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Analysis of condensed phosphates in food products by ion chromatography with an on-line hydroxide eluent generator

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Abstract

An ion chromatographic method with gradient elution using an automated eluent generator was developed for the simultaneous determination of condensed phosphates (CPs) such as orthophosphate (P1), pyrophosphate (P2), polyphosphate, trimetaphosphate and phytate in food products. The linear calibration curves for P1, P2, tripolyphosphate, and tetrapolyphosphate in the range 0.5–500 μM had a correlation factor of 0.999 or better. The precision of the method for the CP peak areas obtained with the hydroxide eluent generator was better than that obtained with potassium hydroxide eluents prepared off-line. This method was applied to the determination of CPs in food products such as ham, fish paste, and cheese extracted by trichloroacetic acid.

Keywords: Food analysis; Gradient elution; Phosphates; Polyphosphates

1. Introduction

Condensed phosphates (CPs) are widely used as food additives in cheese, fish paste products, and ham and sausage to prevent discoloration and to stabilize vitamin C, as well as for other purposes. CPs are classified by their structure; for example, polyphosphates (PPs) have a linear chain structure, metaphosphates a ring structure, and ultraphosphates a long chain and crosslinked structure. They are often combined during the manufacturing process of food products. An excess intake of CPs inhibits the absorption of calcium at the intestine, so it is important to control their concentrations in food products. It is also important to determine the degree of polymerization for each individual PP because the stability of CPs in food is still not well understood.

Numerous analytical methods for determining CPs have been reported (e.g. anion-exchange HPLC with post-column molybdate colorimetric detection [1–3], or with indirect photometric detection [4,5], and capillary electrophoresis [6,7]). However, these methods are not easily applied to food analysis, because of the variety of matrices in foods. Another approach for the practical analysis of long-chain PPs in foods is based on the use of a low-capacity ion-exchange column with a nitrate gradient and post-column detection with iron(III) sulfosalicylate at 500 nm [8,9]. However, this method requires a very complicated system and is unable to detect orthophosphate (P1) and cyclic phosphates.

Dilute hydroxide solutions have been used increasingly as eluents in gradient ion chromatographic separation with suppressed conductivity detection, because they produce a low detection background

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[10–12]. Recently, we reported the simultaneous determination of CPs from P1 to heptapolyphosphate (P7) including phytate (IP6) and trimetaphosphate (P3m) by using gradient IC conductivity detection with sodium hydroxide (NaOH) eluent containing 15% methanol [13]. This method is easy to use and can be used to determine P1 and cyclic phosphate. However, the method has some practical problems, including: (1) the complicated procedure for preparing highly pure NaOH; (2) the need to protect the eluent from carbonate contamination [14]; (3) the safety issue associated with using high concentrations of NaOH as an eluent; and (4) the decrease in sensitivity when methanol is added to the eluent to improve the separation of P3m.

A commercial, automated, on-line eluent generation system (EG40 eluent generator module, Dionex, Sunnyvale, CA, USA) was recently developed [15]. The EG40 eluent generator system electrolytically produces high-purity potassium hydroxide (KOH) eluents using deionized (DI) water as the carrier stream at the point of use. In this paper, we report the results of a study in which we compared the use of hydroxide eluents generated by the EG40 KOH eluent generator system to the use of hydroxide eluents prepared off-line for high precision, sensitive determination of CPs in food samples. KOH eluents generated by the EG40 eluent generator are free of carbonate contamination. The use of the EG40 hydroxide eluent generator leads to negligible baseline shifts during the hydroxide gradients, greater retention time reproducibility, and better method precision for target analytes.

2. Experiments

2.1. Apparatus

A DX-500 ion chromatography (IC) system (Dionex) consisting of a gradient pump, a 10-μl sample loop, and a conductivity detector was used in this study. Dionex IonPac AS11 (2×250 mm) and IonPac AG11 (2×50 mm) columns packed with anion-exchange resin were used as the separation columns. The Dionex ASRS-Ultra anion self-regenerating suppressor was operated in the external water mode at 300 mA. The Dionex PeakNet workstation was used for data processing.

A Dionex EG40 eluent generator equipped with an EGC-KOH cartridge was used. The EGC-KOH cartridge was placed between the pump outlet and the EG40 degas assembly inlet. The degas assembly outlet was connected to the sample injector. A high-capacity, anion-exchange column was placed at the pump outlet to remove the small amount of dissolved carbon dioxide and carbonate in the DI water.

2.2. Water and reagents

DI water with a specific resistance over 18 MΩ from a Milli-RO/Milli-Q system (Millipore, Bedford, MA, USA) was aspirated for degas and used to prepare all eluents and standards. Trichloroacetic acid (TCA), KOH, disodium orthophosphate (P1), and tetrasodium pyrophosphate (P2) were purchased from Wako (Osaka, Japan), and pentasodium tripolyphosphate (P3), hexaammonium tetrapolyphosphate (P4), trisodium trimetaphosphate (P3m), inositol hexaphosphoric acid (IP6), and myoinositol 1,3,4,5,6-pentakisphosphate (IP5) were from Sigma (St. Louis, MO, USA). The stock solutions (20 m*M* each) of the target analytes were prepared in DI water and then stored in a refrigerator for further use. Working standard solutions were prepared daily by appropriate dilution of the stock solution.

The previously reported sample treatment procedure [10] was used. Samples (5 g each) were homogenized with cooled 4% TCA, centrifuged for 10 min with 3000 rev./min, diluted ten times with DI water, and passed through a 0.45-μm membrane filter.

3. Results and discussion

3.1. Column selection

Our previously developed method [13] used a Dionex OmniPac PAX-100 column. It was necessary to add methanol to the hydroxide eluent to improve the peak resolution and to induce the early elution of highly retained anions. Unfortunately, the use of methanol caused a reduction of the peak response

and an increase in baseline noise. Baluyot and Hartford [12] have reported that the AS11 column is a better chromatographic column for characterizing PPs than the PAX-100 column. The AS11 column has a higher hydroxide selectivity than the PAX-100 column, and the adding methanol is not required for separating CPs. In this study, we used 2-mm AS11 columns. The use of 2-mm columns operated under lower flow-rates makes it possible to use higher concentrations of hydroxide eluent with the suppressor. The separation of PPs from P1 to P52 using this microbore column with KOH gradient of 30–200 m*M* over 25 min is shown in Fig. 1.

3.2. Optimization of separation condition

Despite the benefits of hydroxide eluents, their use in IC is often hampered by problems associated with carbonate contamination. Carbonate can be introduced as an impurity from the source chemical or, more frequently, through the adsorption of carbon dioxide from air when preparing and using eluent. Varying levels of carbonate in the hydroxide eluent can cause baseline shift (i.e. an increase in the background conductivity) during hydroxide gradients and affect the retention time reproducibility. Severe baseline shifts during the hydroxide gradients can compromise the quantitation of target analytes. To overcome the problems associated with carbonate contamination, we used the EG40 KOH eluent generator as the source of highly pure, carbonate-free hydroxide eluent. The EG40 eluent generator system electrolytically produces high-purity KOH eluents using DI water as the carrier stream at the point of use. KOH eluents generated by the EG40 eluent generator are free of carbonate contamination.

We optimized the IC gradient condition using the EG40. Since too much TCA used at the sample extraction could interfere with the determination of

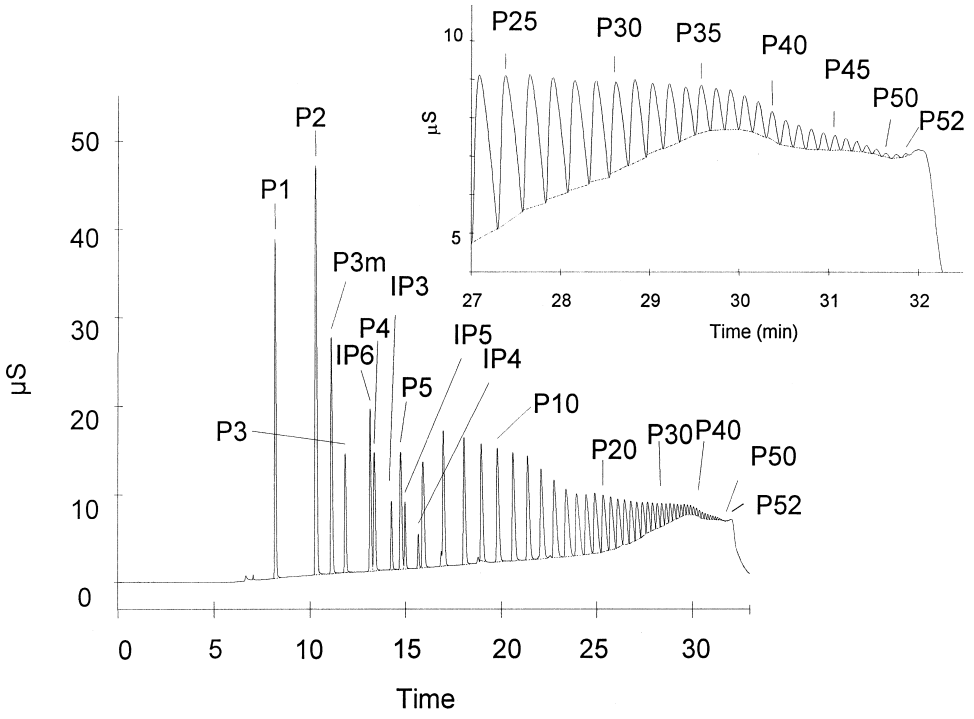

Fig. 1. Typical chromatogram of PPs on AS11 column. IC conditions, eluent gradient 30–200 m*M* NaOH over 30 min; flow-rate, 0.5 ml/min; suppressor, ASRS-ULTRA; oven, 40°C; injection volume, 10 μl. Sample: sodium phosphate glasses type 15 (Sigma) dissolved at 1 mg/ml.

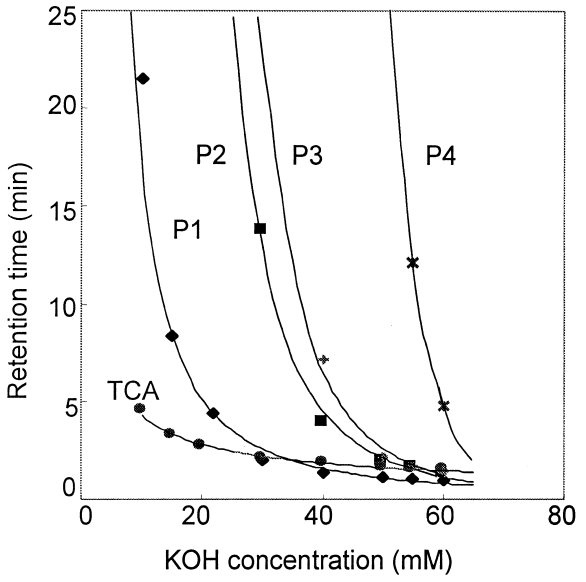

Fig. 2. Effect of initial concentration of the eluent on the separation of PPs and TCA. Eluent: KOH by EG-40, other conditions are the same as in Fig. 1.

P1 because they are closely eluted with the target analytes on the AS11 column, we optimized the initial eluent concentration to improve the separation between TCA and P1. When the eluent concentration was less than 15 m*M* KOH (see Fig. 2), TCA and P1 were well separated. The optimal gradient conditions include an isocratic separation at 15 m*M* KOH for 8 min until P1 is eluted, and then a linear hydroxide gradient from 15 to 100 m*M* KOH over 17 min so that IP6 and P3m are completely separated from the PPs.

We compared the performance of the IC method for determining CP using KOH eluents generated by the EG40 or prepared off-line, and obtained better peak area and retention time reproducibility for each target analyte with the EG40. Table 1 summarizes the peak area and retention time precision data for CPs. The relative standard deviations ($n=7$) of peak areas of P1, P2, P3, P4, P3m, and IP6 (10 μ*M* each) were less than 2.0% with the EG40, and ranged from 1.4 to 11.3% with conventionally prepared KOH. The relative standard deviations ($n=7$) of retention times ranged from 0.01 to 0.41% with the EG40 and 0.07 to 0.75% with conventionally prepared KOH. Using EG40, we determined that the linear calibration curves for P1, P2, P3, P4 had correlation coefficients of 0.999 or higher in the concentration range of 1–500 μ*M*. As shown in Fig. 3, the slopes of calibration curves for PPs were nearly proportional to the phosphorus numbers. Although we do

Table 1
Comparison of KOH solution and EG40 as the eluent in repeatability of retention time and peak area

	P1	P2	P3	P4	P3m	IP6
KOH prepared off-line						
Retention time						
Average (min)	8.51	17.28	19.30	21.19	17.67	21.63
RSD (%)	0.75	0.08	0.07	0.07	0.12	0.16
Peak area						
Average (counts)	24 694	61 680	88 447	65 018	66 685	133 480
RSD (%)	11.26	7.99	6.35	7.11	8.17	1.40
KOH generated by EG-40						
Retention time						
Average (min)	7.78	16.10	18.00	19.65	16.52	20.45
RSD (%)	0.41	<0.01	0.08	0.09	0.06	0.12
Peak area						
Average (counts)	29 098	66 760	100 451	81 760	82 436	149 478
RSD(%)	1.28	1.77	1.77	1.64	1.51	1.45

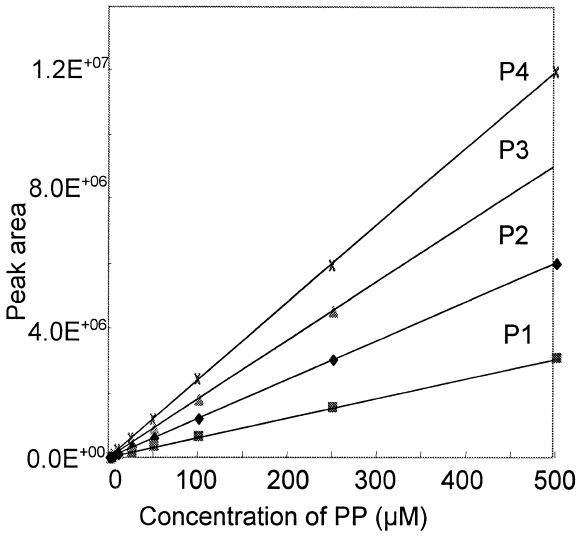

Fig. 3. Calibration curves of PP. Experimental conditions see text.

not understand why the responses for PPs appear to be proportional to the phosphorous number, the results suggest that it may be possible to use the PPs of low-phosphorous numbers as the standard to quantitate the PPs of high-phosphorus numbers for which standards are not available. As expected, the hydroxide gradient using EG40 yielded minimal baseline shift (about 0.08 μs) during the gradient and other hand, the baseline shift was about 10 μs with KOH eluents prepared conventionally. Thus, we were able to accurately determine components strongly retained on the column, even at low concentrations. These results suggest that the EG40 improves the performance of the IC method in determining PPs. KOH concentration generated by EG40 is confined to 100 m*M*. PPs over degree of polymerization 8 (DP8) cannot be eluted from the AS11 column under this condition. However, either PPs over DP8 do not exist in food products or their concentrations are very low [9]. Thus, the IC method using EG40 described in this paper is adequate for determining common PPs in food samples.

3.3. Applications to samples

Food products like cheese may contain high concentrations of calcium. When calcium exists in a sample solution, peak distortion and retention time shortening were observed for P4 and IP6. Moreover, their peak areas decreased with the addition of more calcium (Fig. 4). The sample solutions were therefore passed through the cartridge packed with cation-exchange resin to remove the calcium prior to injection. Fig. 5 shows typical chromatograms where the proposed method was applied to ham, fish paste and cheese samples. An isocratic condition of 15 m*M* KOH was used to separate TCA and T1, so the peak shape of P1 was tailing. In this condition, the detection limits ($S/N=3$) of each CP were 0.5, 0.16, 0.12, 0.16, 0.16, and 0.19 μ*M* for P1, P2, P3, P4, P3m, and IP6, respectively.

In conclusion, use of the IC method in conjunction with the EG40 described above is suitable for the simultaneous determination of CPs in food products by gradient IC. Use of the EG40 hydroxide eluent generator leads to negligible baseline shifts during the hydroxide gradients, greater retention time reproducibility, better method precision, and lower method detection limits for target analytes in the analysis of CPs in food samples.

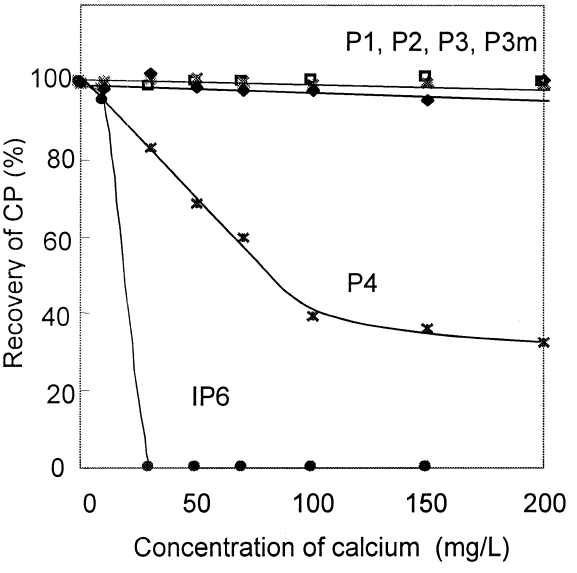

Fig. 4. Effect of calcium concentration on peak area of CPs. Concentrations injected are 10 mg/l each.

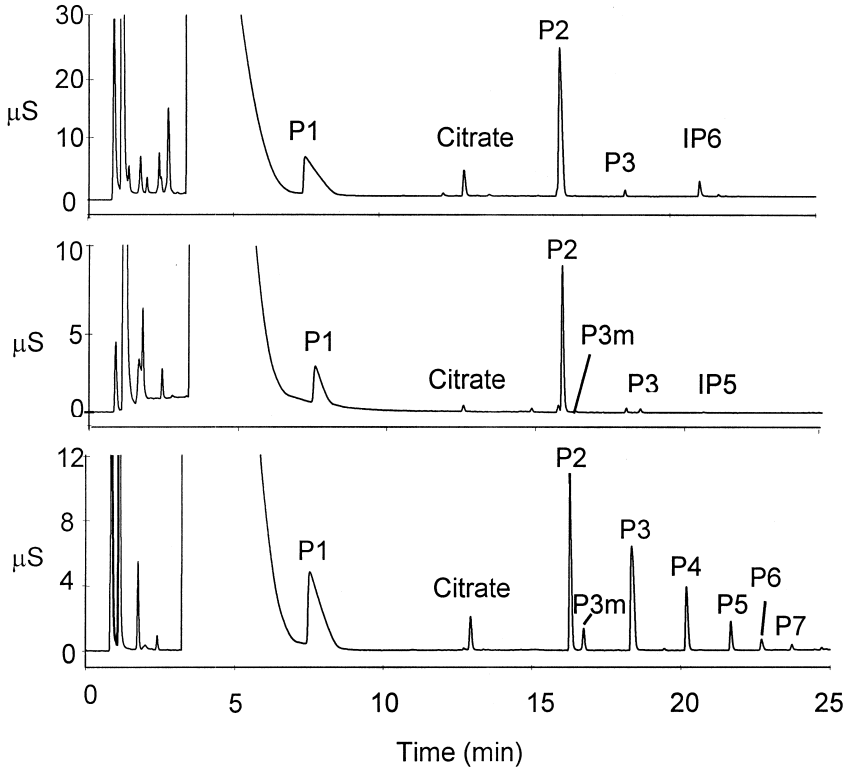

Fig. 5. Chromatograms of extracts from ham (upper), fish paste (center) and cheese (lower). Analytical conditions see text.

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