>
<td>0.013</td>
<td>0.054</td>
</tr>
<tr>
<td>C (mango)</td>
<td>0.100</td>
<td>0.261</td>
<td>0.393</td>
</tr>
</tbody>
</table>

\(^a\)Average of three trials.
\(^b\)The difference between total phosphate and ortho- plus polyphosphates are attributed to phospholipids, nucleotides and other phosphorus compounds contained in the precipitates.

Contrary to this, no polyphosphate was detected from grape-flavor soft drinks in the present survey.

REFERENCES